International Congress on Transposable Elements

Saint Malo - France

April 21-24, 2012

ICTE 2012

GDR 2157

Société Française de Génétique

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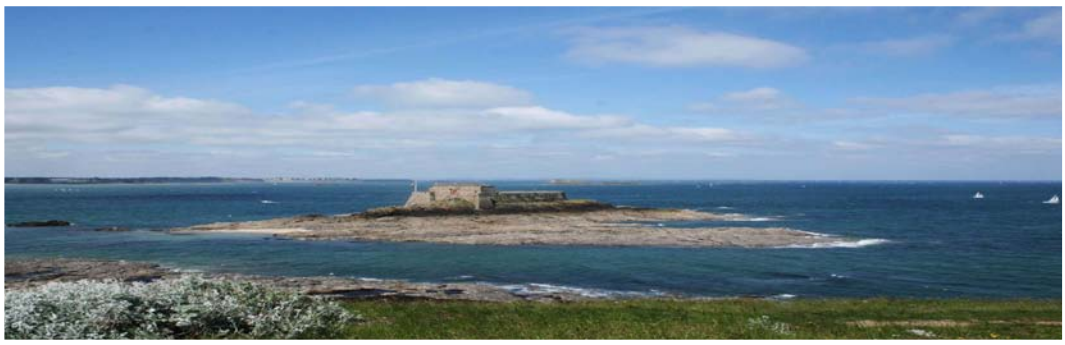
Mobile DNA

ICTE2012: International Congress on Transposable Elements



The French Society of Genetics and the French Transposition Community (CNRS) are happy to announce the 2nd International Congress on Transposable genetic elements. Transposable elements are at the heart of biology. Since their discovery in the 1940's, they have been shown to contribute significantly to genome structure, evolution and function. Their study aims at understanding their biological success and to characterize their contribution to fundamental biological functions. The increasing interest in these elements is strongly linked to our recent knowledge of genome organization and function. They are major players of phenotypic diversity but they are also involved in diverse human pathologies. Transposable element studies cover a broad spectrum of organisms and a large variety of biological processes and methodologies. The aim of this international congress unique in Europe is to integrate recent knowledge to design new concepts and to identify future orientations of research. Several topics will be covered such as, the evolution and activity of transposable elements, their dynamics within genomes and populations, their mechanisms of transposition and control and their impact on genomes.

Organisers:

K. Ainouche
M. Bétermier
M. Chandler
R. Cordaux
G. Cristofari
J.M. Deragon
H. Quesneville
C. Vitte
P. Lesage
D. Mazel
C. Vaury
C. Vieira

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Scientific Program - ICTE 2012

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8:30 - 11:00 pm	**Welcome Reception**

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9:00 am - 3:20 pm

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Chair: Cédric Feschotte, Clémentine Vitte

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9:50 am	New insights into the association of ISs with conjugative elements and transposition specificity: characterization of new IS*256* related IS families **Philippe Glaser**
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3:50 - 5:00 pm

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Chair: Chantal Vaury, Cristina Vieira

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Elena Casacuberta

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Claudia Marcia Aparecida Carareto

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Vincent Parissi

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Bernard Hallet

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Clément Monot (Cristofari lab)

4:50 pm — *BARE* retrotransposons are translated and replicated via distinct RNA pools
Alan Schulman

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Fred Dyda

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6:00 pm — Single strand (HuH) transposases and genome dynamics
Bao Ton-Hoang

6:30 pm — Emergent properties of a DNA looping reaction: lessons from mariner transposition
Ronald Chalmers

6:50 pm — Programmed DNA elimination in Tetrahymena – recognition and excision of chromatin by the PiggyBac-like transposase Tpb2p
Alexander Vogt

7:10 pm — Remnants of Tc/*mariner* elements are excised precisely by a *piggyBac* domesticated transposase and the NHEJ double-strand break repair pathway in the ciliate *Paramecium*
Mireille Bétermier

8:30 pm — Banquet

Deep sequencing of yeast Ty1 and human L1 retrotransposon integration targets

Jef D. Boeke, Loris Mularoni, Lisa Cheng Ran Huang, Yulian Zhou, Tyson Bowen, Sunil Gangadharan, Jared Steranka, Anne Pulver, Sarah J. Wheelan, and Kathleen Burns

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Saccharomyces cerevisiae Ty1 elements target regions upstream of tRNA genes and other Pol III-transcribed genes when retrotransposing to new sites. We used deep sequencing of Ty1-flanking sequence amplicons to characterize Ty1 integration. Surprisingly, some insertions were found in mitochondrial DNA sequences, presumably reflecting insertion into mitochondrial DNA segments that had migrated to the nucleus. The overwhelming majority of insertions were associated with the 5' regions of Pol III transcribed genes; alignment of Ty1 insertion sites revealed a strong sequence motif centered on but extending beyond the target site duplication. A strong sequence-independent preference for nucleosomal integration sites was observed, in distinction to the preferences of the Hermes DNA transposon engineered to jump in yeast and the Tf1 retrotransposon of *Schizosaccharomyces pombe*, both of which prefer nucleosome free regions. Remarkably, an exquisitely specific relationship between Ty1 integration and nucleosomal position was revealed by alignment of hotspot Ty1 insertion positions regions to peak nucleosome positions, geographically implicating nucleosomal DNA segments at specific positions on the nucleosome lateral surface as targets, near the "bottom" of the nucleosome. The specificity is observed in the three tRNA 5'-proximal nucleosomes, with insertion frequency dropping off sharply 5' to the tRNA gene. The sites are disposed asymmetrically on the nucleosome relative to its dyad axis, ruling out several simple molecular models for Ty1 targeting, and instead suggesting association with a dynamic or directional process such as nucleosome remodeling associated with these regions.

Deep sequencing has also been used to carry out screens of human genomes for polymorphic insertions of human non-LTR retrotransposons (retrotransposon insertion polymorphisms). We find a wide variety of such RIPs across a broad swath of human populations. However, frequencies of individual presence/absence alleles vary quite dramatically. We are also evaluating frequencies of RIPs in schizophrenic and control populations.

The primate mobilome

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Primate mobile elements (SINEs, LINEs and SVA elements) belong to discrete subfamilies that can be differentiated from one another by diagnostic nucleotide substitutions. An analysis of several recently integrated mobile element lineages was undertaken to assess mobile element associated primate genomic diversity. Our screening of the mobile elements resulted in the recovery of a number of "young" Alu, L1 and SVA elements with different distributions throughout the primate lineage. Many of the mobile elements recovered from the human genome were restricted to the human lineage, with some elements that were polymorphic for insertion presence/absence in diverse human populations. The distribution of Alu, L1 and SVA elements throughout various primate genomes makes them useful tools for resolving population genetic relationships and non-human primate phylogenetic relationships. We have also characterized the structural genomic variation associated with the insertion of recently integrated mobile elements in primate genomes along with post insertion recombination based events. These genomic deletions are yet another source of mobile element associated genetic variation within the primate lineage.

Alu/Alu Non-Allelic Recombination

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Alu elements are the most abundant retroelements in the human genome, with over 1,000,000 copies. Thus, they represent the single most common cause of non-allelic homologous recombination (NAHR), which contributes significantly more to human genetic instability than does their insertional mutagenesis. There are a number of genes that appear to be subject to recurrent Alu/Alu recombination events that contribute to disease. These include recurrent deletions or duplications within the MLL, MSH2, VHL, and BrCA1 genes contributing to cancer. The exact reasons for some regions to be more prone to this form of recombination are unknown but may involve the density and relative similarity of Alu elements in these genes, as well as potential broader chromosomal contributions.

We have created a novel cassette-based recombination assay system that for the first time allows the evaluation of a broad range of potential Alu-related sequence factors for their impact on mutagenic recombination. This cassette can be altered and replaced at the same genomic site in specific cells, allowing the testing of multiple variant constructs in the same chromosomal and cellular environment. By expressing the I-SceI endonuclease in these cells, we can create and measure a high level of Alu/Alu recombination. Replacing one of the Alu elements with variations having different percentage and distribution of mismatches demonstrates a strong dependence on sequence identity, as well as distribution. Analysis of the recombination junctions showed that the recombination events were spread fairly evenly across the Alu element, with no major hotspots. Having a single longer region of homology increased the recombination rate, but surprisingly the recombination events did not map specifically to that longer region. With perfectly matched Alu elements, our system detects only Alu/Alu NAHR events. However, as mismatch is increased, deletions caused by non-homologous end joining (NHEJ) become dominant. Surprisingly, at moderate levels of mismatch (5-10%), both NAHR and NHEJ recombination in our vector is suppressed. Thus, there is a complicated interplay of stimulation and suppression of local recombination caused by Alu elements.

Ongoing studies are testing other variables that are likely to contribute to Alu/Alu NAHR with the ultimate goal of predicting genomic regions that are likely to be most sensitive to this form of genetic instability.

Keywords : Recombination, endonuclease, LINE-1, Alu

New insights into the association of ISs with conjugative elements and transposition specificity: characterization of new IS*256* related IS families

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Although most transposable elements (TE) commonly used to construct insertion mutant libraries show weak insertion specificity, transposable element target sites are generally not random. TEs display variable degree of selectivity, and have also often evolved to minimize the burden of their insertion onto the host. We have identified and characterized a diverse family of integrative and conjugative element (ICE) called Tn*GBS* which is widespread in the *Streptococcus* genus. These complex modular elements were the first described ICE which associates a DDE transposase derived from Insertion Sequence (IS) for their integration into host chromosome and not a serine or a tyrosine recombinase of phage origin. Analyses of the Tn*GBS*s and related IS natural insertion sites and transposition under laboratory conditions demonstrate the unique insertion specificity of these TEs in bacteria: upstream σA promoters. Although these transposases were not detected as similar to previously characterized transposases by BLAST, iterative psi-blast permits to link this new family both to different clades of unannotated transposases and to the distantly related and widespread mutator like IS*256* family. We identified by multiple sequence alignments three conserved domains in Tn*GBS* related transposases and by inference based on experimental data from IS*256* we accurately predicted the catalytic DDE residues and a potential DNA binding domain probably implicated in the recognition of the inverted repeats. Interestingly among the different new transposase lineages identified only two distant clades share the insertion specificity upstream promoters. Thus, by differential sequence similarity comparison between transposases sequences from distant lineages we identified a sequence signature potentially implicated in the insertion specificity. Strikingly, although the large majority of element coding for these transposase are encoded by IS, we identified several cases of transposase recruitment by different conjugative elements. This observation shows the compatibility of conjugation with transposition mechanism of this TE family implying a double stranded circular intermediate.

Keywords : ICE, IS, transposase, transposition specificity

Integration profiling; a genome-wide map of functional sequence

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Next generation DNA sequencing has greatly accelerated the discovery of coding and noncoding genes, and determining their function represents one of the greatest challenges of genomic and systems biology. We describe here integration profiling, a novel approach to identify the function of eukaryotic genes based upon dense maps of transposon integration. As a proof of concept, we generated a large library of transposon Hermes insertions in *S. pombe* cells. With integration profiling, large populations of cells with single insertions of Hermes are grown for many generations. Cells with insertions in genes important for growth, become depleted in the culture. Deep sequencing of insertion sites reveals which insertions cannot be tolerated under a specified growth condition. In these experiments we determined which genes are required for cells to grow in defined medium. Our culture of *S. pombe* with Hermes insertions was grown for 80 generations and from these cells we sequenced 360,000 independent insertions. This represents one integration for every 29 nt of the genome. ORFs fell into two classes with one group having significantly higher densities of integration. Using essentiality designations from the deletion consortium (1), we found the ORFs with the higher density of integration corresponded to the nonessential genes while the ORFs with low numbers of insertions corresponded to the essential ORFs. Importantly, nonessential genes important for reaching full colony size had intermediate levels of insertion. This result indicated that integration profiling can not only identify which genes are essential for growth but can also measure how much the nonessential genes contribute to growth rates. Discrepancies between the genes designated nonessential by the deletion consortium and our integration data revealed that approximately 10% of the consortium deletion strains still retain ORFs said to be deleted. We are now using integration profiles to identify genes that contribute to other functions. Close examination of chromatin structure at insertion sites revealed Hermes has a preference for nucleosome free positions. As a result, insertions within ORFs exhibited a 150 bp pattern of periodicity that correlated to sites between positioned nucleosomes.

Keywords : integration profile, transposon, Hermes, *Schizosaccharomyes pombe*

1. Kim et al, Nat Biotechnol. 2010 Jun;28(6):617-23

Studies of the Human LINE-1 RNP Complex

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Long INterspersed Element-1s (LINE-1s or L1s) are autonomous non-LTR retrotransposons that comprise approximately 17% of human genomic DNA. The vast majority of L1 copies cannot mobilize (*i.e.,* retrotranspose) to new genomic locations due to the accumulation of mutations that occur either during or after retrotransposition. However, a small cohort of approximately 80-100 L1s retains the ability to retrotranspose in the average human genome. Indeed, ongoing L1 retrotransposition continues to impact human genetic diversity.

More than twenty years ago, the isolation of a retrotransposition-competent L1 (RC-L1), identified as the progenitor of a *de novo* mutagenic L1 insertion that caused a sporadic case of hemophilia A, opened the door for a detailed mechanistic examination of L1 retrotransposition. The subsequent development of a cultured cell assay has provided unprecedented insight into the molecular mechanism of L1 retrotransposition. A round of retrotransposition initiates when an RC-L1 is transcribed from its original genomic location to generate an L1 mRNA containing two non-overlapping open reading frames (ORF1 and ORF2). This bicistronic L1 mRNA is notable as most eukaryotic mRNAs are monocistronic. ORF1 encodes an RNA-binding protein and ORF2 encodes a high molecular weight protein containing functional endonuclease and reverse transcriptase activities; both ORF1p and ORF2p are essential for L1 retrotransposition. Once translated, ORF1p and ORF2p bind their encoding mRNA, a phenomenon known as *cis*-preference, and assemble into a ribonucleoprotein (RNP) complex. Components of the L1 RNP then gain access to the nucleus where L1 integration occurs at a new chromosomal location by a mechanism termed target-site primed reverse transcription (TPRT).

In this presentation, I will discuss our ongoing studies of ORF1p, ORF2p, and the formation of the human L1 RNP complex. I will also present recent findings that help elucidate the unconventional translation mechanism of ORF2.

KAP1 control of *cis*-acting activators derived from endogenous retroviruses is essential to preserve pluripotency transcriptional networks

Helen M. Rowe, Adamandia Kapopoulou, Andrea Corsinotti, Johan Jakobsson, Liana Fasching, Todd Macfarlan, Stephane Viville, Baraah Bakhubie, Sonia Verp, Sandra Offner and Didier Trono

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KAP1 (also called TRIM28) is a co-repressor highly expressed in embryonic stem (ES) cells, which together with its enzymatic partner the histone methyltransferase ESET (or SETDB1) is responsible for the transcriptional silencing of endogenous retroviruses (ERVs). KAP1 has also been implicated in preserving the self-renewal capacity of ES cells, since upon KAP1 depletion these cells die or differentiate, while KAP1 knockout embryos fail to develop. We therefore asked whether there was a link between ERV control and maintenance of transcriptional networks in ES cells.
We first observed that many of the genes upregulated in KAP1 knockout ES cells were tissue-specific, harboring so-called bivalent or poised promoters, which in wild-type cells bore both the activating H3K4me3 and the repressive H3K27me3 histone marks. Furthermore, we found that these genes lay significantly closer to ERVs than genes that were downregulated or unaffected when KAP1 was deleted.
To look into the mechanisms of this apparent interplay between KAP1-mediated ERV silencing and transcriptional control of neighbouring genes, we mapped chromatin and DNA methylation marks across one such ERV (IAP575) and its adjacent poised gene, *Zfp575*, located upstream in the same orientation. Results showed that KAP1 and ESET bound to the IAP575 sequence, which was highly DNA methylated, but not to *Zfp575*, even though the repressive chromatin marks H4K20me3 and H3K9me3 did spread from the IAP several kilobases into the *Zfp575* gene body, with loss of these marks upon KAP1 deletion. H3K9me3 chromatin immunoprecipitation followed by sequencing (ChIP-Seq) then confirmed the KAP1-dependent deposition of this mark at hundreds of ERVs, along with its short-range spreading into nearby genes. Remarkably, KAP1 knockout induced chromatin signatures present at active enhancers over the sequences of multiple IAPs including IAP575. Providing functional support to this observation, KAP1-targeted IAP sequences acted as enhancers in reporter assays in cells that had lost KAP1-repression activity.
These data indicate that ERVs are transcriptional landmines, the KAP1-mediated control of which is essential to preserve the transcriptional networks that are at the heart of ES cell pluripotency. Regulation of retroelements is, therefore, critical not just to prevent retrotransposition, but more broadly to safeguard the timely activation of genes through development.

Keywords : Endogenous retrovirus, KAP1, poised genes, pluripotency, active enhancers

Complex relationships between ERVs, heterochromatin spreading and genes

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Transposable elements (TEs) are often thought to be harmful because of their potential to spread heterochromatin (a repressive chromatin state) into nearby sequences. However, there are few examples of spreading of heterochromatin caused by TE insertions, even though TEs are often found in regions of repressive chromatin. We developed a model to study heterochromatin induction by TEs in a natural system. We studied two mouse embryonic stem cell lines from different mouse strains harboring polymorphic retrotransposons, such that one cell line possesses a particular TE copy (full site) while the other cell line lacks the copy at the same genomic location (empty site). We then compared the chromatin state of these full and empty sites. From all the LINEs and ERVs analyzed, IAP ERVs are the strongest inducers of repressive histone marks, which extend for at least 1 kb from the insertion site and can be detected up to 5 kb away in some cases. We found only one gene promoter that was partly silenced in ES cells by induced heterochromatin of a nearby polymorphic IAP insertion. The lack of such cases suggests a strong negative selection against IAP insertions near genes. We wondered if genomic environment could influence ERV heterochromatin as already observed in *A. thaliana* and for human *Alus*. We compared the state of DNA methylation of ERVs in different genomic environments, for both IAP and ETn/MusD copies. While IAPs are uniformly heavily methylated in adult tissues, ETn/MusDs show decreased DNA methylation when near transcription start sites (TSSs) of actively transcribing genes. Furthermore, we found that full-length ERVs present a bias of DNA methylation such that the LTR (long terminal repeat) nearest the TSS of a gene is hypomethylated compared to the other LTR. DNA methylation spreading from highly methylated ERV copies to nearby genes is not observed, with the regions between TEs and genes likely acting as boundaries protecting the unmethylated gene promoter. The boundary region seems to bind CTCF and is enriched in permissive histone marks as H3K4me3. The presence of unmethylated copies near genes is associated with exaptation of the LTR promoter and increase expression of the nearby gene. Therefore, the epigenetic states of ERVs and genes can exert influence on each other and the forces implicated in this equilibrium remain unknown.

Keywords : Epigenetics, ERVs, heterochromatin spreading

The role of genomic repeats in gene regulation

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Next-generation sequencing (NGS) technologies (e.g. ChIP-Seq, RNA-Seq) are now supplanting array-based technologies because of their accuracy, comprehensiveness and cost. Notably, these technologies enable an unbiased look at the functional contributions of the genome including the contributions of repetitive regions. We will present results that demonstrate the ubiquitous role that play repeats in gene regulation. In particular, we will show that species-specific transposable elements have been an important source of new regulatory elements and have contributed more than 20% of the binding sites of key transcription factors in human ES cells. We will also present results from the analysis of DNaseI hypersensitivity and ChIP-Seq ENCODE datasets in normal, embryonic and cancer cell lines that demonstrate that in all cell types between 8% and 12% of the open chromatin regions have been contributed by transposable elements with a significant over-representation of endogenous retroviral (ERV) repeat families. Interestingly, we further show that hundreds of these transposons-derived sequences are activated in a cell-type specific manner. Although we find that most of this tissue-specific activity on transposon-derived sequences is detected in embryonic stem cells and cancer cell lines, a few repeat families are also open in distinct differentiated cell types. Given that various repeat families are polymorphic across individuals, characterizing their regulatory activity is likely to be critical to understand functional variation and susceptibility to disease.

LTR retrotransposons control fruit-specific, cold-dependent accumulation of anthocyanins in blood oranges

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The derivation of blood orange has been an outstanding question in horticulture for several centuries. Consumer interest in blood oranges has risen significantly in recent years with the demonstration that consumption of blood orange juice promotes health, perhaps most strikingly in the demonstration that it prevents obesity in mice fed a high fat diet. Despite increasing consumer interest, the supply of blood oranges, especially as juice, has not increased in recent years because of production limitations resulting from a heavy dependency on low temperatures for the development of strong red pigmentation in fruit.

We have been investigating the molecular basis for the blood orange trait and shown that all Mediterranean blood oranges are derived from a single event involving insertion of a copia-like retrotransposon in a gene encoding a transcription factor that regulates anthocyanin biosynthesis. We show that the regulatory gene has come under the control of the retrotransposon LTR that provides a promoter with a TATA box, a transcriptional start and a 5' donor splice site for the first intron in the transcript.

The cold-dependency of fruit pigmentation reflects the induction of expression of the retroelement by this abiotic stress.

Amongst blood orange accessions in China we have discovered an event that has occurred independently of the blood oranges of Mediterranean origin; it is caused by a related retrotransposon and promotes anthocyanin biosynthesis through inducing expression of the same regulatory gene through a parallel but distinct mechanism. This new retroelement is inserted in opposite orientation and provides an upstream activator sequence that controls the expression of the regulatory gene.

Our analyses shed light on the derivation of blood oranges and, through defining the molecular mechanisms underpinning the blood orange traits, offer insight into how the temperature dependency could be overcome in future crop improvement strategies.

Different accessions of blood orange demonstrate the high levels of recombination and transposition associated with retroelements and suggest that they may be responsible for generating much of the diversity available in Citrus.

Keywords : LTR retrotransposons, anthocyanins, Citrus, transcription factors

Comparative genome-wide analysis of LTR-retrotransposition in plant kingdom

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LTR-retrotransposons (LTR-RTs) are the most abundant transposable elements in most eukaryotic genomes and especially in plants. These elements contribute to genome structure and evolution in such an extent that they are considered as the main factors of genome size variation. The genomic expansions associated with the activity of LTR-RTs have been studied in several genome species. These usually occur through transpositional bursts that lead to large increases of their copy number. Following such bursts, these copy are quickly eliminated through deletions. Consequently, most of the TE-associated genomic structural changes appear to be recent (mostly within the last 2 million years), although it is clear that such process must have recurrently shaped plant genomes for much longer time. Several questions regarding the dynamics of retrotranspositional bursts remain unanswered. In particular, whether one or few families contribute to genomic amplifications or rather many of the hundreds that populate the genome.
We develop a new pipeline for the precise identification of LTR-RTs (using LTR-harvest) and their annotation using a clustering strategy (using silix program). We applied this pipeline to all sequenced plant genomes and clearly show that in all cases only a limited number of families contribute to recent genomic expansions, regardless the genome size of the species. We discuss these results in the light of the recent advances in the knowledge of the TE silencing pathways in plants and propose a new model for TE-driven plant genome evolution.

Keywords : LTR-retrotransposons, comparative genomic, TE annotation

Impact of transposable elements on transcriptome: the example of Drosophila melanogaster

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We study transposable elements (TEs) impact on gene transcription using high-throughput sequencing (RNA-Seq). TEs, which turn out to be expressed in euchromatin as well as in heterochromatin interact with genes at different levels.
Some –usually short– TE copies bear a transcription start site (TSS) that do not necessarily correspond to their canonical TSS. First, these active TEs seem to accumulate in the 5' upstream regions of the genes, possibly providing a mechanism of cis-regulation of the nearby genes. Indeed, in some cases, the transcript is co-linear to the gene, overlapping it, providing a longer alternative transcript as well as a new transcription start site.
Second, apart from the 5' upstream regions, most active TEs seem transcribed on the same strand as the gene. They could also be at the origin of new transcription start sites and new exons. Conversely, few anti-sense-TEs with respect to the matured transcript are observed. This suggests that anti-sense TEs insertions have a disruptive action and are counter-selected. However, anti-sense-TEs seem to accumulate in introns, where they could provide another complex way of gene regulation, and in the 3' downstream region, where other mechanisms akin to siRNAs could take place if the gene and the TE transcripts overlap.
Third, we noted that for some TE families, only small regions may contain putative TSSs, and these regions seem to be over-represented in the genome. This could imply that some specific TE fragments may have been positively selected by the host genome.
Finally, we tried to infer a biological function for this gene-TE interplay. It seems that protein binding genes are over-represented among the genes which contain specific TE insertions with cryptic-TSSs in their close vicinity.
All together, these results suggest that active transposable copies influence transcription in the host genome. It is likely that some features of transposable elements have been exaptated and provide new functions to genes.

Mechanisms of transcriptional gene silencing and heterochromatin formation in Arabidopsis thaliana : roles of the RNA-directed DNA methylation (RdDM) pathway and more surprises!

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Heterochromatin, whose formation requires conserved pathways of histone and DNA modifications, participates in numerous nuclear processes, including centromere function, gene silencing and nuclear organization. An important recent breakthrough was the discovery that the RNA-interference pathway (RNAi) is implicated in heterochromatin assembly and transcriptional gene silencing (TGS) in eukaryotes. Indeed, mutations affecting various RNAi components, including Argonaute proteins, have been shown to impair heterochromatin assembly and TGS at repeated sequences in fungi and plants. Moreover, numerous short-interfering RNAs have been identified that correspond to these sequences, suggesting that siRNAs are responsible for targeting heterochromatin modification to specific genomic locations. Recent studies performed in plants have added another level of complexity to this pathway by showing the existence of plant-specific homologs of DNA-dependent RNA polymerase II, PolIV and PolV, that mediate siRNA accumulation and DNA methylation-dependent silencing of endogenous repeated sequences. Recent results concerning the function of these novel polymerases in the pathway of RNA-directed DNA methylation in Arabidopsis will be presented.

Endogenous regulation of L1 elements by RNAi pathways in mouse ES cells

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A basal network of gene regulation orchestrates the processes ensuring maintenance of cellular identity and genome integrity. Eukaryotic small RNAs generated by the ubiquitous RNAse-III Dicer have recently emerged as central players in this network, by mediating gene silencing at the transcriptional or post-transcriptional level via RNA interference (RNAi). To gain insight into their potential developmental functions in mammals, we have characterized small RNA expression profiles during Embryonic Stem (ES) cell differentiation, a stereotypical model for early mammalian development. Long interspersed elements 1 (LINE-1 or L1) are non-long-terminal-repeat retrotransposons that dominate the mouse genomic landscape, and are expressed in germ cells or during early development and ES cells. Based on clear precedents in plants and fission yeast, we investigated in this study a role for RNAi and other epigenetic pathways in the regulation of L1 transcription and mobilization. This work has uncovered the existence of novel small (s)RNAs that map to active L1 elements. Some of these sRNAs have characteristics of cognate siRNA populations, while others display strand biases and length heterogeneity that evoke their biogenesis through RNA surveillance pathways, in a Dicer-independent manner. We further found that genetic ablation of Dicer or the sRNA effector proteins AGOs has complex and profound consequences on L1 transcription and mobilization in ES cells, indicating that endogenous RNAi pathways indeed maintain genomic integrity against L1 proliferation. These observations demonstrate the value of ES cells in uncovering novel sRNAs repertoires, their dynamics and functions in mammals where studies in early embryos are challenging.

Regulation of human retrotransposons in human cancer

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Long Interspersed Element-1, LINE-1, is a family of retrotransposable elements distributed throughout mammalian genomes. Each human genome contains about 500,000 copies of these elements, the majority of which are 5' truncated and, therefore, are nonfunctional. However, there are almost 4,000 L1 loci that are full-length and at least a hundred of these are expected to be fully functional. L1 elements amplify in the human genome through a RNA intermediate, a process known as retrotransposition. This amplification requires production of the full-length L1 mRNA and functional L1 proteins. One of these proteins, L1 ORF2, encodes an endonuclease (EN) domain responsible for the introduction of the first-strand nick in the host DNA and a reverse transcriptase (RT) domain required for the cDNA synthesis.

LINE-1elements are currently active and contribute to genomic instability through insertional mutagenesis resulting from retrotransposition of itself as well as other human retroelements such as Alu and SVA. LINE-1 expression can also damage host DNA via introduction of DNA double-strand breaks (DSBs) that are generally known to be highly toxic and mutagenic. In order to minimize the damage these elements can impose on the genome L1 activity is attenuated by multiple mechanisms.

By using *in vivo* approaches we identified that L1 expression in human cancers exhibits a circadian rhythm that can be disrupted by the light exposure at night. Analysis of L1 expression in a tissue-isolated xenograft model indicated that circulating melatonin controls L1 expression. Tissue culture experiments confirmed that activation of melatonin signaling significantly affects L1 retrotransposition in cancer cells.

Our findings suggest that retrotransposition and DNA damage induced by human retrotransposable elements may be regulated by host exposure to light. Our findings have significant implications for retroelement contribution to genomic instability in shift-workers, older individuals, and any other subpopulations that are likely to have disrupted circadian system.

Keywords : LINE-1, Alu, cancer, retrotransposition, melatonin

Human LINE-1 restriction by APOBEC3C is CDA-independent and mediated by an L1 RNP interaction causing inhibition of L1 reverse transcription

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LINE-1 (L1) retrotransposons are mobile genetic elements which are proliferating extensively in the human genome. The generation of ~34% of the human genome is a consequence of the activity of L1 elements which was also shown to cause genetic disorders and tumorigenic diseases by local genomic destabilization. Mammalian cells have adopted several strategies to restrict mobility and potentially deleterious consequences of uncontrolled retrotransposition. The human APOBEC3 (A3) protein family of cytidine deaminases (CDAs) includes seven proteins that take part in intracellular defence against retroelements and inhibit L1 retrotransposition by 35-99%. However, genomic L1 retrotransposition events that occurred in the presence of L1-inhibiting A3 proteins are devoid of detectable G-to-A hypermutations suggesting an L1 restricting mechanism that is deaminase-independent. We set out to uncover the mechanisms which are responsible for the reported APOBEC3C (A3C)-mediated inhibition of L1 retrotransposition rates by up to 75%.
We show that A3C-mediated L1 inhibition requires both A3C dimerization and an intact RNA binding pocket domain, indicating that an interaction between A3C dimers and RNA is essential for L1 inhibition. Cofractionation of L1 open reading frame 1 protein (L1 ORF1p) and A3C after density gradient centrifugation of L1 ribonucleoprotein particles (L1 RNPs) suggests an interaction between A3C and L1 RNPs. Consistently, A3C and L1 ORF1p colocalize with stress granule marker proteins implying the sequestration of L1 RNP-A3C complexes into cytoplasmic stress granules. We confirmed a direct interaction between L1 ORF1p and A3C by co-imunoprecipitation experiments. We conducted LEAP (L1 element amplification protocol) assays using L1 RNPs from HeLa cells overexpressing A3C , and found that the presence of A3C reduces the L1 reverse transcription (RT) rate by ~50%. This RT inhibition is consistent with the observed restriction of L1 retrotransposition by 40-75%. Sequencing of LEAP products that resulted from reverse transcription in the presence of A3C confirmed the absence of G-to-A hypermutations and validated A3C-mediated L1 inhibition by a CDA-independent mechanism. Taken together, our results suggest that A3C-mediated L1 restriction occurs via an L1 mRNA-bridged interaction between A3C and L1 ORF1p in L1 RNPs which inhibits processivity of L1 RT during the target-primed reverse transcription (TPRT) process.

Keywords : LINE-1, APOBEC3, intracellular defense, LINE-1 restriction factors

"*P*-element repression, homology-dependent silencing and paramutation in *Drosophila*"

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A paramutation is an interaction between two alleles of a locus, through which one allele induces a heritable modification of the other allele without modifying the DNA sequence. The paramutated allele retains the epigenetically-acquired properties in the succeeding generations and becomes itself paramutagenic. Although well characterized in plants, stably-inherited paramutations had not, until now, been described in animals. Here, using *Trans*-Silencing Effect (TSE), a homology-dependent repression mechanism discovered in the course of study of *P* transposable element repression, we show the existence of a fully penetrant and stable (>40 generations) paramutation in *Drosophila melanogaster*. In TSE, a *P*-transgene inserted in heterochromatin has the capacity to repress in *trans*, in the female germline, a homologous *P-lacZ* transgene located in euchromatin. Phenotypic and genetic analysis have shown that TSE exhibits variegation in ovaries, displays a maternal effect as well as epigenetic transmission through meiosis and involves heterochromatin components (including HP1) and the PIWI-Interacting RNAs (piRNAs) silencing pathway. We show that clusters of *P*-element derived transgenes exhibiting a strong capacity to induce TSE can convert other homologous transgene clusters stably incapable of TSE into strong silencers. This conversion is mediated by maternal inheritance of cytoplasm carrying piRNAs homologous to the transgenes. The paramutated cluster, previously unable to produce piRNAs, is converted into a piRNA-producing locus and is itself fully paramutagenic. TSE in *Drosophila* shows that paramutation can occur without pairing of the paramutagenic and the paramutated loci, in contrast to what has been proposed in plants. Our work provides a model to analyze the emergence of piRNA loci, as well as *trans*-generational epigenetic effects observed in transposable element repression.

Germline silencing of Idefix retrotransposon from *Drosophila melanogaster*

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It has become clear from many recent studies that germ line and somatic cells are equipped with different retrotransposons silencing mechanisms. In the Drosophila germline, retrotransposons are silenced by the PIWI-interacting RNA (piRNA) pathway. Interactions between transcripts processed by the RNAi system and components of the chromatin structure exist in the process of TE silencing in various organisms. However it is still unclear whether it is coupled (or not) to a transcriptional silencing (TGS). Idefix is a retrotransposon known to be silenced in somatic tissues. Using sensor transgenes carrying a fragment of Idefix, we show that this retrotransposon is also silenced in the germline. A set of mutations in genes implicated in the piRNA pathway has been tested for their ability to derepress the euchromatic sensor-transgene. Proteins of the piRNA pathway concentrate in a cytoplasmic structure close to the nuclear membrane called the nuage where the ping-pong cycle operates. We have found that these proteins are required to continuously silence Idefix at a posttranscriptional level along the whole drosophila oogenesis. We further show that proteins associated to compact heterochromatic structure are not deposited on the sensor transgene that only displays marks of open chromatin. Overall, our results suggest that the piRNA-mediated repression of Idefix is acting only at a post-transcriptional level in the Drosophila germ line.
Interestingly, our experiments further reveal a spatial and temporal sensitive window in the germarium where the sensor transgene silencing is lost. The results of on-going experiments driven to caracterise the specificity of this small patch of germ cells will be discussed.
Overall, our data help to better understand the struggle established between TE to propagate and their host genome to survive.

Systematic RNA expression analysis of the battle between Transposons and the piRNA pathway in *Drosophila* ovaries

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The genomes of all higher organisms contain large fractions of selfish mobile elements, called transposable elements (TEs). In animal gonads, uncontrolled TE expression and transposition leads to mutagenesis, genomic instability and sterility. To prevent TE induced damage many organisms have developed intricate control mechanisms that silence resident TE populations. In recent years, a specific small RNA pathway based on Piwi proteins and their associated Piwi interacting RNAs (piRNAs) has emerged as the major TE control mechanism in the animal gonad.
In *Drosophila*, Piwi clade proteins bind piRNAs that originate from transcripts of large heterochromatic loci, called piRNA clusters. These loci are mainly composed of broken, inactive TEs. Active piRNA/Piwi complexes control TE expression either by transcriptional or post- transcriptional silencing. The piRNA pathway acts differently in the two tissues of *Drosophila* ovaries. In somatic follicle cells only Piwi is expressed and a linear, primary piRNA pathway module is active. Besides the primary pathway, the germline additionally expresses Aubergine and Argonaute3 that form a secondary adaptive piRNA pathway module, called the ping-pong cycle. Roughly 100 TE families populate the *Drosophila* genome and their spatio-temporal expression varies widely. Also piRNA clusters show differential expression in follicle cells and germline cells and this correlates with the transposon identity of the contained transposon fragments.
To obtain a systematic overview of which TEs are silenced by the somatic and the germline piRNA pathways, we are performing genome wide sequencing of RNA isolated from ovaries and early eggs of selected tissue specific piRNA pathway mutants. Our analysis shows that the soma specific piRNA pathway controls several soma born retroviral-like transposons that cross the soma/germline boundary if not silenced. Conversely, in germline specific piRNA pathway mutants a distinct set of TEs is de-repressed and transmitted to early eggs. Our RNAseq data establishes a genome wide survey of TE regulation in *Drosophila* ovaries and will provide the basis for further systematic dissections of the piRNA pathway and transposon biology.

Keywords : Drosophila, ovary, piRNA pathway, RNAseq, endogenous retroviruses

Tye7 regulates yeast Ty1 retrotransposon sense and antisense transcription in response to adenylic nucleotides stress

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Transposable elements play a fundamental role in genome evolution. It is proposed that their mobility, activated under stress, induces mutations that could confer advantages to the host organism. Transcription of the Ty1 LTR-retrotransposon of *Saccharomyces cerevisiae* is activated in response to a severe deficiency in adenylic nucleotides. Here, we show that Ty2 and Ty3 are also stimulated under these stress conditions, revealing the simultaneous activation of three active Ty retrotransposon families. We demonstrate that Ty1 activation in response to adenylic nucleotide depletion requires the DNA-binding transcription factor Tye7. Ty1 is transcribed in both sense and antisense directions. We identify three Tye7 potential binding sites in the region of Ty1 DNA sequence where antisense transcription starts. We show that Tye7 binds to Ty1 DNA and regulates Ty1 antisense transcription. Altogether, our data suggest that, in response to adenylic nucleotide reduction, *TYE7* is induced and activates Ty1 mRNA transcription, possibly by controlling Ty1 antisense transcription. We also provide the first evidence that Ty1 antisense transcription can be regulated by environmental stress conditions, pointing to a new level of control of Ty1 activity by stress, as Ty1 antisense RNAs play an important role in regulating Ty1 mobility at both the transcriptional and post-transcriptional stages.

Keywords : Ty1 retrotransposon, environmental stress conditions, adenylic nucleotides, transcription regulation, Ty1 antisense RNA

Cis- and trans-acting determinants of Ty1 RNA fate

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The RNA of long-terminal repeat retrotransposons serves three distinct functions as the template for translation of Gag and Pol proteins, the genome for virus-like particles (VLPs) and the template for reverse transcription within VLPs. To understand how Ty1 RNA is partitioned among these functions, we are analyzing the role of *cis*-acting sequences and host co-factors that influence Ty1 RNA fate in *S. cerevisiae*. We used SHAPE analysis to determine the secondary structure of a 615-nucleotide 5' fragment of *in vitro*-transcribed Ty1 RNA that includes the UTR, translation start site and essential packaging and reverse transcription sequences. The RNA folds into three highly structured domains delineated by a pseudoknot. Stable secondary structure in the 5' UTR and translation initiation region is expected to inhibit translation of Ty1 RNA and favor packaging. Accordingly, the results of our mutational analysis suggest that the pseudoknot is a determinant of Ty1 RNA packaging. To further define the requirements for Ty1 RNA translation and packaging, we characterized ~40 Ty1 co-factors that act between Ty1 transcription and cDNA accumulation. One class of co-factors that includes RNA helicases and other RNA binding proteins is required for the formation of retrosomes, which are microscopically distinct Ty1 Gag/RNA foci where VLP assembly is proposed to occur. Remarkably, this class of co-factor mutants has normal or increased levels of Ty1 Gag, indicating that Ty1 RNA and Gag are not sufficient for retrosome formation. Moreover, maturation of Gag in the absence of retrosomes in these mutants suggests that retrosome formation is not necessary for VLP assembly. Thus, retrosome formation requires specific host co-factors and may play a role in proper packaging or reverse transcription of Ty1 RNA.

Keywords : retrotransposon, Ty1, RNA structure, retrosome, co-factor

NUCLEOTIDE EXCISION REPAIR LIMITS LINE-1 RETROTRANSPOSITION

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The non-viral retrotransposon, Long INterspersed Element-1 (LINE-1), is the most abundant and active mobile element in the human genome. Although the mechanism of insertion of a new LINE-1 copy is not fully understood, a large number of double strand breaks (DSBs), a highly mutagenic form of DNA damage, is observed after the induction of LINE-1 expression. In addition, studies have reported that DNA repair machinery control LINE-1 retrotransposition and might interfere with and/or participate in different steps of the LINE-1 insertion process in order to limit or fix LINE-1 caused damage.
In the early steps of this process, a 3' flap intermediate is generated resulting from the elongation of the LINE-1 cDNA at the insertion site. Our laboratory showed that the enzymatic complex ERCC1-XPF, which specifically recognizes and cleaves 3’ flap structure, limits LINE-1 retrotransposition. This structure-specific endonuclease is involved in DSB repair, telomere maintenance, and nucleotide excision repair (NER) pathways, which repair a broad spectrum of DNA lesions that cause distortion of the helical structure. To further understand the regulation of LINE-1 retrotransposition by the ERCC1-XPF complex, we investigated if other components of the NER pathway are able to control LINE-1 retrotransposition. The data showed that not only the core proteins of NER pathway, XPD and ERCC1-XPF, but also the lesion-binding protein, XPC, limits LINE-1 mobility.
Therefore, the ERCC1-XPF complex seems to be recruited to the LINE-1 insertion site through the NER sensing pathway. Our findings show a new function for NER in the maintenance of genome integrity: control of LINE-1 proliferation in the human genome.

Keywords : LINE-1, retrotransposition, DNA repair, Nucleotide excision repair

Imprinted retrogenes situated in introns of host genes impact on host transcription and polyadenylation

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While retrogene insertion can contribute to genome evolution by providing novel gene copies upon which selection can act, they can also impact on the genome by triggering local effects on chromatin architecture and gene transcription at their site of integration. A subset of retrogenes are subject to genomic imprinting, which results in them being expressed from only one of the two chromosomes – specifically, from the chromosome derived through the paternal lineage. This is controlled by epigenetic marks, including DNA methylation and post-translational histone modifications. We are intrigued by four imprinted retrogenes that share three common features: 1) a promoter-associated CpG island that is differentially methylated between the parental chromosomes, and is required for imprinted expression; 2) derivation from the X chromosome; and 3) situated within an intron of a multi-exonic 'host' gene. These host genes are also subject to genomic imprinting, but presumably only as a consequence of retrogene insertion. Our detailed examinations of two such loci reveal that the host gene transcripts have multiple polyadenylation sites. On the paternally-inherited chromosome, where the retrogenes are expressed, host transcripts use polyadenylation sites upstream of the retrogene insertion. On the maternally-inherited chromosome, where the retrogenes are silenced by DNA methylation, host transcripts use downstream polyadenylation sites. This imprinted use of polyadenylation sites is lost when DNA methylation at the retrogene promoter is perturbed and the retrogene is expressed from both chromosomes. We are now addressing the mechanism responsible for this effect, and asking whether it is retrogene transcription or DNA methylation directly that influences polyadenylation site choice. These two models may not be mutually exclusive, as we currently have evidence supporting both. Understanding the mechanisms responsible for such effects are likely to be relevant to other examples of intronic repeat elements, such as intracisternal A particles (IAPs), which have been shown to interfere with host transcription. The imprinted retrogenes provide a good model to dissect these mechanisms, because of the allele specificity of the effects. The relationship between retrogene and host illustrates the local impact that retrotransposition can have on chromatin architecture and transcription. In addition, these loci provide some of the first experimental examples of epigenetic marks influencing alternative polyadenylation.

Keywords : retrogene, epigenetics, imprinting, transcription, polyadenylation

Dynamic regulation of a telomere-specific retrotransposon in Drosophila

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Non-LTR retrotransposons populate the telomeric region in Drosophila. These elements transpose exclusively to chromosome ends, even to broken ends during de novo telomere formation on induced DNA breaks. To better understand the mechanism for this sequence-independent targeting to DNA ends, we characterized HeT-A, the most abundant telomeric retro-element in Drosophila. HeT-A contains a single orf, which encodes a 110kD protein with limited homology to Orf1p from other non-LTR elements. This unusually large Orf1p forms multiple foci in proliferating cells, some of which are telomere-associated. Intriguingly, Orf1p foci are present only for a short time during the cell cycle, possibly in G1/early S phase. Under certain genetic backgrounds, possibly ones with elevated HeT-A transcription, Orf1p forms strikingly large spherical structures, most of which engulf one or more telomeres. IP experiments suggest that Orf1p associates with HeT-A transcripts suggesting that these intra-cellular foci/spheres are Orf1 RNPs. Using Orf1p immunolocalization we surveyed a number of Drosophila mutants defective in telomere protection, and identified novel factors essential for the end-targeting of Orf1 RNP. Using a genetic assay for identifying new HeT-A transposition events, we discovered that one branch of the DNA damage checkpoints is essential for inhibiting HeT-A attachment to broken ends. We have also generated transgenic lines carrying a genetically marked HeT-A element. Although this element has been placed at non-telomeric locations, it is nevertheless subjected to piRNA-mediated silencing in the germline similar to endogenous elements located at telomeres. We have recovered potential transposition events from this marked element. Molecular characterization of these events is underway and promises to yield new insights into how these elements are targeted specifically to chromosome ends.

Transposable elements, small RNA and the evolution of plant genome size

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DNA methylation is common in all eukaryotes but particularly prevalent in plants. Plants methylate the DNA of both transposable elements (TEs) and genes. For the former, DNA methylation generally suppresses TE activity, acting as a critical component to prevent TE proliferation and concomitant genome size expansion. However, the methylation of TEs near genes affects gene expression, leading to a trade-off between the largely beneficial effects of TE suppression and potentially deleterious effects on gene expression. The function of DNA methylation on coding genes is not yet fully elucidated, but there is growing evidence both that methylation targets genes of essential function and that such targeting is evolutionary conserved. Altogether, the methylation of TEs and genes plays a prominent role in shaping genome size, structure and function; these effects on genomes from the genera *Arabidopsis*, *Oryza* and *Zea* will be discussed.

Host Factors, L1 Proteins and Retroelement Evolution

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The human L1 element can be ancestrally traced back to over 100 million years ago (mya), with different L1 families active during different periods of time. L1 subfamily evolution follows a linear pattern, whereby a single L1 lineage proliferates, differentiates, and is eventually replaced by a new dominant subfamily. Although arising more recently, a similar linear subfamily evolution can be observed for Alu elements. The observations of the co-extinction of human L2 and its proposed SINE partner, MIR, and L1 and B1 in sigmodontine rodents are evidence of the SINE dependence on LINEs. Two evolutionary periods of LINE and SINE subfamily progression are of particular interest: the first, ~55-35 mya when Alu and retropseudogene amplification peaked and L1PA8 was one of the dominant active L1 subfamilies; and second, ~25 mya when L1 proteins experienced a rapid and significant increase in amino acid evolution during the transition from L1PA5 to L1PA4.
We have successfully reconstructed the consensus sequences of the extinct L1PA4 and L1PA8 subfamilies. The three L1 subfamilies, including the modern L1PA1, are retrocompetent in human cells, with L1PA8 being the most efficient. They also support Alu subfamily retrotransposition, but at different levels of efficiency. In rodent cell lines, L1PA1 and L1PA4 show equivalent retrotransposition rates. However, the retrotransposition rate of the older L1PA8 subfamily is reduced by an order of magnitude in rodent cell lines relative to human cell lines. Using PA1-PA8 chimeric proteins, we have defined a region within the L1 coding sequence that is responsible for the observed low retrotransposition rates in rodent cells. Analysis of protein expression determined that differences in expression levels do not account for the low L1PA8 retrotransposition rates in rodent cells. We have also preliminary data from the ORF reconstruction of the older L1PA13A in our analysis. Although we observe that subfamily-specific ORF2 proteins can differentially affect the retrotranspositional efficiency of older vs. younger Alu subfamily constructs, our data suggests that host factors may have played a more significant role in contributing to the subfamily evolutionary patterns observed.

Keywords : Alu, LINE-1, ORF1, retrotransposition, subfamily evolution

Minigene study of ALU elements exonization controlled by competition of hnRNP C with the U2AF^{65}

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Heterogenous nuclear ribonucleoprotein C1/C2 (hnRNP C) was shown to silence alternative exons, most likely by binding to long uridine tracts at the 3' splice sites of pre-mRNAs. Using genome-wide approach combining individual-nucleotide resolution UV cross-linking and immunoprecipitation (iCLIP) and RNAseq methods, we determined that its silencing is mediated through direct competition with the core splicing factor U2AF^{65} for the binding to polypyrimidine tracts. Due to observed binding at "deep intronic sequence" positions, we could further show that hnRNP C silences the inclusion of cryptic exons, of which ALU elements, as the most abundant mobile elements in humans, are a major source of exonized sequences. Exonization process is known to be important as an evolution tool as well as in prevention of cryptic exon inclusion that might lead to a disease development, mainly by introduction of premature stop codons or frameshift mutations in mature mRNAs. Therefore, we determined that exonization of ALU elements is directed by competition of hnRNP C with the U2AF^{65}. In order to study that, we performed reporter assays using minigenes, so far the most effectual choice to study regulation of alternative splicing by RNA-binding proteins *in vivo*. We used bioinformatically derived list of predicted ALU exon changes from combined iCLIP and RNAseq data. Cryptic exonization under hnRNP C regulation was verified in control and hnRNP C knock-down conditions. In order to study its competition with U2AF^{65} in details, we constructed mutated minigenes in the hnRNP C binding sites with aim of disrupting uridine tracts to prevent hnRNP C binding and maintain binding of U2AF^{65}. Introduced mutations lead to constitutive inclusion of ALU elements even in the presence of hnRNP C, what confirmed that hnRNP C regulates exonization of ALU elements through competition with U2AF^{65}. Moreover, by constructing examples of minigenes with naturally occurring sequence polymorphisms, we demonstrated their role in primate evolution and in disease.

Keywords : exonization, hnRNP C, minigene, Alu elements

The scnRNA pathway: a highly flexible mechanism for non-Mendelian inheritance of mating types in the ciliate *Paramecium aurelia*

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The maternal inheritance of mating types in several species of the *P. aurelia* complex is a classical example of transgenerational epigenetic inheritance. Conjugation between cells of complementary mating types (O and E) produces two genetically identical zygotes ; yet the new somatic macronucleus that develops in each cell after reciprocal fertilization becomes determined for the same mating type as its maternal (cytoplasmic) parent. This process is regulated by scnRNAs, a meiosis-specific class of small RNAs that is required for the elimination of transposable elements during macronuclear development and also mediates the maternal inheritance of alternative patterns of genome rearrangements. We have identified the *mtA* gene, which encodes a trans-membrane protein specifically expressed in E cells, as being responsible for mating-type determination in *P. tetraurelia* : its promoter is excised in O macronuclei but retained in E macronuclei, allowing its expression. However, expression of *mtA* further requires the products of the *mtB* and *mtC* genes, which likely encode transcription factors. This E-specific pathway appears to be conserved in other sibling species, but the mechanism of mating-type determination differs. In *P. septaurelia*, the *mtA* promoter is retained in both mating types, and the pathway is inactivated in O cells by the excision of a segment of the *mtB* coding sequence during macronuclear development. These observations highlight the flexibility with which the scnRNA pathway is naturally used to maintain an essential phenotypic polymorphism in populations of genetically identical cells.

Keywords : small RNA ; genome rearrangements ; non-Mendelian inheritance ; Paramecium; genome evolution

Usual and unusual evolutionary arms races between host and viral genomes

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Poxviruses encode K3L, a mimic of eIF2alpha, which is the substrate of protein kinase R (PKR), an important component of innate immunity in vertebrates. The PKR-K3L interaction exemplifies the conundrum imposed by viral mimicry. To be effective, PKR must recognize a conserved substrate (eIF2alpha) while avoiding rapidly evolving substrate mimics such as K3L. Using the PKR-K3L system and a combination of phylogenetic and functional analyses, we uncover evolutionary strategies by which host proteins can overcome mimicry. We find that PKR has evolved under intense episodes of positive selection in primates. The ability of PKR to evade viral mimics is partly due to positive selection at sites most intimately involved in eIF2alpha recognition. We also find that adaptive changes on multiple surfaces of PKR produce combinations of substitutions that increase the odds of defeating mimicry. Although many viruses adapt to novel environments by rapidly exploring sequence space, poxviruses are also highly adaptable and undergo cross-species transmission by largely unknown mechanisms despite low mutation rates. We studied vaccinia virus evolution by serial infection of human cells, where the viral antagonist K3L is maladapted against antiviral Protein kinase R (PKR). Viruses rapidly acquired higher fitness via recurrent K3L gene amplifications to defeat PKR, despite incurring up to 7-10% increases in genome size. Gene expansions counteracted human PKR and facilitated the gain of an adaptive amino acid substitution in K3L to defeat PKR. Our discovery of poxviral 'gene-accordions' explains how poxviruses can rapidly adapt to defeat different host defenses despite low mutation rates and reveals how classical Red Queen conflicts progress through unrecognized intermediates.

Parasitoid wasp symbiotic viruses: Russian dolls TEs and horizontal transfer between insects

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Bracovirus particles (BVs) are injected along with parasitoid wasp eggs into lepidopteran host body and act by manipulating host immune defenses, development and physiology, thereby enabling wasp larvae to survive in a potentially harmful environment. During the last few years a breakthrough has been made toward understanding the relationship between bracoviruses and the viral world with the characterization of the viral genes involved in particles production. They reside permanently in the wasp genome and are no longer packaged in the particles. The genome packaged in the particles consists in multiple dsDNA circles encoding virulence genes originating probably from the wasp and involved in host manipulation. Functionally bracovirus particles are thus gene transfer agents. The circles packaged in the particles are produced from a proviral form present in the wasp genome, which constitutes also a large target (≈1 megabase) for TEs insertions. In the genome of CcBV (Cotesia congregata bracovirus) we identified remnants of retroelements and large DNA transposons: a Maverick and several copies of a putative new element. DNA circles packaged in the particles were shown recently to integrate into lepidopteran host DNA and analysis of a wasp genome revealed that reintegration of circles back into wasp DNA can also occur. Thus BV circles navigate between genomes of different insect orders, they act like TEs and are potential vehicles for genetic exchanges between genomes. Since several lepidopteran genomes became recently available, it is now possible to evaluate bracovirus contribution to TEs horizontal transmission between Hymenoptera and Lepidoptera.

Keywords : parasitoid wasp, Lepidoptera, large DNA transposon, polydnavirus, bracovirus

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Transposable element evolution in *Wolbachia* bacterial endosymbionts

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Transposable elements (TE) are one of the major driving forces of genome evolution, raising the question of the long-term dynamics underlying their evolutionary success. Long-term TE evolution can readily be reconstructed in mammals thanks to many degraded copies constituting genomic fossil records of past TE proliferations. By contrast, bacterial genomes usually experience high sequence turnover and short TE retention times, thereby obscuring ancient TE evolutionary patterns. The genomes of *Wolbachia*, one of the most abundant bacterial endosymbionts on Earth, are littered with TEs. The question arises as to why there are so many TEs elements in the genomes of this ancient endosymbiont. To address this question, we investigated the dynamics of insertion sequence (IS) TEs using an evolutionary perspective. Our results indicate that several processes may explain TE abundance in *Wolbachia*, including recent activity, along with recurrent invasions through horizontal transfers and gene conversion. Remarkably, we found that 70% of *Wolbachia* IS are nonfunctional. They constitute an unusual bacterial IS genomic fossil record providing direct empirical evidence for a long-term IS evolutionary dynamics following successive periods of intense transpositional activity. The identification of an important IS genomic fossil record in *Wolbachia* demonstrates that IS elements are not always of recent origin, contrary to the conventional view of TE evolution in prokaryote genomes.

Master *mariners*

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The *mariner* transposable element, discovered in the 80s by the research group of Dan Hartl, is the one with the largest specific distribution. Initially described in *D. mauritiana*, this element was rapidly detected in a large spectrum of eukaryotes including many *Drosophila* species but also numerous insects, nematodes, rotifers, cnidarians, platyhelminthes, and vertebrates.
A detailed analysis of all the sequences known (about 5,000), revealed the existence of several Subfamilies, Tribes and Clans. Today, two main groups, *MareNostrum* and *Atlantis*, can be described without any ambiguity. Recently described sequences belonging to the *Atlantis* group seem particularly abundant in actinopterygians, amphibians, echinoderms. An automatic classification based on pairwise comparisons enables us to define 16 Subfamilies.
D. simulans, a sibling of *D. mauritiana*, was particularly investigated because all natural populations of this cosmopolitan species have active copies. In this species, a simple phenotypic test allows us to estimate their somatic activity, and a comparison among these populations reveals a large variability that seems less sensitive to environmental variations than to historical contingencies. This is illustrated by the somatic activity along the Nile route followed during the migration from Africa to Europe and back to North Africa.
The structural evolution of the element shows that most of the elements detected present internal deletions. These deletions are not randomly distributed since they are more frequent in the 5' part of the element. Moreover, most of them (>80%) occur between micro-homologies from 3 to 10 bp, which is probably due to abortive gap repairs after excision.
Detailed analyses of the average transcription level reveal the existence of variation between populations. These variations are correlated with the estimation of the average copy number and to that of the somatic activities (excision test). This could be correlated with the invasion status of the species since low levels of expression are observed in ancestral populations compared to the invasive ones. Otherwise, non-constant activity is observed during the life cycle of the species. A burst of expression is observed in late pupae and is maintained in adults. More precisely, this burst is only due to an over-transcription in male testes.
According to theoretical models, a competition between autonomous and non-autonomous (but *trans*-mobilizable) copies should be observed within the same genome. To test such an assumption, experimental design has been set up allowing us to follow the invasion of an autonomous copy into a population containing only few non-autonomous ones. While a rapid invasion of the autonomous copy is observed at the population level, amplification of non-autonomous copies is prevalent within genomes.

Keywords : mariner, evolution, classification, *Drosophila simulans*

HeT-A_pi1, a piRNA target sequence in the Drosophila telomeric retrotransposon HeT-A, is extremely conserved across copies and species

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The maintenance of the telomeres in *Drosophila* species depends on the transposition of the non-LTR retrotransposons *HeT-A*, *TAHRE* and *TART*. *HeT-A* and *TART* elements have been found in all studied species of *Drosophila* suggesting that their function has been maintained for more than 60 million years. Of the three elements, *HeT-A* is by far the main component of *D. melanogaster* telomeres and, unexpectedly for an element with an essential role in telomere elongation; the conservation of the nucleotide sequence of *HeT-A* is very low. In order to better understand the function of this telomeric retrotransposon, we studied the degree of conservation along *HeT-A* copies. We identified a small sequence within the 3' UTR of the element that is extremely conserved among copies of the element both, within *D. melanogaster* and related species from the *melanogaster* group. The sequence corresponds to a piRNA target that we named HeT-A_pi1. Comparison with piRNA target sequences from other Drosophila retrotransposons showed that HeT-A_pi1 is the piRNA target with the highest degree of conservation among species from the *melanogaster* group. A piRNA target sequence (HeT-A_pi1) within the 3' UTR of *HeT-A* is extremely conserved both within and between species. This high conservation suggests an important function of this piRNA target in the co-evolution of this element and the *Drosophila* genome.

Keywords : HeT-A, Drosophila telomeres, piRNAs, retrotransposons

Drosophilids as a model of recent invasion and ancestral polymorphism of transposable elements

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Transposable elements (TEs) are segments of repetitive DNA that can mobilize and propagate within and between genomes. Due to their ability to mobilize, they are a key source of genomic and epigenomic variation that influence the evolutionary trajectory of the host species. TEs have an evolutionary cycle that combines several processes, as for example, burst of transposition, sequence degeneration, vertical and horizontal transfers. In recent years, we have reported numerous cases of horizontal transfers (HT) of TEs between species of drosofilids. Among the species involved, many are closely related, as species of the *melanogaster* subgroup of the genus *Drosophila*, but an outstanding number of HTs occurred between species that evolved separately by more than 50 Mya, as those of the genus *Zaprionus* and the *melanogaster* subgroup. Since the *Zaprionus* genus and the *melanogaster* subgroup seem to have shared the same age of origin and diversification in tropical Africa, as well as ecological features, our data suggest that they passed through a permissive period of TE invasion during their diversification period. Moreover, losses of active TEs harbored by the ancestor species and reintroduction of active copies (donated by its sister species *D. simulans*), followed by burst of amplification, seems to have happened in *D. melanogaster*. This introduction would have occurred in Africa before the worldwide expansion of the species, probably in the late Pleistocene, a period that these species returned to sympatry in Africa after being diversified apart (Financial support: FAPESP, CNPq)

Keywords : transposable elements, horizontal transfer, ancestral polymorphism, *Drosophila*, *Zaprionus*

How bat genomes stay slim: high rate of DNA loss counteracts transposable element invasions

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Bats are exceptional among mammals for their ability to fly, but also for their relatively small genomes (27% smaller than most other eutherians), two characteristics reminiscent of birds. It has been hypothesized that these two properties are linked as the metabolic cost of flight imposes an upper constraint on cell size and thereby genome size. Transposable elements are major players in the dynamics and evolution of genome size in many eukaryotes. In mammals, DNA is mostly gained through the amplification of TEs, while deletion of ancient inactive TEs provides a baseline to estimate the rate of non-essential DNA removal.

Thus, to shed light into bat genome size evolution, we compare the genomic content and evolutionary dynamics of TEs in the microbat *Myotis lucifugus* and the megabat *Pteropus vampyrum*, which represent the two most deeply diverged lineages of bats. We estimate that TEs occupy ~35% and ~30% of the genome of the microbat and megabat respectively, which is on the lower end of TE amount among mammals. The most striking difference between the two bats lies in their DNA transposons content: while this class of elements shows no signs of recent activity in megabat, they have undergone a recent and massive expansion in the microbat. Next we examine the balance between the amount of DNA gained via lineage-specific transposition events and the rate at which ancestral TEs have been removed in the two bat lineages, as well as in five other lineages of placental mammals (human, mouse, dog, elephant and tenrec).

The results indicate that the smaller genome size of bats is the result of a relatively low level of lineage-specific TE activity combined to a very high rate of DNA loss per generation. The significantly higher level of TE activity in the lineage of the microbat than in the megabat has been counteracted by a greater rate of removal of ancestral TEs in the microbat lineage. We corroborate these data by a study of small DNA deletions (1-30 bp) in multi-species alignments of orthologous genomic regions, which confirms that the two bat lineage have experienced a relatively high rate of small deletions compared to other mammalian lineages examined. However, small deletions can only partially account for the amount of DNA inferred to have been removed in each bat lineage, implying a role also for larger deletions to contract bat genomes. Lastly, we observe that the rate of short DNA deletion was even higher before the divergence of the two bats, which suggests that genome contraction initiated prior to, and perhaps was a pre-requisite for the invention of flight.

Keywords : Bats, Transposable Elements, Genome size, DNA loss

A Transposase Goes To Work

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DNA cut & paste transposons are important contributors to genome structure and function in many organisms, not just because of their effects when moving from place to place, but also because they have been domesticated by their hosts to provide novel functions. The most spectacular example of such domestication is in the vertebrate immune systems where the RAG proteins share many attributes with DNA cut & paste transposons and mediate the combinatorial assembly of gene segments to form many different antigen receptor genes that encode immunoglobulins and T Cell receptor genes during lymphocyte development. It has long been suggested that this recombination system indeed derived from a transposon (1) but the nature of the progenitor has been unclear. It has been more recently suggested that the RAG system and the superfamily of fossil transposable elements called *Transib* that has been identified in insects and some invertebrates share a common progenitor (2) but an intact *Transib* element has not previously been characterized for comparison to the RAG mechanism.
We report here that the transposase from an intact *Transib* element from the cotton and corn bollworm pest *Helicoverpa zea* (3) called *Hztransib*, is active *in vitro* and that its DNA breakage and joining activities mimic those of RAG.

Keywords : cut & paste, transposition, transposase, V(D)J recombination, RAG

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Structure-function studies of the RAG1/2 recombinase/transposase

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The basic function of the RAG1/RAG2 protein complex as the specific "recombinase" in V(D)J recombination is well understood by now, but there has not been enough information about its structure and biochemistry. To gain some insight into the spatial arrangement of proteins and DNA in the first stage of V(D)J recombination, we isolated the post-cleavage complex of RAG1/2 with a pair of its DNA sites. When this "signal end complex", containing two pairs of RAG1 and RAG2 protomers, was studied by electron microscopy, we found an anchor-shaped particle with approximate two-fold symmetry. The two DNA molecules are parallel, and the proteins have a similar parallel arrangement, with both RAG2 units and the catalytic portions of RAG1 at the head end of the anchor, and the nonamer-binding portions of RAG1 at the tail. These first images of the V(D)J recombinase in its post-cleavage state provide a framework for modeling RAG1/2 and its interactions with DNA. We were able to construct a rough structural model.

The EM work was done with the catalytically active "core" parts of RAG1 and RAG2, but missing a large N-terminal piece of RAG1 and a C-terminal piece of RAG2. What are the functions of these parts? The RAG2 C-terminal contains a PHD domain that is known to bind chromatin, specifically histone H3 methylated on R2 and K4 (H3K4Me3R2Me2s). This is probably used to target RAG1/2 to recombinationally active loci, which carry these modifications. The RAG1 N-terminus contains a RING finger with ubiquitin ligase activity, and a primary ubiquitylation site at K233. Both these added portions probably have regulatory functions. We have found that when the full-length proteins are used, the added portions interact with each other to inhibit RAG1/2 activity, but this auto-inhibition is lifted when the complex binds a histone H3 peptide containing K4Me3R2Me2s. This interaction of the PHD domain evidently has a dual role, both targeting RAG1/2 and making it more active. Ubiquitylation of RAG1 also modulates its activity.

Keywords : V(D)J recombination, auto-inhibition, ubiquitylation

Cellular control of HIV-1 integration : Lost in regulation…

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DNA mobility is not only a crucial process for genome evolution and pathogen replication but also a tool for gene transfer. Better understanding is essential for optimizing these strategies. Gene therapy aims to correct the expression of a defective gene by insertion or replacement of an active copy of the gene. For optimal long-term expression of this active copy, its insertion inside the genome and the stability of the integration locus is important. To be efficient the transgene must be expressed at the integration locus and must not induce any new deleterious effect. Consequently the control of integration targeting constitutes a major issue in the field. In recent years, gene therapies using lentiviral vectors have shown much success but also many limitations, especially owing to the lack of data about the cellular behaviour of the transferred gene. Integration selectivity is mainly driven by the retroviral integrase enzyme and its interaction with host chromatin. Our laboratory was involved for long time in the determination of the nucleoprotein complexes required for the HIV-1 integration reaction (Faure et al., NAR 2005, Baranova et al., NAR 2007, Lesbats et al., NAR 2008). More recently we focused our work on the molecular mechanisms for controlling the specificity and the efficiency of these integration complexes in the cells. We have recently shown that HIV-1 integration could be regulated by chromatin structure maintenance (Lesbats et al., PLoS Pathogens 2011) and DNA repair processes (Desfarges et al., NAR 2006, Cosnefroy et al., J. Of Virology 2011). Our project is focused on the understanding of these processes based on data we recently obtained in the new model of integration into polynucleosomal template developed in the lab (Lesbats et al., PLoS Pathogens 2011). This system has been used to study the relationship between chromatin accessibility and retroviral integration in an HIV model. We demonstrated that stable chromatin is refractory to integration and that functional coupling between integrase and the chromatin remodelling complex SWI/SNF restores and targets the integration into the nucleosomal region. This mechanism can be involved in targeting the integration in the infected cells and also in the lentiviral gene transfer procedure. Our data could help to design new strategies for affecting the selectivity of lentiviral integration in order either to direct integration into more or less silenced zone of the genome.

Keywords : Retroviral integration, HIV-1, Gene transfer, integration selectivity

DNA forks hi-jacking during replicative transposition of the Tn*3*-family transposon Tn*4430*

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Transposition reactions generally generate DNA intermediates that need to be processed or repaired by the host cell. However, the mechanisms whereby transpososomes recruit or communicate with the host DNA-processing machineries are poorly understood. Here, we provide a convergent set of genetical and biochemical evidences indicating that replication of the Tn*3*-family transposon Tn*4430* during transposition is tightly coupled to its mechanism of target site selection.
In vivo, the insertion pattern of Tn*4430* into a pSC101-derived plasmid was found to be strongly altered by the presence of arrays of pseudo-palindromic sequences consisting of 5 repeats of the *Escherichia coli lacO* operator. This changed the regional preference of the transposon without affecting its insertion site sequence specificity. Alteration of the insertion pattern depended on the relative positioning of the *lacO* array with respect to the unidirectional replication origin of the plasmid, suggesting a link between transposition targeting and replication.
In vitro, the TnpA transposase of Tn*4430* was found to bind with a high affinity to non-specific replication fork-like DNA structures containing a 3'OH at the branch point. When compared to linear DNA fragments, these structures are efficient substrates for TnpA-catalyzed end transfer. Strand transfer occurred at a highly specific position at the branch point of the forked substrate, poising the transposon for DNA replication.
We thus propose a model in which the Tn*3*-family elements target DNA replication or repair intermediates as a mechanism to recruit the host replication machinery during replicative transposition.

R2 target primed reverse transcription and its control

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R2 non-LTR retrotransposons insert into the tandemly repeated rRNA genes of many animals. Inserts are co-transcribed with the rRNA genes and processed by a self-cleaving ribozyme encoded at the 5' end of the elements. R2 elements encode a single open reading frame producing an endonuclease, specific for the 28S rRNA gene insertion site, and a reverse transcriptase (RT), responsible for the synthesis of new DNA copies from an RNA transcript. Based on the activities of the R2 protein purified from an E. coli expression system, R2 retrotransposition occurs in a symmetric manner by two subunits. One subunit binds the 3' end of the R2 transcripts, cleaves one strand of the DNA target, and using the released 3' end to prime first strand DNA synthesis: target primed reverse transcription (TPRT). The second subunit binds the 5' end of the R2 RNA, cuts the second strand of the target site and synthesizes the second DNA strand. To study RNA bindings the R2 protein was mutated at conserved amino acid residues upstream of the RT domain. This conserved region is present in many other non-LTR retrotransposons, including mammalian L1s. Mutations were identified which did not affect DNA cleavage or simple RT activity but dramatically prevented the TPRT reactions. Thus a domain immediately N-terminal to the RT domain appears to play a major role in binding of both 5' and 3' RNA. The domain structure of the R2 proteins has a number of interesting similarities to that of telomerase.

A snap-velcro model for flexible priming of L1 reverse transcription at extended and imperfect AT-rich regions

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L1 retrotransposons have a prominent role in reshaping mammalian genomes. To replicate, the L1 ribonucleoprotein particle (RNP) first uses its endonuclease (EN) to generate a nick in the genomic DNA, which it then uses to initiate reverse transcription. Accordingly, most L1 insertions occur into the L1 EN recognition sequence (A/TTTT). However, these sites are often degenerate and contain much longer stretches of imperfect AT-rich sequences. Using a direct assay with native L1 RNPs, we now define the preferential rules of L1 reverse transcription initiation. First, efficient priming is detected with as little as 4 matching nucleotides at the primer 3' end. Second, L1 RNP can tolerate terminal mismatches if they are compensated by an increased number of upstream matching nucleotides. Third, efficient priming in the context of duplex DNA requires a 3' overhang, suggesting the possible existence of additional DNA processing steps, which generate a single-stranded 3' end to allow L1 reverse transcription. Based on these data, we propose that the terminal bases of the primer act as a specific snap and the upstream ones as a weaker velcro strap allowing efficient and flexible retrotransposition into imperfect AT-rich regions as is observed *in vivo* in mammalian genomes.

Keywords : genome plasticity, retrotransposon, ribonucleoprotein, reverse transcriptase, LINE-1

This study was supported by grants to G.C. from ARC (#4854), INSERM and INCa (Avenir program), and the European Research Council (ERC Starting Grant 'Retrogenomics'). M. K. and C. Monot are supported by PhD fellowships from the Ligue Nationale Contre le Cancer and from the French Ministry of Research, respectively.

BARE retrotransposons are translated and replicated via distinct RNA pools

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Retrotransposons, also called Class I transposable elements, and their fragments can constitute considerably more than 50% of higher plant genomes. The most abundant subclass, the Long Terminal Repeat (LTR) retrotransposons, are transcribed from promoters in the LTRs and encode proteins for packaging of the transcripts, their reverse transcription into cDNA, and integration of the cDNA back into the genome. In addition, the genome contains non-autonomous LTR retrotransposons, which utilize the proteins of autonomous elements for their replication and propagation. A major question for both retrotransposons and retroviruses is how the two conflicting roles for their transcripts, translation and reverse transcription, are managed. Here, we show that the *copia*-like *BARE* retrotransposon of barley, despite its simple organization into only one open reading frame, produces three distinct classes of transcripts, one that is capped, polyadenylated, and translated but which cannot be reverse transcribed and another that is not capped or polyadenylated, but destined instead for packaging and ultimate reverse transcription. The third class is capped, polyadenylated, and spliced to favor produce a subgenomic RNA encoding only Gag, the capsid protein that forms the virus-like particles (VLPs). Moreover, the *BARE2* subfamily, which cannot synthesize Gag and is dependent on *BARE1* for this protein, does not produce the spliced sub-genomic RNA but does make the replication competent transcripts, which are packaged into *BARE1* particles. To our knowledge, this is first demonstration of distinct RNA pools for translation and transcription for any retrotransposon.

Keywords : LTR retrotransposon; replication; transcription; translation

One ring to rule them all: The 3-dimensional structure of *Hermes*, a *hAT* family DNA transposase complexed with transposon ends

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Hermes is a *hAT* family DNA transposase from the housefly, *Musca domestica*. We have previously shown that in addition to an RNaseH-like catalytic domain, the transposase enzyme also contains an intertwined dimerization domain and an insertion domain that interrupts the RNaseH-like fold.
We have now solved the crystal structure of *Hermes* complexed with a DNA sequence representing the very end of the transposon using X-ray crystallography at 3.4 Å resolution. Consistent with biochemical data, the complex is a large assembly, a tetramer of four transposase dimers. Each dimer binds two transposon ends. The transposase dimers interact through reciprocal interactions, resulting in a large, 560kDa ring shaped assembly.
In each of the dimers, the transposon ends are located such that the 3' OH ends of the transferred strands point into a large, hydrophilic cavity that appears to be suitable to accept target DNA. The two 3' OH ends are 36 Å apart, consistent with the expected 8 bp target site duplication characteristic of *Hermes*. When compared to the structure of DNA-free *Hermes*, there has been a large movement of the insertion domain relative to the RNaseH-like domain. The insertion domain forms a number of intimate contacts with the transferred strand, and its ability to move relative to the RNaseH-like domain and therefore relative to the nuclease active site suggests that its movement might be related to *Hermes'* need to switch nuclease activity from the non-transferred strand to the transferred strand. The structure also suggests that there is only one active site involved with one transposon end and that strand switching is likely accomplished without releasing and rebinding each transposon end in a different orientation.
The reciprocal interactions holding the dimers together in a ring shaped assembly that appears to be important for transposition activity in cells. As isolated dimers display hyperactivity *in vitro*, this suggests that the large assembly may also serve to regulate or inhibit transposition activity.

Single strand (HuH) transposases and genome dynamics

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We address the central role of a functionally related group of transposable elements called "HuH elements" in structuring and shaping bacterial genomes. We have identified and characterised a widespread class of IS (IS*200*/IS*605* family) quite different from classical TEs: they use obligatory single strand DNA intermediates and have ends with subterminal imperfect palindromes (IP) which are recognised and bound by their Tpases. These Tpases ($TnpA_{IS200/IS605}$), with a catalytic site containing a single Tyr and a His-u-His amino acid triad, are members of a larger "HuH" endonuclease superfamily including RCR Rep proteins (Rolling Circle Replication), relaxases (conjugal plasmid transfer) and Tpases of the IS*91* family. All create a covalent 5'-phosphotyrosine enzyme-substrate intermediate. The Tpase binds the subterminal IP located some distance from the cleavage sites. Remarkably, cleavage sites are not recognised directly by the protein but by short "guide" sequences 5' to the IP foot. Recognition involves a network of canonical and non-canonical base interactions similar to those found in RNA structures. We have demonstrated the importance of the lagging strand template for activity of some members and our *in silico* genomic analysis suggests that all IS*200*/IS*605* family members have evolved a mode of transposition that exploits ssDNA at the replication fork. Recently, a new clade of these Tpases ($TnpA_{REP}$) was found associated with short intergenic multicopy palindromic regulatory sequences REPs/BIMEs. It has been suggested that $TnpA_{REP}$ is responsible for REP proliferation over genomes. We analysed and compared REP distribution in numerous available *E. coli* and *Shigella* strains. Phylogenetic analysis clearly indicated that $tnpA_{REP}$ was acquired early in the species radiation and was lost later in some strains. We also studied *E. coli* K12 $TnpA_{REP}$ activity *in vitro* and demonstrated that it catalyses cleavage and recombination of BIMEs. While $TnpA_{REP}$ shared the same general organization and similar catalytic characteristics with $TnpA_{IS200/IS605}$, it exhibited distinct properties potentially important in the creation of BIME variability and in their amplification. $TnpA_{REP}$ may therefore be one of the first examples of transposase domestication in prokaryotes.

Emergent properties of a DNA looping reaction: lessons from mariner transposition

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Autoregulation of transposons is well known in bacteria. However, certain aspects of eukaryotic cell biology make it impossible for many of the documented mechanisms to operate. Here we present a biochemical analysis and a computer simulation, which together show how the autoregulation of mariner transposition arises from the transposase subunit architecture by a simple and elegant mechanism.

Biochemical analysis has shown that the mariner transposase exists as a homodimer in solution in the absence of DNA. The dimer recruits the naked transposon ends into a synapsis, within which all of the subsequent events take place. At the start of a genomic invasion, when the transposase concentration is low, the pre-formed transposase dimer maximizes the probability of productive interactions with the transposon ends. However, once the transposon is established, and the transposase concentration begins to rise, the competition for transposon ends suppresses an otherwise unsustainable exponential increase in the number of transposons. The competition for transposon ends is enhanced by allosteric coupling between the transposase subunits: the transposase dimer binds quickly and tightly to the first transposon end, but in so doing the affinity of the complex for the second transposon end is sharply reduced. This potentiates the competition for transposon ends and reduces the rate transposition still further.

We also present a computer simulation that shows how the competition for binding sites controls the kinetics of a genomic invasion. The simulation reveals how the emergent properties of the transposase subunit architecture adjust the rate of transposition to match the size of the genome and antagonize any RNAi silencing response on the part of the host. It also provides a general framework for understanding other transposition reactions in terms of the transposase subunit architecture. The competition for binding sites, embodied in the simulation's underlying kinetic model, provides a general mechanism of regulation applicable to any homotypic DNA looping reaction. Taken together our results illustrate the ways in which the mariner transposon is exquisitely adapted for survival in eukaryotic genomes.

Keywords : transposition, regulation, simulation, mariner

Programmed DNA elimination in Tetrahymena – recognition and excision of chromatin by the PiggyBac-like transposase Tpb2p

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During sexual reproduction the new developing macronucleus of *Tetrahymena* undergoes massive programmed DNA rearrangement, where over 30 % of the genome is eliminated. This process involves the sequence specific recognition of internal eliminated sequences (IESs) by an Argonaute-scan RNA (scnRNA) complex followed by heterochromatin formation including the accumulations of methylated histone H3 at Lys9 and Lys27 and the chromodomain protein Pdd1p. This heterochromatin formation eventually leads to the formation of distinct heterochromatin bodies in which DNA elimination is believed to occur.

Recently we have reported that the PiggyBac-like transposase Tpb2p is recruited to the heterochromatin bodies. Depletion of *TPB2* by RNAi caused defects in DNA elimination and heterochromatin body formation (Cheng, Vogt et al. 2010). Furthermore, the recombinantly expressed Tpb2p from bacteria can recognize and cut boundaries of different IESs in vitro when they are placed in the middle of an artificially designed and synthesized oligo (Cheng, Vogt et al. 2010). All together, Tpb2p is the most probable candidate to be the enzyme catalyzing the excision of IESs. In this study, we aimed to further investigate how the precise elimination of IESs can be mediated by Tpb2p.

We first analyzed the nuclease activity of recombinant Tpb2p in further details. Using synthesized oligo DNAs where every position of the reported left boundary of the R IES element (sequence AGTGAT) was individually mutated, we found that the third and fourth positions in the boundary sequence were important for efficient cleavage by Tpb2p. Furthermore, an in vivo study confirmed that these two positions were crucial for the precise elimination of the R IES element. Therefore, some DNA sequence preference of Tpb2p clearly contributes to the precise elimination of IESs.

On the other hand, because Tpb2p is a component of heterochromatin and is required for heterochromatin body formation, heterochromatin interaction with Tpb2p might also be involved in the precise IES elimination. Tpb2p has an endonuclease domain and a zinc finger domain. We found that the zinc finger domain, but not the endonuclease domain, was essential for heterochromatin body formation. Therefore, the zinc finger domain of Tpb2p may directly interact with some of the heterochromatin components and this interaction, in combination with the DNA sequence preference of Tpb2p, might mediate the precise elimination of IESs.

Keywords : domesticated transposase, DNA rearrangement, Tetrahymena, heterochromatin

Remnants of Tc/*mariner* elements are excised precisely by a *piggyBac* domesticated transposase and the NHEJ double-strand break repair pathway in the ciliate *Paramecium*

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Ciliates harbor two distinct nuclei in their cytoplasm. The micronucleus (MIC) undergoes meiosis during sexual processes and transmits the germline genome to the next generation. The somatic macronucleus (MAC) is responsible for gene expression, but it is lost during sexual events. At each sexual cycle, new MICs and MAC differentiate from the zygotic nucleus. The MAC genome was entirely sequenced and harbors very few repeated sequences, while analysis of individual MIC regions indicated that a substantial fraction of germline-restricted DNA is eliminated in a programmed manner during MAC development. Repeated DNA (transposons, minisatellites) is eliminated and thousands of single-copy, non-coding Internal Eliminated Sequences (IESs) are removed precisely from coding and intergenic regions. IESs are excised between two conserved flanking TA dinucleotides and a single TA is retained on somatic chromosomes. This reaction is initiated by the introduction of DNA double-strand breaks (DSB) at each end, and depends on PiggyMac (Pgm), a domesticated *piggyBac* transposase. The Ligase IV-Xrcc4p complex, a core component of the non-homologous end joining (NHEJ) DSB repair pathway, carries out the closure of IES excision sites and the circularization of excised IESs.

To gain insight into the mechanisms involved in the precise excision of IESs, we sequenced the genomic DNA extracted from Pgm-depleted cells during development of their new MACs, in which IESs are expected to be retained. We identified a genome-wide set of ~45,000 IESs and observed that 47% of genes contain at least one IES in the MIC. Sequence analysis provided support to the hypothesis that IESs have evolved from Tc/*mariner* transposons, under strong constraint for their precise excision from the MAC genome. Most IESs are very short (93% are <150 bp) and exhibit a striking 10.2-bp periodicity in their size distribution, which coincides with the helical pitch of double-stranded DNA. This is fully consistent with our previous suggestion that assembly of a transpososome-like synaptic complex is an early step during IES excision.

Bringing IES ends together before DNA cleavage would ensure that the correct MAC-destined fragments are joined in the final repair step. To investigate whether DNA cleavage and repair are coupled, we used RNAi to deplete *Paramecium tetraurelia* in Ku70/Ku80, which channels broken ends towards the NHEJ pathway. All IESs that we tested at the molecular level were retained and amplified in the new MACs of Ku-depleted cells and no DSB could be detected at their ends, a similar phenotype to that of *PGM*-silenced cells. Our results suggest that Ku may be part of the cleavage complex.

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Impact of transposable elements and their dynamics in Mediterranean and African *Lupinus* genomes

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The role of TEs in genome size variation which accompanies adaptive and diversification processes in plants is explored. Particular attention is focused on comparative genomic analyses of closely related species of ecological and agronomical interest from genus *Lupinus* (Fabaceae), which are adapted to contrasted ecological conditions and have remarkable genome size differences. Based on molecular cytogenetics and transposon-display approaches, our studies revealed a high diversity of retrotransposons of ancient and recent origins, but also showed differential-lineage specific amplification of TEs among the Mediterranean and African lupines. Using high-throughput sequence data generated by NGS technologies, a first evaluation of the repeat compartment has been performed on the nuclear genome of *Lupinus* using similarity graph-based clustering and sequence characterisation methods. The results provide a most accurate identification and estimation of the diversity of TEs (mainly gypsy and copia elements) and their dynamics in the Old World lupin genomes.

IG2

Repeat-Induced Point mutation (RIP) and the impact of transposable elements on fungal genomes

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Fungi have an efficient mechanism to inactivate multi-copy sequences called RIP (Repeat-induced point mutation). It acts as a defence mechanism against transposable elements (TEs) invasion. RIP mutates irreversibly C into T in duplicated sequences.
We took advantage of several international whole fungal genome sequencing projects where we were involved in, and of powerful bioinformatic automated analysis tools to perform a comparative genomic analysis of their TEs and RIP mechanisms.
We used the REPET package (Quesneville et al. PLoS Comp Biol 2005; Flutre et al. Plos One 2011) to efficiently detect, classify and annotate TEs, in ten genomes. In addition, we used RIPCAL (Hane et al. BMC Bioinformatics 2008) and locally developed scripts to detect and analyse RIP signatures among TEs copies.
This analysis shows that the invasion success of TEs is dependent of no or reduced RIP efficiency. Furthermore, we show a correlation between RIP efficiency and the RIP-targeted di-nucleotide frequency. These analysis also revealed traces of ancient RIP in small and degenerated TE copies in less TE-invaded genomes. Finally, we show how the genome structure can be affected by the interplay of both TE invasion and RIP, forming AT-rich isochores.

Keywords : Transposable elements, RIP, fungi

IG3

Two Types of Penelope-like Elements (PLEs) in Rotifers of the Class Bdelloidea

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Endonuclease-deficient *Penelope*-like elements (PLEs) associated with telomeres were initially found in bdelloid rotifers and a few other species from diverse taxa. It is becoming increasingly clear that such elements are much more widespread than previously thought, and can be found in numerous fungi, protists, plants, and invertebrates. Moreover, a detailed analysis of telomere-associated PLEs in the bdelloid rotifer *Adineta vaga* on a genome-wide scale brings unexpected twists to the previously observed PLE distribution patterns, and renews our interest in evaluating their roles in telomere maintenance. These intron-containing *Athena* retroelements bear a much closer resemblance to single-copy genes than to mobile genetic elements, and form a multigene family in which selected lineages may have the potential to generate telomeric repeats in the absence of telomerase.

Representatives of another, more conventional type of PLEs, which are intronless and carry a C-terminal GIY-YIG endonuclease domain, are not as widely represented in bdelloids as their endonuclease-deficient counterparts. Relatively few sequences of this type, mostly decayed, were identified in the *A. vaga* genome. Many of these sequences, while having originated from transposition, as evidenced by target-site duplications and typical PLE-type terminal repeat structures, had most of their coding sequence completely erased and transformed into an extremely asparagine-rich ORF, which preserves very few of the previously recognizable conserved motifs. This results in disruption of element functionality and would prevent genome-wide proliferation of deleterious copies potentially capable of endonuclease-mediated integration into internal genomic locations. Together with other genome defense mechanisms, these findings help to reconcile the relative paucity of transposon insertions in the core chromosomal regions with high rates of acquisition of foreign genetic material by bdelloid genomes.

Keywords : reverse transcriptase, GIY-YIG endonuclease, retroelements

IG4

REPET v2 : TEs detection and classification improvements

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The REPET package (Flutre et al, 2011) integrates two pipelines, which are constantly improving : TEdenovo and TEannot for transposable elements (TEs) detection and annotation. In the last release TEdenovo changed significantly.
The TEdenovo pipeline strategy is to find as much as possible potential TEs, and then to classify putative TEs in order to filter out false positives. The pipeline starts by the detection of repeated sequences comparing by alignments the genome with itself. These alignments are independently clustered according to different tools (RECON, GROUPER, PILER).Then, it builds multiple alignments from the clusters, from which a consensus sequence is derived. These consensus are classified according to TE features and redundancy is removed. Finally, there is the possibility to remove false-positives according to the classification (SSR, host genes, rDNA and under-represented unclassified consensus).
Two steps have been improved in REPET v2:
1) A structural TE detection approach is now implemented : LTRharvest (Ellinghaus et al, 2008) is used to search for LTR retrotransposons, using structural features of this TE category. It allows catching TEs present in only one or two copies in the genome. Potential TEs thus detected and all other derived consensus are put together before the classification and redundancy removal step.
2) Classification benefits from improvement too with the integration of PASTEC, a new classifier that we have developed. It tests all TE classifications and each result is weighted according to the evidences found. In addition to similarities to known TEs in Repbase Update and the search for repeated structures, it also uses HMM profiles, which are interesting to classify TEs and to detect host genes. PASTEC gives precisions about completeness and indicates if TEs are potential chimeras.
For illustrations of these two pipelines and associated tools, in the frame of genome annotation, see Véronique Jamilloux's poster.

Keywords : transposable elements, pipelines, detection, classification

IG5

Characterization of transposons isolated from insects

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Transposons are mobile DNAs spread in most organisms including some viruses. The ability of these sequences in mobilizing can be a decisive factor in evolutionary processes. In Eukarya, transposable elements (TEs) are a significant percentage of genomes showing a great diversity in gene content, size and mechanism of transposition. According to the above, TEs are classified into two main groups: Class I (retrotransposons) and Class II (DNA transposons).
Insect cells are the best system to study and produce baculoviruses, a pathogen used as bioinsecticide, protein expression system and gene therapy or vaccine vectors. Baculoviridae is a large family of insect viruses that infect and kill different species of Lepidoptera, Hymenoptera and Diptera. Baculoviruses have double-stranded circular DNA genomes of 80,000-180,000 bp, containing between 80 to 180 genes. These variations in gene number suggest that structural mutations are one of the major sources in the emergence of new viral species. Some of these genes (around 30, known as *core genes*) are shared by all members with known sequence (58 viruses). Meanwhile, remaining genes might have been "obtained" from other genomes. The high similarity between baculovirus and insect sequences or between baculovirus and other pathogens could be the consequence of transposition events that were selected during evolution of baculoviruses.
In fact, transposons have been detected within baculovirus genomes. One of the transposons most widely used in biotechnology is probably piggyBac, an insect DNA TE. This sequence was identified in AcMNPV (*Autographa californica* Multiple nucleopolyhedrovirus) propagated in TN-368 cell line. Later, other reports acknowledged the presence of other TEs, including the description of TED -a retrotransposon in AcMNPV- and TC14.7 -a DNA TE in CpGV- (*Cydia pomonella* Granulovirus). Two additional DNA TEs have been described, one from CpGV designated TCp3.2, and the other, a new piggyBac-related transposon isolated from AgMNPV (*Anticarsia gemmatalis* Multiple nucleopolyhedrovirus) and designated IDT. Considering these evidences, gene transfer processes could be more common than initially realized.
With the aim to discover and characterize new active transposons from insects, we transfected prokaryotic plasmids in insect cell lines (Sf9 and Sf21 from *Spodoptera frugiperda*, Hi5 from *Trichoplusia ni* and UFL-Ag-286 from *Anticarsia gemmatalis*) and then we recovered modified plasmids with DNA insertions by *Escherichia coli* transformation.
The proposed strategy has allowed isolating and sequencing 4 TE´s never before described and a variant of Piggybac, a transposon with many applications in biology.

Keywords : Transposon, baculovirus, piggybac

IG6

Identification of an active Helitron in domesticated wheat

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Polyploidization and differential proliferation of transposable elements (TEs) have particularly played an important role in the dynamic evolution of wheat species genome (*Triticum* and *Aegilops* genera). Increasing number of studies have shown that the wheat species genomes are composed of more than 90% repetitive DNA. These include various types of class 1 and class 2 TEs. However, no helitrons were yet described in these important wheat genomes. These DNA types of transposons were discovered one decayed ago (Kaptinov and Jurka, 2001 PNAS 98: 8714-8719) and are proposed to move through a mechanism, called rolling circle transposition, that includes DNA replication and strand replacement. They have been shown to be particularly active in the Maize genome and sequence analysis show that are ubiquitous in eukaryotes.

In the course of characterization of domestication gene (*Q/q*), we identified the first active helitron in hexaploid wheat. This helitron insertion is of 9,536 bp and occurred only very recently as it was traced in only two cultivars. Intron/exon structure of the helitron predicted a putative RepHel gene of four exons. The RepHel encoded protein is of 1,405 amino acids and shows 76% amino acid similarity to that encoded by Helitron4 Os of rice (*AC105746.1*,) and 87% amino acid similarity to a putative *Brachypodium distachyon* helicase (Bradi5g20077). A comparison of the 5' and 3' end sequences of the identified helitron shows a typical AT insertion site, the 5'TC, the3'CTAG and the sequences leading to the hairpin structure formation at 3' ends, show high conservation with rice and maize Helitron consensus sequences. Moreover, a gene encoding a 128-amino acid fragment of a glyoxalate reductase-like protein interrupted by a frame-shift is also present in the helitron, , indicating characteristic features of gene movement through helitrons. Analysis of expression shows that the entire helitron sequence was presumably transcribed with two to four additional exons spliced out of a 9,535 bp helitron.

We are now characterizing this helitron at the whole genome level and retracing its activation history and conditions by comparing different related species.

IG7

Ty elements related intra-specific variability in *S. cerevisiae*

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Along with single nucleotide polymorphism and chromosomal rearrangements, transposable element (TE) related polymorphism provides the richest sources of genetic variability. TE contents vary between individuals of the same species by the number of insertions and their location. The presence of mutated or degenerated elements represents an additional polymorphism, with the solo-LTR that are left by the deletion of LTR retrotransposon coding-sequences being a particular case. LTR retrotransposons belonging to five families called Ty1 to Ty5 are the only TEs present in the genome of the model yeast *Saccharomyces cerevisiae*. The whole genomic sequences of several *S. cerevisiae* strains have been recently made available but, except for a very few of them, the precise map of their Ty insertions was not resolved. Actually, TEs still remain a challenge for assembling genome sequences because of their repetitive nature and length exceeding that of a sequence read. Investigating the Ty polymorphism in *S. cerevisiae* may be useful to better understand its phenotypic variability and to get insights into the dynamics of Ty elements (expansion, persistence, extinction). Here we present for several strains, the partial Ty maps that have been obtained from the analyses of the available public sequences and confirmed by PCR. We will also present the development of a method that combines sequence capture with high-throughput sequencing in order to map all the Ty insertions of a given strain.

Keywords : Polymorphism, Ty LTR retrotransposon, *Saccharomyces cerevisiae*, Sequence capture

IG8

Unraveling the Life Dynamics of Sirevirus LTR Retrotransposons: Major Players in the Organization and Evolution of the Maize and Other Angiosperm Genomes

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Sireviruses is an ancient, and with a unique genome structure, LTR retrotransposon genus of the Copia superfamily, and the only one that has exclusively proliferated within plant genomes. However, as a result of receiving little research interest until now, the extent of their colonization and impact on their host genomes remained unclear. Here, aided by the recent development of a purpose-built algorithm, we report that Sireviruses have infiltrated most phylogenetic branches of the plant kingdom, extensively colonizing genomes such as that of soybean, sorghum and lotus. In maize, Sireviruses reached massive numbers to form a plethora of autonomous and non-autonomous families with distinct genome characteristics, some outlined herein for the first time. Sireviruses occupy 21% of the maize genome and comprise 90% of the Copia population, experiencing intense amplification during the past 600,000 years, and mediating the formation of gene islands by targeting their own genomes in chromosome-distal gene-rich areas. Intriguingly, this spatial and temporal integration pattern is not universal, as evident by their pericentromeric preference in soybean and by their much older amplification burst in cacao. Maize Sireviruses are constantly recycled by host mechanisms, exhibiting a significantly higher solo LTR formation rate than previously reported for known maize LTR retrotransposons. Their LTRs are heavily methylated, whilst they also form recombination hotspots by producing vast numbers of indels with specific lengths of 19-22bp. Finally, there is evidence for a palindromic consensus target sequence. To support further studies into these infiltration patterns, their evolutionary depth and impact on their hosts, and also facilitate genome annotation projects, we developed a highly curated database that catalogues Sireviruses in eleven fully-sequenced plants, currently housing approximately 16,200 elements. Overall, this multi-faceted work brings for the first time Sireviruses, together with a unique set of tools and data for the scientific community, under the spotlight (http://bat.infspire.org/sireviruses/).

IG9

Associations between LTR-retrotransposons and genes in the transcriptome of tobacco (*Nicotiana tabacum*) and their expressions in response to stress conditions

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Transposable elements (TEs) are a source of structural and functional diversification. They can disrupt genes, remodel chromosomal landscape and modulate gene expression. The retroviral-type LTR elements are major components of plant genomes and they represent promoter/regulatory capsules that can drive the production of chimeric co-transcripts with adjacent genes in response to their own specific expression pattern. The expression of the best-known plant LTR-retrotransposons, such as Tnt1 of tobacco, is linked to stress pathways and may be involved in global stress response.

The objectives of our study aim at evaluating the importance of TE-gene associations in the tobacco transcriptome and the impact of retrotransposon activation in the modulation of the expression of adjacent genes, such as via the production of chimeric co-transcripts in this species. The first bioinformatic analysis revealed that ESTs containing TE-related sequences represent at least 2% of tobacco transcriptome, with retrotransposons predominant. Many TE hits are associated with tobacco ESTs produced in stress-related conditions such as *in vitro* cell cultures or senescent leaves. We have identified many co-transcripts originating from the LTR transcriptional start of retrotransposons. These co-transcripts extend into downstream adjacent sequences, including genic sequences. Our experimental analysis of the expression of the retrotransposons and their associated co-transcripts show that the LTRs can drive the- synchronous expression of the co-transcripts in conditions where retrotransposons are transcriptionally activated, such as microbial elicitors or wounding.

Our current hypothesis is that plant retrotransposons may act as intermediates of stress stimuli, redirecting messages towards cellular functions in stress conditions. This may be of key significance for plant adaptability to environmental changes, as plants can not move to avoid them and have evolved complex and highly coordinated responses to biotic and abiotic challenges.

IG10

The drastic reshaping of the *Chondrus crispus* genome by transposable elements

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The genome of *C. crispus* has suffered a major reshaping as has been shown by our analysis of the tranposable elements that compose ~73% of its104Mb genome. The most abundant transposable elements (TEs) are class I LTR retrotransposons making up a total of 58 Mb, while non-LTR retroelements (LINEs and SINEs) were not found in the *Chondrus* genome. The search for Class II elements determined 21 families of terminal inverted repeat elements, representing 13 Mb of the genome as well as one active helitron family with 16 recent copies that represent 1 Mb of the genome. The retrotransposon component of the genome is extremely complex, not only due to the enormous number of recently transposed elements pertaining to different families, but also because each family has members that have diverged significantly and continue transposing actively. We found evidence for a very recent, ongoing burst of transposition activity, that is responsible for at least 18 Mb of the genome produced by over 2,600 complete copies. The analysis of the similarity between LTRs shows that the transposition of all elements (copias, gypsys and LARDs confounded) occurred concomitantly and is very recent. The mean similarity is 98%, with well over 100 elements exhibiting completely identical LTRs.This allows us to situate the transposition events in the last 330.000 years. We also observed that the sizes of the copia and gypsy reference elements are remarkably conserved, an observation that is valid for their related elements as well, suggesting a low ratio of insertions/deletions.

Furthermore we found a region that accumulates insertions/deletions and mutations faster than the rest of the element. This region is passed to the new copy but diverges much faster than the rest of the element.

LTR retroelements are a major force in reshaping the genome of *Chondrus* and have contributed to the increased size as compared to other red algae as well as the reduced gene number. *Chondrus crispus* presents a unique opportunity to look into the dynamics of an ongoing transposable element burst which also shows a mechanism of diversification that we have not observed on other species where a determined region diverges faster than the rest of the element.

IG11

De novo retrotransposition of endogenous L1 elements in cultured human transformed cells

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L1 retrotransposons are major contributors of germline structural variation in humans, sporadically causing heritable diseases by jumping into or close to genes. A number of human primary or transformed cultured cells are permissive to L1 retrotransposition, when a genetically marked L1 element is ectopically expressed. However the extent of endogenous retrotransposition in human cultured cells remains unexplored. Here we found that L1 ribonucleoprotein particles (RNP) can be detected in a broad range of human cell lines of various origins. We comprehensively mapped by next-generation sequencing L1 elements in one of these L1-expressing lines, HEK-293T, revealing tens of insertions not present in the human reference genome. To explore further their natural history, we examined their presence in the parental cell line, HEK-293, which do not express detectable L1 levels, and discovered several *bona fide* L1 retrotransposition events. This demonstrates for the first time that endogenous L1 elements can jump in cultured human transformed cells. In addition, the presence of L1 RNPs in a wide range of cell lines suggests that endogenous retrotransposition in somatic transformed cells might be more frequent than previously anticipated and could lead to continuous genome remodeling.

This work was supported by the following grants to GC: ARC (#4854), INSERM and INCa (Avenir program), and the European Research Council (ERC Starting Grant 'Retrogenomics'). MK was a recipient of a PhD fellowship from the Ligue Nationale Contre le Cancer.

IG12

Annotating transposable elements in the 1 Gb wheat chromosome 3B

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Because of its giant size (1C=17 Gb), a hexaploid composition and a transposable element (TE) content close to 85%, the wheat genome sequence remains largely unexplored.
To overcome these issues, a chromosome-by-chromosome approach has been established by the International Wheat Genome Sequencing Consortium. After building the first physical map of a wheat chromosome (the 3B, 1 Gb), we have recently undertaken its complete sequencing by combining Roche/454 sequencing of BAC pools with whole chromosome shotgun using Illumina technology. This provides an opportunity to study the dynamics of TEs at a scale never reached so far. Thereby, analysis of the distribution and classification of these elements will able elucidate the evolution of TEs and their impact on the genome structuring. Also, by using gene annotation and expression data, this work will provides information about the impact of TEs on gene expression.
The first challenge we face is to annotate precisely ca. 800 Mb of nested TEs. For that purpose, we are using the REPET pipeline (http://urgi.versailles.inra.fr/index.php/repet). With a reference set of 13 large sequences manually annotated and curated, we want to evaluate and improve the accuracy of the pipeline by adapting parameters to the wheat genome features. The accuracy and relevance of the first automated annotation of the 3B chromosome will discussed.

Keywords : wheat genome, annotation, transposable elements, evolution

IG13

Tnt1 retrotransposon expression is associated with the modulation of genes in tobacco

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Retrotransposons are abundant components of plant genomes, able to generate structural and functional variability. Nevertheless, up to now very little is known about functional roles of these elements. In order to investigate this question we generated transgenic tobacco plants with constructs directed to modulate Tnt1 expression, a retrotransposon present in tobacco genome as hundreds of copies. Pleiotropic phenotypes were observed, such as necrotic spots on leaves, no development of roots and, in some cases, severe developmental abnormalities in the photosynthetic parts. RNA-Seq based analyses revealed global changes in gene expression. These genes take part in several processes in plants and are candidates to be related to the phenotypes observed. Among them, two interesting candidates are NAC-domain-like and Acidic chitinase-like genes. RT-qPCR assay confirmed that both genes are induced. NAC is a family of transcription factors, related to several processes in plants such as senescence, ethylene and auxin signaling pathways, cell wall and lateral root development. Acidic chitinase is a pathogenesis-related protein, responsive to salicilic acid signaling pathway. These results indicate a Tnt1-mediated regulation of many sets of genes, affecting several pathways, and suggest a central role of Tnt1 in gene regulation in tobacco.

Keywords : Tnt1, retrotransposon, gene regulation, tobacco

IG14

Functional analysis of the domesticated piggyBac transposase involved in developmentally programmed genome rearrangements in Paramecium

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In ciliates, the germline and somatic genomes are physically separated between two distinct nuclei, the micronucleus and the macronucleus, respectively. At each sexual cycle, extensive genome rearrangements contribute to the differentiation of a new macronucleus from the germline. In *Paramecium*, these rearrangements include the imprecise elimination of repeated DNA (transposons, minisatellites) and the precise excision of ~45.000 Internal Eliminated Sequences (IESs). *Paramecium* IESs are invariably flanked by two 5'-TA-3' dinucleotides and are thought to have derived from ancestral Tc1/*mariner*-related transposons. Their elimination from the somatic genome leaves a single TA at each excision site and this precision is essential for the assembly of functional genes and the recovery of viable sexual progeny. IESs excision is a "cut-and-close" mechanism, with a 4-bp staggered double-strand cleavage around the conserved TA at each end.

DNA cleavage was recently shown to require the developmentally induced production of PiggyMac, a domesticated *piggyBac* transposase encoded by the *Paramecium* somatic genome that localizes specifically to the developing new macronucleus at the time IES excision takes place (Baudry, Malinsky *et al.* 2009). Transposons of the *piggyBac* family were shown to undergo precise excision between two flanking TTAA repeats. The DNA cleavages catalyzed *in vitro* at transposon ends by the *T. ni piggyBac* transposase exhibit the same geometry as those observed at *Paramecium* IES ends. RNAi-mediated inactivation of the *Paramecium PiggyMac* gene results in 100% lethality of sexual progeny and in a complete inhibition in genome rearrangements. This correlates with the absence of any detectable DNA double-strand breaks at IES ends and the retention of germline sequences in the genome of silenced cells. The protein PiggyMac and the *piggyBac* transposase share their catalytic domain, but some differences can be noticed in the other domains of Pgm, such as the addition of a large coiled-coil domain at the C-terminus of the protein and changes in the Cys-rich domain.

We conducted two parallel approaches to further characterize the role of the domains of PiggyMac: (1) as it is not possible to make mutants in *Paramecium*, we performed a functional complementation assay in cells silenced for *PiggyMac*, with an engineered transgene designed to escape RNAi against the endogenous gene. Complementation was monitered by following the localization of the protein and the progeny survival. We have established that the transgene is functional and able to escape RNAi. As a control, mutation of the catalytic residues is currently tested, followed by the deletion of the coiled-coil or the Cys-rich domains. (2) We try to reconstruct an *in vitro* IES excision assay, in order to characterize Pgm activity, and to test the different mutated or deleted versions of the protein.

IG15

Expression of *mariner-like-element* in the marine microalgae *Phaeodactylum tricornutum*

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Diatoms are present in all aquatic environments. They are a major component of phytoplankton and thus play a key role in the life of marine ecosystems. They are the source of the food chains of many species. About 100 000 species are listed, but it could be many more. The model diatom Phaeodactylum tricornum is an unicellular marine microalgae, measuring from 2 to 100 µm. Its culture conditions are controlled in the laboratory. Its physiology is well known and its genome was completely sequenced (http://genome.jgi-psf.org/Phatr2/Phatr2.home.html). Within its genome, the characterization of mariner-like-elements or MLE was undertaken. They belong to the second class of transposable elements or transposons and move through a "cut & paste" mode. The complete MLE element are about 1.5 to 4.7 Kbp in plants and include inverted terminal repeats (ITR) of about 20-40 bp.In order to characterize MLE in P. tricornutum, and especially transcribed MLE, we submitted the algal cultures to 3 different temperatures (8, 16 and 24 °C) for short (5 hours) and long exposure (8 days). If 16 °C corresponds to the usual temperature of culture in the laboratory, 8 and 24 °C, correspond to average temperatures in which microalgae can live in marine environments. RT-PCR was used to assess MLE expression in P. tricornutum under these different thermal condition . Primers were defined according to a consensus sequence that allowed the characterization of the MLE in P. tricornutum (Hermann et al.; submitted). Although not quantitative, this technique indicates the presence or absence of MLE expression. As a control for RNA extraction quality and RT-PCR, both actin and 18S ribosomal housekeeping genes and superoxide dismutase gene were used. The latter was chosen for the difference of length between genomic DNA and complementary DNA sequence. This difference is due to the presence of an intron in genomic DNA. MLE expression was observed at all temperatures after 5 h whereas no MLE expression was found after 8 days.As a result, temperature changes do not seem to lead to induction of MLE expression whatever the length of exposure. By contrast MLE expression has been highlighted in short term culture of P. tricornutum and it could be sensitive to cell density.

IG16

Selecting Hyperactive Integrases Through Synonymous Gene Evolution

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Integrons are gene recruitment platforms that allow for rapid bacterial evolution, playing a major role in the acquisition of antimicrobial resistance genes. The integrase is a tyrosine recombinase that catalyzes the reaction between *attC* and *attI* sites. However, its activity is finely tuned to preferentially process *attI* x *attC* and *attC* x *attC* reactions, as they allow for the acquisition and rearrangement of cassettes, rather than the *attI* x *attI* reactions that lack an evolutionary meaning. Being a less frequent phenomenon, the structural basis of *attI* recognition by the integrase has yet remained elusive.
Random mutagenesis is a convenient method for evolving sequences and proteins. Nevertheless, evolution of a protein is limited to the evolutionary landscape (EL) of its coding sequence, i.e. the number of different amino acids attainable by each codon through point mutation. The *Evolutionary Landscape Painter* (ELP) is an algorithm that allows for the rational design of sequences that, although synonymous, have access to a different protein sequence space. Thus, ELP helps enlarging the evolutionary potential of a gene.
We have used ELP to design two *intI1* alternative sequences maximizing the evolutionary landscape of the integrase. We are evolving the three sequences, namely WT, alt1 and alt2, through random mutagenesis and selecting for *attI* x *attI* efficiency using a previously developed in-mass suicide-conjugation assay. Selection of enhanced activity mutants from the three synonymous sequences will lead to the identification of the key positions and, virtually, all the residues that can enhance the *attI* x *attI* activity of the integrase. This will help shed light into the structural features governing the recognition of the *attI* site, and the processing of the *attI* x *attI* recombination by the integrase.

Keywords : Integron, site-specific recombinase, attI, mutagenesis, synonymous sequence

IG17

Genome-wide assessment of transposable element mobilization in Arabidopsis

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Transposable elements (TEs) and their relics are major components of eukaryotic genomes. TEs are potentially highly mutagenic as their proliferation can cause chromosomal rearrangements, disrupt genes or affect gene expression through transcriptional interference. However, few TEs are usually mobile within genomes at any one time thanks to potent mechanisms that restrain their activity, such as DNA methylation in mammals and plants. Thus, in the flowering plant Arabidopsis, severe loss of DNA methylation caused by mutations in the chromatin remodeler gene *DDM1* triggers massive accumulation of transcripts corresponding to TEs. Yet, comparatively few TEs appear to be mobilized as a result. To address this issue in more detail and to determine the distribution of new TE insertions as well as their consequences on the expression of neighboring genes, we have sequenced the genome of over 60 epigenetic Recombinant Inbred Lines (epiRILs) that were derived from a cross between a wild type and an isogenic *ddm1* mutant line. After backcrossing of the F1 and selection of the progeny homozygous for wild-type *DDM1*, the epiRILs were propagated through six rounds of selfing. The epiRILs therefore permit a detailed assessment of transposition events soon after they have occurred. Using Illumina sequencing of mate pairs, we could show that both retroelements and DNA transposons are mobilized in *ddm1* and the epiRILs. However, mobile TEs belong to only a dozen or so of the >300 TE families identified in the Arabidopsis genome. Furthermore the rate and dynamics of transposition vary dramatically both within and between TE families, suggesting additional mechanisms of control. Finally, our analysis indicates that new insertions are distributed with no overt bias throughout the genome and in numerous cases disrupt genes or interfere with their expression. These findings provide compelling evidence that natural selection is the main force shaping the over-accumulation of TEs within pericentromeric regions in Arabidopsis.

Keywords : Genome resequencing, DNA methylation, Arabidopsis, TE mobilization

Withdrawn

IG19

Transposable elements *gypsy*, *412*, *P*, *Galileo* and *hobo* and the breakpoints of chromosomal inversions in natural populations of *Drosophila willistoni*

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Transposable elements (TEs) have been strongly identified as generators of chromosomal rearrangements in various organisms. In the genus *Drosophila*, the most widespread type of rearrangement is the inversion. In this sense, the Neotropical *Drosophila willistoni* is one of the species with the highest number of chromosomal inversions occurring in natural populations. Currently this species has an improved photomap of their polytene chromosomes (n = 3), subdivided into 302 subsections, as well as a review of the breakpoints of 51 inversions segregating in 30 different populations. Considering these information, this study aims to analyze the association between the *in situ* hybridization sites of TEs *gypsy*, *412* (Class I elements), *P*, *Galileo* and *hobo* (Class II elements) obtained from nine populations, with the breakpoints of inversions known in the polytene chromosomes of *D. willistoni*. It was considered as association the hybridization sites of TEs that were in the same subsection of the breakpoint. Our results, considering all chromosomes, showed a total of 86 hybridization sites to the TE *gypsy*, of which 29 corresponded to breakpoints; 100 hybridizations sites to the TE *412*, of which 33 corresponded to breakpoints; 112 hybridization sites to the TE *P*, of which 44 corresponded to breakpoints; 99 hybridization sites to the TE *Galileo*, of which 23 corresponded to breakpoints and finally, 105 hybridization sites of the TE *hobo*, of which 35 corresponded to breakpoints. Within the 23 subsections that showed hybridization signals for all the five TEs analyzed, eight corresponded to breakpoints of inversions. Statistical analysis by $\chi 2$ Test, which obtained the expected number of association by the product between the total number of hybridization signals of a given TE and the total number of breakpoints, divided by the total number of subsections, did not suggest a significant association for any of the TEs with analyzed breakpoints. However, this result may have been influenced by the number of subsections, once that the photomap of the species in each subsection covers two or more chromosomal bands and the accuracy of this inference is limited. This analysis is part of an extensive study of the breakpoints and the origin of inversions in *D. willistoni*, which will include the refinement of the photomap, subdividing the current subsections.

Grants and fellowships: CNPq, FAPERGS, PRONEX-FAPERGS, PPGBM.

Keywords : *Drosophila willistoni*, inversion, breakpoints, transposable elements

IG20

Transposition bursts and expression rates in interspecific hybrids of *Drosophila buzzatii* and *D. koepferae*

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The comprehension of the real impact of transposable elements (TEs) on genomes is one of the biggest challenges in Biology. One major unsolved point is the mechanisms contributing to the activation of the TE transposition in eukaryotes. Activations of TEs by interspecific crosses has been extensively studied in plants and associated to the induction of new variability. In the case of Drosophila data were limited to the study of a single element. In this work we present the effects of interspecific hybridation on TEs transposition rates using a whole genome approach and analyzing the changes of expression patterns of one of the elements implied in hybrid mobilization.

Species crosses were followed for three generations throughout introgression by serially backcrossing *D.buzzatii* males into the *D. koepferae* genome. The introgressed hybrids were analysed for new transposed genome regions using AFLP markers. Subsequent analysis of transposition rates of the two main classes of mobilized elements, were performed using the technique of transposon display. Our results show that about 70% of the detected hybrid instability originates by mobilisation of TEs. From a total of 34 TEs mobilised in the hybrid genome, 50% belong to LTR retrotransposons, 30% to non-LTR retrotransposons, and 20 % to DNA transposons. Rates of transposition of *Osvaldo* (a LTR retrotransposon) and *Helena* (a non-LTR retrotransposon) in hybrids are one order of magnitude higher (10^{-2}) than those in *D.buzzatii* (10^{-3}). Expression patterns of the retrotransposon *Osvaldo* in somatic and germinal lines show a general tendency to an increase of expression levels in hybrids compared to parental species. In the germinal line, in particular, the levels of expression tend to be higher in hybrid males compared to the hybrid females and differences between families and generations of hybrids were observed.

These results constitute the first approach to study the regulation mechanisms of *Osvaldo* in the genome and to understand the transpositional activity of different TEs in the whole genome of hybrids during hybridization events. High genomic instability originated by transposition in hybrids could highly increase the evolutionary potential of species leading to the emergence of new phenotypes on which selection could act.

Keywords : interspecific hybrids, transposition, Drosophila, instability

IG21

Modular evolution and functional diversity of Integrative and Conjugative Elements of the Tn*GBS* family

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Integrative and conjugative elements (ICEs) have a major impact on gene flow and genome dynamics of bacteria. Unlike most ICEs characterized to date that use a phage like integrase for their mobility, Tn*GBSs* use a DDE transposase. Tn*GBS*1 and Tn*GBS*2 in *Streptococcus agalactiae* strain NEM316 were the two first identified ICEs of this family. By systematic genome analysis we identified 56 different Tn*GBS*-related ICEs, all but one being inserted in streptococcal genomes. These ICEs contain two types of conjugation modules, typified either by Tn*GBS*1 or Tn*GBS*2. Each of these conjugation modules, comprising a type four secretion system, a VirD4 coupling protein, a relaxase and its cognate *oriT* site, is shared with distinct families of Firmicute's conjugative plasmids. The Tn*GBS*s also share replication modules with different plasmids and we show that both Tn*GBS*1 and Tn*GBS*2 combine replication and transposition to promote their spreading and stabilization. Phylogenetic analysis allows to reconstruct the successive recruitment by two conjugative elements of different origins of a transposition module probably derived from insertion sequences (IS). Despite an evolutionary successful horizontal spreading of Tn*GBS*-related ICEs in the Streptococcus genus, these ICEs have a restricted host range. We show that for both Tn*GBS*1 and Tn*GBS*2, this host restriction is not due to a transfer incompatibility linked to the conjugation module, but most probably to the modules implicated in replication and/or to their transposition specificity upstream SigmaA dependent promoters, which is unique among bacterial transposable elements. Taken together, our experimental data and phylogenetic analyses lead us to propose a model for the evolution of conjugative elements in which conjugation modules recruit and exchange replication and/or integration modules of different origins, adapting vertical maintenance and spreading capacity of the elements to different hosts.

Keywords : ICE, transposase, transposition specificity, replication, evolution

IG22

Copy number and genomic distribution of *PIF/Harbinger* elements in wild and cultivated *Medicago truncatula*

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Elements belonging to *PIF/Harbinger* superfamily are characterized by 14-25 bp-long terminal inverted repeats (TIRs), 3 bp-long target site duplications (TSD) and carry two open reading frames, one coding for a DDE transposase and the other comprising a *Myb*-like domain. Previous analyses of the *Medicago truncatula* genome revealed 27 elements classified in the *MtPH-M* family, divided into three subfamilies. Here we report on a comparison of the copy number and insertion sites polymorphism of *MtPH-M1* transposons among plants representing a *M. truncatula* Gaertn. core collection and cv. A17 'Jemalong'. Southern hybridization with a probe specific to the *MtPH-M1-Ia* element identified one copy of the element in the wild ecotype L554, two copies in other ecotypes and three copies in A17'Jemalong' and L213. A probe anchored in the internal region common to *MtPH-M1-Ia* and *MtPH-M1-IIa* produced five to six signals in the wild ecotypes, while nine signals were observed in A17 'Jemalong'. Probes anchored in terminal sequences of elements belonging to the *MtPH-M1* subfamily generated up to 13 and over 30 signals for the 5' and 3' end, respectively. Differences in the copy number were accompanied by size differences of restriction fragments detected by the probes, indicating variability in the genomic localization of *MtPH-M1* elements, which was further investigated using a splinkerette-PCR-based approach. Five *MtPH-M1* insertion sites, not present in the reference genome of A17'Jemalong', were identified in wild ecotypes. Higher number of hybridization signals observed for A17 'Jemalong' and different genomic localization of *MtPH-M1* insertions, as compared to wild ecotypes, suggests transpositional activity of *MtPH-M1* elements in the course of domestication. The research project was financed from the Polish Ministry of Science and Higher Education grant no. N N301 013436.

Keywords : *PIF/Harbinger*, *Medicago truncatula*, transposition, diversity

IG23

LTR Retrotransposons in the Robusta coffee genome (*Coffee canephora*): A BAC-end view

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Coffee is one of the most important international trade commodities and is ranked as the second most valuable primary commodity exported by developing countries. Two species are used in commercial production: *Coffea arabica*, a perennial allotetraploid species (2n = 4x = 44; ~1250 Mbp), known as Arabica coffee and *Coffea canephora*, a perennial diploid species (2n = 2x = 22; ~710 Mbp) known as Robusta coffee. Recently, 136,027 BAC-end sequences (BES) were generated from *C. canephora* BAC libraries, representing 7.5% of the genome size (unpublished results).

Here we present the first large-scale identification of LTR Retrotransposons in *C. canephora* based on the *in silico* analysis of 94 Mbp of BES. Sixteen percent of BES show significant similarity to known transposable elements in Repbase (www.girinst.org). A large majority of them (93%) belong to the Class I LTR-RTs. Interestingly the *Gypsy* superfamily of LTR-RTs outnumbers clearly the *Copia* superfamily, with a *Gypsy:Copia* ratio of 3**:**1. This ratio appears similar to rice BES (*O. sativa;* 2.87**:**1) but differs significantly from other model plant species. We use the Assisted Automated Assembler of Repeat Families algorithm (AAARF; DeBarry et al., 2008) to assemble the 136,027 BES into 700 contigs of repeated sequences. 24 contigs ranged from 13,433 to 4,257 bp show similarities to LTR-RTs and eight of them were annotated as full-length elements. Among the 24 families, 17 and 5 belong to the *Gypsy* and *Copia* LTR-RTs superfamilies respectively.

Twenty-one LTR-RTs families were found conserved in *C. canephora* EST sequences available in GenBank, suggesting that LTR-RTs are potentially expressed. Leaves and fruits cDNA libraries from *C. canephora* (Mahesh et al. 2006) were used as template for PCR amplifications in RT and LTR regions of sixteen LTR-RT families. Eight of them were found potentially transcriptionally active in *C. canephora* leaves or fruits.

In summary, we demonstrate the potential of BES to identify and characterize full-length LTR-RTs. In *Coffea canephora*, LTR-RTs appear redundant and transcriptionally active suggesting that they contribute significantly to the genome structure and evolution.

IG24

Target immunity and transpososome activation during Tn*4430* transposition

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Tn*4430* is a transposon of the Tn*3* family, a widespread family of replicative transposons in bacteria. The transposase protein (TnpA) of this family of elements is the key enzyme that catalyses the DNA cutting and re-joining reactions allowing the transposon to move from one DNA location to another. The protein is also responsible for target immunity, a process that prevents multiple insertion of the element in the same DNA molecule.
In this study, we have achieved a decisive step toward the understanding of the transposition and target immunity mechanisms of Tn*4430* by developing sensitive biochemical assays reproducing the activities of the TnpA protein *in vitro*. These assays were used to characterize TnpA mutants that are defective in conferring immunity. These mutants showed promiscuous activity compared to the wild type transposase. They formed higher-order nucleoprotein complexes with paired ends of the transposon (PEC) more efficiently than the wild type protein. This correlated with higher cleavage activity at the 3' ends of the element. PEC formation by wild type TnpA was facilitated on pre-cleaved DNA substrates and both the wild type and mutated TnpAs exhibited similar strand transfer activity. The data support a model in which TnpA activation is tightly regulated at the level of transpososome assembly and that mutations affecting immunity have 'unlocked' the transposase making it less demanding with respect to the activation signal.
Most unexpectedly, all the examined mutants were also proficient at cleaving the non-transferred strand at the 5' ends of the transposon, generating specific double-strand breaks in the DNA. Sequential cleavage at the 3' and 5' ends occurred with no apparent hairpin intermediate, suggesting a missing link between classical replicative and "cut-and-paste" transposition mechanisms.

IG25

Epigenomic Limits of Productive Retrovirus Integration

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Integration of retroviruses and retrotransposons occurs in most genomic regions with weak but statistically significant preferences for target site sequences. HIV-1 preferentially targets genes, particularly the transcriptionally active ones. Avian sarcoma and leukosis viruses (ASLV) integrate with only slight preference for genes while murine leukemia virus uniquely favors integration in close proximity to upstream or downstream transcription start sites. Genomic features as well as the "epigenetic landscape" at the site of integration are crucial for the outcome of each integration event, i. e. stable long terminal repeat-driven expression of the provirus or silencing and transcriptional suppression. The current availability of assembled genome sequences with epigenomic characteristics and reliable cloning of retrovirus integration sites enables to study the interplay between transcription signals of the retrovirus and enhancing or suppressive influences of the adjacent cellular DNA.
We studied the integration sites of ASLV-derived vectors integrated into normal or DNA methylation-deficient human cells. Integration sites from cell clones containing single proviruses were cloned and their genomic features as well as epigenetic landscape were correlated with expression or silencing of the provirus. Dnmt3b turned out to be the major player in silencing the long terminal repeat-driven transcription. Proviruses integrated into H3K4me3-rich CpG islands associated with promoters of active genes display long-term stability of expression and are resistant to the transcriptional silencing after overexpression of Dnmt3b. Stability of expression and resistance to the silencing decreases with the distance from transcription start site. By contrast, proviruses integrated into the intergenic regions tend to the spontaneous transcriptional silencing even in DNA methyltransferase-deficient cells.
In conclusion, we propose a general model of the crosstalk between the integrating mobile element and surrounding chromatin at the target site, where the long-term expression or gradual silencing are to a great part predetermined by local epigenomic features.This work was supported by the Czech Science Foundation.

Keywords : Retrovirus integration, provirus silencing, epigenomics

IG26

Transposition activity in recent evolution of *Cucumis melo* elucidated by whole-genome resequencing of melon varieties

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Transposable elements are major components of all eukaryote genomes studied to date where they drive genome and gene evolution. We have performed a detailed analysis of the transposon content of the recently sequenced melon genome. Using homology and structure-based methods we have annotated 19.7% of the genome space as transposable elements, all of which are attributed to Class I or II. The majority (87%) are classified according to the major superfamilies of elements previously described in plants and further grouped into families. The retrotransposon elements described account for 14.7% of the genome whereas DNA transposons represent 5.0%. A similar analysis of the closely related cucumber genome revealed that most melon transposon families are specific to the melon lineage, and thus are recent. Dating insertion times of LTR retrotransposons shows that their highest activity was around 2 MYA, notably after the split with the cucumber lineage. We are at present exploiting resequencing data of 7 different melon varieties to gain insight into transposon activity in the short evolutionary time scale that corresponds to melon domestication and breeding. Using mapping data to detect vacant sites in the resequenced lines with respect to the reference, we have identified 1787 polymorphic sites that putatively correspond to transposon movement. Over 50% of these are attributed to retrotransposons, which, taken with the fraction they occupy and insertion dating, confirms that these elements have had a salient role in the recent evolution of this genome. This approach also permits us to explore the role of TEs in gene evolution, by studying the relationship of these polymorphic sites with genes. For example, 285 of these sites are found within annotated genes. We are still refining the methods of detecting TE movement using this data, notably detecting insertions as well as absences in the resequenced samples. Given the promising results of these preliminary analyses, we are confident that the approach of using resequencing data from varieties of different origin and under different selective pressures will allow us to obtain a genome-wide picture of the impact of transposition on recent melon genome evolution.

Keywords : transposable element, genome annotation, genome resequencing, genome evolution

IG27

The chordate *Oikopleura dioica*: confrontation between a compact genome and its new invaders

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The pelagic tunicate *Oikopleura dioica* was recently established as a new model organism near the transition between invertebrates and vertebrates. It has a very small genome (70 Mb) but a relatively large number of annotated genes (~18,000). Among profound changes accompanying the genome compaction, most ancestral families of transposable elements (TEs) are absent in *Oikopleura*. In contrast, *Oikopleura* hosts a flourishing family of *gypsy*/*Ty3* LTR retrotransposons, the *tor* elements. Each *tor* element is present in one or only a few polymorphic copies in the genome, but the families to which these elements belong are remarkably diversified. Part of *tor* elements contains a gene encoding a candidate viral envelope (*env*) in addition to *gag* and *pol*. Many elements have kept intact coding sequences and they are efficiently transcribed from their own promoters located in the LTR and, for some elements, upstream the *env* gene. Taken together, these observations suggest a recent acquisition of *tor* elements by horizontal transfer.

Our research aims at understanding how *tor* elements were acquired and how their proliferation is controlled in the ultra-compact genome of *Oikopleura*. The genotyping of wild and cultured individuals for the presence of genomic *tor* insertions shows low-frequency, hemizygous insertions. Because the effective population size of *Oikopleura* is very large we suspect that *tor* insertions can persist for a certain time in the genome, even when they are disrupting transcriptional units. Recombinant *tor* Env proteins expressed in mammalian cells were found glycosylated and co-purify with cellular membranes. Such biochemical properties are critical for a function as surface receptors and these results are consistent with the proposed role of Env in the horizontal transfer of *tor*. We have also observed a strong tissue-specific expression of *env* genes during the embryogenesis, which suggests a function in the development of *Oikopleura*.

According to the recent developments on the regulation by non-coding RNA, *Oikopleura* could make extensive use of small RNA to preserve its highly compact genome from TEs. We have characterized an abundant class of piRNA-like molecules, present in *Oikopleura* eggs and which includes RNA guides against *tor* sequences. Gene expression profiling indicates that several genes of the *ago / piwi* family are expressed in the male and female gonads, where they could be involved in the biogenesis of *Oikopleura* piRNA. Our future work will aim at clarifying the role of piRNA in the control of gene and TEs expression and the functions of the diverse *argonaute / piwi* genes in the small RNA metabolism of *Oikopleura*.

Keywords : retrotransposon, tunicate, envelope, piwi

IG28

Factors affecting paramutation in *Drosophila*

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The study of ***P* transposable element** repression in ***Drosophila melanogaster*** led to the discovery of a homology-dependent repression mechanism operating in the germline, called ***Trans*-Silencing Effect** (TSE): a *P*-transgene inserted in heterochromatin (called **Silencer**) can repress a homologous *P*-transgene inserted in euchromatin (called **Target**), irrespective from the genomic location of the latter. TSE is strongly sensitive to mutants affecting **heterochromatin components** (e.g. **HP1**) and the **PIWI-interacting RNA silencing** mechanism (**piRNAs**) which was shown to be involved in TEs repression in the germline. In addition, production of piRNAs itself was shown to be sensitive to HP1 dose, indicating that a RNA silencing pathway can depend on heterochromatin components. These results therefore suggest that TSE involves a positive loop between heterochromatin formation and the piRNA pathway.
One of the strong TSE Silencer locus corresponds to a cluster of seven *P*-transgenes, generating a local heterochromatic sector inside euchromatin and producing piRNAs. Strikingly, another cluster of transgenes located at the same locus is stably devoid of *trans*-silencing capacities and does not produce piRNAs. We have tested if this cluster which is not silencer can be converted into a silencer locus, simply by maternal inheritance of the cytoplasm produced by a female carrying a silencer locus. We have obtained a complete and stable **epigenetic conversion** (paramutation) since the previously non-silencer locus has acquired strong silencing capacities. In addition, the paramutated locus has also become paramutagenic and has stably acquired a *de novo* capacity to produce piRNAs. **Paramutation** in *Drosophila* is induced by a cytoplasm carrying piRNAs and is associated with **emergence of a piRNA producing locus**.
We have started to investigate the **molecular mechanism** of this paramutation by a candidate gene approach of **mutants** affecting this process. We report the effects, on repression elicited by a paramutated locus, of mutants affecting heterochromatin components or various RNA silencing pathways. Mechanism of establishment and maintenance of paramutation are discussed.

Keywords : *P* element, piRNA silencing, epigenetics, paramutation, *Drosophila melanogaster*

IG29

An iterative process for TEs annotation in large genomes

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The recent successes of new sequencing technologies allow today the sequencing of very large genomes at reasonable costs. Transposable elements (TEs) constitute the most structurally dynamic components and the largest portion of their nuclear genomes, e.g. 85% of the maize genome (Schnable et al. 2009), and 88% of the wheat genome (Choulet et al. 2010). Therefore, TE annotation should be considered as a major task in genome projects. However, it still remains a major challenge, since a good TE annotation relies critically on an expertly assembled reference sequence set, data that currently cannot be obtained in an automatic fashion. This crucial step is now a bottleneck for many genome analyses.

In this context, we scale up for the new high-throughput sequencing technologies, a repeat annotation pipeline. Hence, we improve, in the v2.0 release - see poster "REPET v2" (Arnoux et al. 2012) in this conference -, the REPET package (Flutre et al. 2011). REPET, gathers two pipelines: TEdenovo build a TEs library and TEannot annotate TE copies in the genome. In addition, we test a new strategies dedicated to these very large genomes. One of these uses an iterative approach:

1) Detection of young TEs with stringent parameters able to find quickly only the less degenerate ones to build a first TE library.
2) TE annotation and extraction of the corresponding sequences from the initial contigs. We obtain a reduced genome sequence.
3) Detection of the other TEs with sensitive parameters on the reduced genome sequence to build a second TE library.
4) Annotation of the original contigs with the concatenation of the two TE libraries.

The rational is that these large genomes are made of mostly few TE families that recently invade. They will be detected in the first step and this will allow reducing the genome by an important factor. We tested this approach on *A.thaliana* using TEdenovo and TEannot with very stringent settings in the first round and with default settings in the second round. We will present the benchmarks obtained on this well annotated genomes. We will also present preliminary results on the wheat, an allohexaploid with three homoeologous genomes. It has one of the largest plant genomes ~17Gbp, and with 88% of TEs is very repetitive {Choulet, 2010 #158}. We start with the 3B chromosome (293890 contigs, 975Mbp) the first fully sequenced.

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IG30

Differential proliferation of TEs in the A and C genomes of the recent allotetraploid oilseed rape (*Brassica napus*)

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Polyploidization and differential proliferation of transposable elements (TEs) have played a major role in the dynamics of plant genome evolution. Within the family of *Brassicaceae*, the genus *Brassica* includes species which genomes have been recurrently duplicated during their evolution, by frequent polyploidization events. Among these, oilseed rape (*B. napus*) has been formed through a relatively recent allotetraploidization event between *B. rapa* (A genome) and *B. oleracea* (C genome). It represents an important crop that provides about ~13 % of edible vegetable oil world-wide. In the course of sequencing the highly duplicated genome of oilseed rape, we have characterized proliferation of TEs in its A and C genomes. Annotation of TEs has been done based on structural features, *de novo* prediction of repeats as well as similarity search. Class 1 TEs represent globally ~17 % of the genome sequences whereas class 2 TEs represent ~4 % ; important differences were observed between the A and C genomes in term of dynamics of TE proliferation as well as distribution along the chromosomes. TEs have much more proliferated in the C genome than in the A genome, and now explain a size difference of 135 Mb. Comparison with parental species indicates that TE proliferation occurred most likely before the A and C genome "joined" in the oilseed rape genome. These comparisons should also shed light on the homogenization process of the A and C genomes in oilseed rape, through "cross-transposition". The significance of differential proliferation of TEs between the two genomes for the stability of the allopolyploid will be discussed.

Keywords : *Brassica*, oilseed rape, polyploidy, genome evolution, proliferation of transposable elements

IG31

Testing the parasitoid route for transposon horizontal transfer using NSG

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The Horizontal Transposon Transfer (HTT) is now a well documented phenomenon but the vectors and mechanisms involved are yet poorly understood. *Drosophila* has been the main model for HTT studies and 54% of all events described so far are in these organisms. Among the suggested vectors or intermediaries for HTT in *Drosophila* are the parasitoid wasps and their intricate virus mechanism to control the host immune system (polydnavirus). For testing the hypotheses of HTT among *Drosophila* and its parasitoid we have designed an experiment involving comparisons in two separated parasitoid-host systems. One is composed by *D. incompta* and its parasitoid wasp. *D. incompta* is a flower breeding fly that presents a restricted ecology, using flowers of *Cestrum* genus as unique sites of oviposition, development and feeding. The other system is composed by five *Drosophila* species and their parasitoid wasps. These species are generalists using decayed fruit as ecological resources. Four different pools of genomic DNA were prepared (2 for the flies systems and 2 for their parasitoid wasps) and sequenced using Solexa-Illumina HiSeq2000 New Sequencing Generation (NSG). For each sample were generated about 3 Gb of sequences. The Transposable Elements (TEs) were screened by Blast using the Repbase TEs database and were clustered in four TE datasets (1-*D.incompta*; 2-pool of generalist flies; 3-wasp of *D. incompta* and 4- wasp of pool of flies). Initially these TE datasets were compared by Blast among each other and with the Repbase database. The sequences with significant Blast hits were aligned and the similarities of sequences analyzed in ways of looking for putative HTT examples. No significantly similar sequences were observed among the flies and their parasitoid wasps. However, when TEs dataset of the flies with different ecological restrictions are compared, at least 31 potential HTT events were observed, 21 cases involving retrotransposons from gypsy superfamily (*Gypsy*, *Tabor* and *412*), 2 cases of retroposons and 8 cases involving DNA transposon. Also interesting is the high identity (97.8%) observed between a *mariner* element SMAR25 from *Schmidtea mediterranea* (planaria) and an element present in *D. incompta*. These results suggest that the intricate ecological interaction between *Drosophila* species and their parasitoid wasps do not promote the passage of TEs between this set of species. Moreover, the HTT events found among *Drosophila* species and between *Drosophila* and other taxas suggest that other vectors are a more important route to promote HTT of *Drosophila* TEs.

IG32

The dynamics of microsatellite births and deaths within transposable elements (Tes)

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Microsatellites – tandem repeats of short DNA motifs – are abundant in the human genome, and have high mutation rates. Microsatellite instability is implicated in numerous genetic diseases, but the molecular processes involved in their emergence and disappearance are still not well understood. Microsatellites are hypothesized to follow a *life cycle*, wherein they are born and expand into adulthood, until their degradation and death. Here we identified microsatellite births/deaths in human, chimpanzee and orangutan genomes, using macaque and marmoset as outgroups.

The previously proposed importance of TEs in microsatellite genesis was evaluated here on a genome-wide basis for the two most copious and recently active TE groups – *Alu*s and L1s. Remarkably, 36.3% of microsatellites present at orthologous positions in human, chimpanzee and orangutan, were likely acquired due to insertion of TEs *"upon arrival"*, with approximately equal contribution of *Alu*s and L1s. Additionally, among microsatellite births and deaths occurring in the human, chimpanzee, orangutan or human-chimpanzee common ancestor lineages, 26.3% of births and 24.3% of deaths occurred within TEs *following their integration*. Moreover, although *Alu*s and L1s together cover ~25% of the aligned human genome, they harbor as many as 41% of all interrupted microsatellites, implying that TEs are important players at all stages of the microsatellite life cycle.

Based on the distribution of microsatellite births and deaths along the length of *Alu* elements in younger and older subfamilies, we propose the following model: *Alu*s give birth to AT-rich microsatellites at 3' ends upon integration because their 3' poly-A tails, essential for transposition, frequently possess long, uninterrupted $[A]_n$ stretches. As mutations accumulate in older *Alu*s, 3' poly-A tail microsatellites die due to interruptions and/or deletions, while the middle A-stretch becomes the hotbed for births and, subsequently, deaths. Remarkably, in *Alu*s microsatellite births and deaths tend to balance out, keeping the overall number of microsatellites constant over time.

A different model emerges for L1s. They frequently give birth to microsatellites upon and after their integration; however, unlike *Alu*s, L1s do so evenly throughout their length, potentially because their whole sequence is AT-rich. Since L1s are on average older than *Alu*s, they may have experienced a substantially greater number of mutations, generating more protomicrosatellites. Interestingly, microsatellites in L1PAs are being eroded, likely by an excess of deaths over births; older L1PA subfamilies are depleted in stationary microsatellites and enriched in deaths.

Keywords : microsatellite births, microsatellite deaths, L1s, *Alu*s, transposable elements

IG33

Evidence that LINE-1 activity underlies fetal oocyte selection

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Attrition of fetal oocytes has a profound impact on ovarian oocyte reserves in humans[1], macaques and rodents. Surprisingly little is known about the underlying cause of this phenomenon besides its coincidence with meiotic prophase I. Here we present evidence implicating LINE-1 (L1) retrotransposons in attrition of fetal oocytes of mice. Typically repressed, L1 elements become active during epigenetic reprogramming of sexually differentiating germ cells. L1 expression is promptly downregulated in fetal prospermatogonia but persists in meiotic prophase I oocytes. We discovered differential accumulation of L1ORF1p, a L1-encoded protein and critical component of L1 ribonucleoproteins, in the nuclei of fetal oocytes. Elevated nuclear L1ORF1p levels strongly correlated with DNA damage, meiotic defects and oocyte elimination. Furthermore, L1 upregulation in *Maelstrom*-null oocytes interfered with meiotic crossover formation leading to oocyte and embryo aneuploidy. These results suggest that L1 activity underlies fetal oocyte attrition and contributes to maternal errors in meiotic chromosome segregation. We propose that fetal attrition serves the purpose of selecting oocytes with minimal L1 activity, thus best suited for the next generation.

IG34

Transposable Elements in Diatoms

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Oceans contribute ~50% to net primary productivity (NPP) on Earth, which is essentially attributed to photosynthesis activity achieved by phytoplankton. Diatoms, which evolved after a secondary endosymbiosis event, are the most successful and diversified groups of unicellular algae with possibly over 100,000 extant species. Their contribution to marine NPP has been estimated to be around 40%, playing a key role as a biological carbon pump as well as in other biogeochemical cycles. Diatom genomes can size up to several gigabases and transposable elements (TEs) are thought to be major contributors to genome size and dynamics in these species. The genome of three diatoms (*Phaeodactylum tricornutum* ~27 Mb, *Thalassiosira pseudonana* ~32 Mb, *Fragilariopsis cylindrus* ~80 Mb) have been sequenced.

We have analyzed the TE content in these genomes. We describe the panorama of TEs detected and their comparative contribution to diatom genomes. For example, we show that LTR-retrotransposons (LTR-RT), especially Ty1/Copia elements, are the most abundant TEs in diatom genomes. Phylogenetic reconstructions indicate that these constitute diatom-specific lineages called Codis that belong to the recently postulated 'Branch 1' of Ty1/Copia-like LTR-RT. Similar sequences are also abundant in marine metagenomes.

Interestingly, the expression of some CoDis from *P. tricornutum* is activated in response to specific stresses such as nitrogen starvation, suggesting that CoDis generate genetic variability in response to challenging environmental conditions. This is relevant considering that nitrogen is the most common and widespread limiting nutrient for marine phytoplankton.

Furthermore, analysis of genome-wide DNA methylation profile in *P. tricornutum* shows that spreading from TEs appears to be a common mechanism resulting in the methylation of flanking genes. We also describe the presence in *P. tricornutum* of a family of non-autonomous Class II elements with internal captured gene which likely impacts the epigenetic regulation of the cognate host gene.

IG35

Transcription profiles of loci of the human endogenous retrovirus group HERV-K(HML-2) in melanoma

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Human endogenous retroviruses (HERVs) and derived elements account for ~8 % of the human genome. The HERV-K(HML-2) group, in short, HML-2, is of interest because of its possible involvement in certain types of cancer. While the HML-2 group was initially formed approx. 28 million years ago, several HML-2 loci were formed very recently in evolution. There are ~30 full-length HML-2 loci in the human genome, some of which still exhibit open reading frames for proviral proteins Gag, Pro, Pol, Env and the accessory protein Rec. Previous studies detected antibodies against Gag and Env proteins and upregulated transcription of HML-2 in patients suffering from germ cell tumors or melanoma. As previous studies on HML-2 in melanoma mainly focused on general differences in transcription profiles, we addressed which specific HERV-loci are transcribed in melanoma. By RT-PCR, we amplified conserved proviral sequence portions from cDNA from 4 melanoma cell lines, 3 melanoma tissue samples, and 2 melanocyte cell lines. PCR primers were optimized towards amplification of as many HML-2 loci as possible. RT-PCR products were cloned and ~1200 cDNA clones were sequenced and assigned to genomic HML-2 loci on the basis of characteristic nucleotide differences. The investigated samples showed characteristic transcription profiles. We identified 19 HML-2 loci to be transcribed, several of which have not been described as transcribed before. Based on relative cloning frequencies that roughly correspond to transcriptional activity of individual loci, HML-2 transcript is generated in most samples from few high-level transcribed loci, with up to 90% of transcript being derived from one locus. Some low-level transcribed loci were detected only in one or another melanoma cell line. No definite pattern of transcribed HML-2 loci distinguished cancer from non-cancer samples. Several of the transcribed HML-2 loci feature ORFs for proviral proteins and there was at least one Rec-encoding locus transcribed in each sample. We also investigated in more detail the reported upregulation of HML-2 transcription in melanoma following UV irradiation. Exposure to UVB changed relative transcription levels of HML-2 loci in all cell lines. Our analysis reveals transcription profiles of HML-2 loci in melanoma and melanocyte cell lines and tumor tissue that are furthermore affected by UV irradiation. Transcribed HML-2 loci will be of particular interest when investigating the potential involvement of HML-2 and encoded proteins in melanoma.

Supported by DFG

IG36

Comprehensive analysis of human endogenous retrovirus W (HERV-W) transcription patterns in multiple sclerosis lesions and control brain tissue using next generation sequencing

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The multi-copy human endogenous retrovirus group HERV-W comprises ~650 loci in the human genome of which ~280 harbor internal gag, *pro*, *pol,* or *env* gene sequences. The majority of these loci are defective due to numerous mutations. However, the HERV-W-derived ERVWE1 gene encodes a complete envelope protein, named Syncytin-1, which likely plays an important role during placental development. Previous studies suggested a potential role of HERV-W and Syncytin-1 in the pathogenesis of multiple sclerosis (MS). HERV-W transcription has been found upregulated in brain samples from patients with MS, and Syncytin-1 RNA levels were reported to be higher in MS brain lesions compared to control brain tissue. Here we report on the genomic loci from which HERV-W transcripts in MS brain lesions and normal control brain tissue originate. We performed a detailed analysis of HERV-W transcription on a larger scale by 454/FLX amplicon sequencing of HERV-W derived cDNAs. HERV-W-specific PCR-primers located in the *env* gene were optimized towards amplification of as many HERV-W loci as possible. RT-PCR products were generated from MS and normal brain samples. Generated cDNA sequences were mapped to particular genomic HERV-W loci employing characteristic nucleotide differences. We generated several 10,000 sequences and identified ~120 HERV-W loci to be transcribed in the different samples. Relative frequencies of assignable cDNAs suggest (very) low transcription levels for most loci. The majority of transcripts derived from <10 different HERV-W loci. On average, 40% of transcripts derived from the ERVWE1 locus, and 23%, 12% and 9% derived from loci on chromosome 14q21.3, 6q21, Xq22.3 (ERVWE2), respectively. Overall, transcription profiles did not differ strikingly between MS and control samples, but some HERV-W loci are candidates for very low level transcription in MS brain only. This is the first study to comprehensively analyse HERV-W transcription in MS using next generation sequencing. Established transcription profiles and transcribed HERV-W loci will be important for further assessing the role of HERV-W in the pathogenesis of MS.

Supported by DFG

IG37

Importance of Transposon-Based Genomic Changes in Natural and Synthetic *Nicotiana* Allotetraploids

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Allopolyploids originate from hybridization between divergent genomes (i.e. different species) associated with chromosome set doubling. This phenomenon occurred recurrently during the history of plant speciation, and is thought to be one of the driving force of evolution in plants. Allopolyploid genomes may undergo a wide range of structural, epigenetic and functional changes, leading to species isolation. As transposable elements (TEs) are major components of plant genomes, they may play a key role in the genetic and functional modifications produced by the allopolyploidy process.
We are currently studying the *Nicotiana* genus as a model system to investigate the extent of genomic change associated with TEs in the allopolyploidization process. The *Nicotiana* genus contains many allopolyploids, the most recent ones being *N. rustica*, *N. arentsii* and *N. tabacum* (tobacco). *N. tabacum* is an allotetraploid generated by hybridization between two distantly related *Nicotianae*, while *N. rustica* and *N.arentsii* originated from crosses between more closely related *Nicotiana* species – belonging to closely related sections for *N. rustica* and the same section for *N. arentsii*. To unravel the extent of TE-associated structural changes, we performed comparative analysis of SSAP (Sequence-Specific Amplification Polymorphism) profiles obtained for seven different endogenous TE populations in both natural and synthetic accessions of *N. tabacum*, *N. arentsii* and *N. wigandoides* as well as their diploid progenitors. We assessed for each allopolyploid *Nicotiana* species the importance of the genomic changes (non-additive SSAP bands) associated with each TE according to the nature and evolutionary distance of their parental genomes.

Keywords : *Nicotiana*, allopolyploidy, transposable elements, SSAP

IG38

Preferential targeting of TE into microsatellites and the role of DNA conformation

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Chromosomal distribution of transposable elements (TEs) is either result of selection processes or targeting into specific loci. In some species, TEs are associated with other genomic repeats, like microsatellites. In other species, TEs are localized in non-repetitive DNA. We analyzed surrounding of TEs in human and found higher frequency of microsatellites. The neighborhood of LTR retrotransposons, DNA transposons and LINEs was enriched with A microsatellites upstream and T microsatellites downstream of the TE insertion site. In SINEs, A microsatellites were abundant upstream and T, AT, TA, TAA and AC downstream of TE insertion. Microsatellites were enriched in neighborhood of TEs also in other model species but the microsatellite motifs were species-specific. It is known that A-tracts cause strongest DNA bending. Additionally we showed using circular dichroism (CD) spectroscopy that some microsatellites adopt unusual DNA conformations. We propose a model where microsatellites adopt unusual DNA form which is directly or indirectly (using chromatide code) recognized by TEs. In this way, microsatellites as conformationally flexible DNA could represent genomic tool with the potential to modulate the frequency of TE insertions.

Keywords : transposable elements, microsatellite, targeting

IG39

Selfish genetic elements in neotropical Drosophila species: the link between symbiont dynamics and host speciation in an ancestral reservoir of *P*-transposons and intracellular *Wolbachia*

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Neotropical Drosophila belonging to the *willistoni* and *saltans* group species are prime model systems for understanding tempo and mode of speciation *in statu nascendi*, as well as the complexity of long-term host-symbiont dynamics of mobile elements and endosymbiotic bacteria, i.e., *P* element transposons and the two bacterial reproductive parasites *Spiroplasma* and *Wolbachia*. These so-called selfish genetic elements (SGEs) are in the intense focus of past and current research mainly in the model system of *D. melanogaster*, which is, however, a host species of very young associations with all three SGEs. Based on earlier studies, *P* elements, *Spiroplasma* as well as *Wolbachia* were quite recently acquired by Middle-American *D. melanogaster* populations via independent horizontal transfer events from native neotropical Drosophila species (Daniels et al. 1990; Montenegro et al. 2005; Miller & Riegler 2006; Miller et al. 2010).

Here we will compare their respective temporal & spatial infection dynamics, global spreading mechansims, and their functional host-interrelations of both symbiont-types, i.e., nuclear endoparasites (TEs) and cytoplasmic *Wolbachia* bacteria, in their ancestral reservoir host species and in *de novo* hosts. We finally will discuss their replication dynamics in hybrid backgrounds of *D. paulistorum* group species, where naturally obligate mutualistic *Wolbachia* transform into pathogens that trigger hybrid inviability & stertility, plus massive changes in sexual mating behaviour and thereby foster speciation.

IG40

euL1db: a comprehensive database of L1-HS retrotransposon insertions in humans

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LINE-1 (L1) elements are autonomous retrotransposons that replicate through a copy-and-paste mechanism, and constitute about 17% of the human genome. They are actively generating new copies from a human-specific subfamily (L1-HS), thus contributing to inter-individual genomic variation. They also undergo somatic retrotransposition, which results in somatic mosaicism. We present here euL1db (european L1 database), an integrated and interactive database of human-specific L1 retrotransposon insertions. Notably, euL1db contains L1-HS insertions discovered by recent next generation sequencing experiments (~ 10000), including the 1000 Genome Project, and polymorphic insertions found by lower throughput experiments, including L1-HS present in a general database of retrotransposon insertion polymorphisms, dbRIP (~ 450). euL1db also propose a curated set of reference L1-HS elements present in the reference human genome. We provide interactive and user-friendly data mining options and integration into the UCSC genome browser. Users can compare L1 integration sites accross multiple datasets or against gene and genomic features. euL1db is an effort to provide curated and comprehensive datasets of known L1-HS insertions in the next-generation sequencing era and will be a useful resource for researchers working in the area of human genomics.
Work in the laboratory of G.C. is supported by INSERM and INCa (Avenir program) and by the European Research Council (ERC Starting Grant 'Retrogenomics').

IG41

AZORIZ : Specificity of the phytostimulatory cooperation between *Azospirillum lipoferum* and rice

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The establishment of mutualistic or pathogenic associations usually involves partner recognition and requires a specific molecular crosstalk between the plant and the invading microorganism. By comparison, the associative symbiosis (cooperation) between crop plants and PGPR bacteria (plant growth-promoting rhizobacteria) are considered as "simple" interactions, involving low or no specific responses. Nevertheless, a differential response following inoculation by the PGPR Azospirillum was reported based on the survey of several cereals species(Charulyu *et al.*, 1985 ; Arsac *et al.*, 1990). In maize, Walker *et al.*, 2010 showed that the inoculation led to a modification on plant secondary metabolite profiles . Furthermore, *Azospirillum* response to the presence of plant extracts is characterized by a specific induction of certain genes (Pothier *et al.*, 2007). AZORIZ project aims at characterizing the molecular basis of the specificity of associative symbiosis between *Azospirillum lipoferum* strains and their corresponding host rice cultivar. With a simplified and checked system of inoculation, the following analyses were conducted seven days after inoculation : (i) the plant growth-promoting effect of each strain on the two rice cultivars; (ii) the rice root colonization patterns; (iii) the metabolomic response of the host; (iv) the transcriptomic response of the plant and in particular that of transposable elements (TEs); (v) the transcriptomic response of *Azospirillum.*The results obtained concerning the parameters of growth show a differential response of the plant according to the inoculated strain and indicate a stronger effect when a strain is tested on its cultivar of origin. Moreover, significant modifications of the secondary metabolism on both rice cultivars were shown in response to the inoculation, with higher effect for extracts from roots (by comparison to extracts from leaves). We provide the first results of the transcriptomic responses of both partners in order to identify the potential genetic determinants defining the specificity of interaction and to test at the whole-genome scale the genes/TE interactions in response to these biotic interactions.

Keywords : Rice, *Azospirillum,* PGPR, Transposable Element

IG42

Next generation sequencing reveals the impact of LTR retrotransposons on genome dynamics in a clade of increasingly parasitic angiosperms

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Repetitive sequences, such as ribosomal rRNA genes, satellite DNA, and dispersed mobile genetic elements, play an essential role in genome evolution. They are major contributors to genome size increase, gene regulation, and gene expression. Even so, there has been little comparative research on repetitive DNA because large-scale genome sequencing is required to obtain an objective view of the types and relative abundances of repetitive DNA. We are undertaking a comparative characterization of the evolution of repetitive DNA in the plant family Orobanchaceae, which includes several transitions towards parasitism, accompanied by partial or complete loss of photosynthetic ability. We used next-generation sequencing to characterize the genomes of nine species of known relationships and life style (autotrophic, hemiparasitic, holoparasitic), and including one tetraploid species. The species vary in genome size from 0.46 to 4.38 pg, with the tetraploid having 2.10 pg. Highly- or moderately repetitive DNA makes up between 25% and 60% of the genomes, making it the major contributor to genome size variation. Genome size, however, does not correlate completely with the genomic fraction consisting of repetitive DNA: the tetraploid (*O. gracilis*) has one of the smallest genomes but highest proportion of repetitive DNA. Overall, Ty3/Gypsy elements comprise 2 to 28% of the nine genomes, Ty1/Copia elements 8 to 23%. The accumulation of Ty3/Gypsy retrotransposons appears related to a higher diversity of repeat families. Unexpectedly, however, the tetraploid genome has lost numerous of these families. Finally, the larger genomes of the seven obligatorily parasitic species fit a hypothesis of larger genomes in parasites, perhaps because of relaxed selection; such an increase could be associated with an accumulation of transposable elements.

Keywords : Repetitive DNA, polyploidy, genome-downsizing, genome size, Orobanchaceae

IG43

Endogenous activity of L1 retrotransposons in human embryogenesis

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L1 retrotransposons are the only autonomous mobile elements in the human genome and are responsible for nearly 40% of our DNA. However, until recently little was known about the activity of endogenous L1 retrotransposons, due to a reliance on cell culture based retrotransposition assays. The main reason for this situation was the lack of robust and sensitive techniques to study the mobilisation of endogenous elements *in vivo*. However, the application of high-throughput sequencing to isolate endogenous L1 retrotransposon insertion has provided a new platform to expand our knowledge of L1 diversity and activity. In addition, a recent study using a fosmid-based end-sequencing approach revealed the extent of L1 activity in contemporary human genomes, reporting a large number of novel L1 retrotransposons of which, more than half were highly active in cell culture (Beck *et al*., 2010). This illustrates the previously unappreciated scale of L1 retrotransposition potential and its role in genomic evolution and disease. Indeed, investigations by Iskow *et al*. (2011) and Baillie *et al.,* (2011) demonstrated that *de novo* somatic endogenous L1 retrotransposition occurs in human lung tumours and human brain cells respectively. While somatic activity may have consequences for disease and intercellular genetic diversity, L1 retrotransposition, either in early development or in the germline, is required for the evolutionary persistence of these elements. With the exception of very rare disease-causing insertions arising from somatic mosaicism there is no direct evidence of *de novo* L1 retrotransposition in early human embryogenesis. One reason for this is likely to be the low rate of L1 retrotransposition at this stage of development, coupled to the lack of a robust and sensitive assay to detect rare or *de novo* L1 insertions in small numbers of cells.
Here we report the development of a robust assay using the Roche 454 high-throughput sequencing platform to isolate single-molecule L1 retrotransposition events. Using this technique we have discovered many candidate novel L1 retrotransposition events in single human embryonic blastomeres, a proportion of which are likely to be of *de novo* origin. This is the first direct demonstration of *de novo* endogenous L1 retrotransposition in single cells of human embryonic blastomeres, and indicates that *de novo* endogenous L1 retrotransposition is a feature of normal early human embryogenesis, *i.e.* that it is not only associated with disease-causing insertions (Rahbari *et al., manuscript in preparation*).

Keywords : L1 retrotransposons, human embryogenesis*, de novo* retrotransposition

1. Beck *et al*., 2010: PMID: 20602998
2. Iskow *et al*., 2011: PMID: 20603005
3. Baillie *et al.,* 2011: PMID: 22037309

IG44

Testing a role for HMGB protein family in developmentally programmed rearrangements in Paramecium

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At each sexual cycle, the ciliate *Paramecium* undergoes a developmentally programmed genome rearrangement. The development of the new macronucleus is accompanied by precise elimination of single copy, short (26-882 bp) noncoding sequences (IESs). PiggyMac (Pgm), a domesticated transposase, participates in IES excision. Recent high-throughput sequencing of total genomic DNA from *PiggyMac*-silenced autogamous cells allowed the identification of ~45,000 IESs (O. Arnaiz *et al*., unpublished) and revealed a 10-11 bp periodicity in their size distribution, which coincides with the phase of the DNA double helix. We therefore propose that the excision process involves the assembly of the two IES ends in a Pgm- containing nucleoprotein complex. The observed periodicity in IES size distribution may reflect the DNA constraints on the assembly of the excision complex. Previous genetic evidence of a crosstalk between IES ends before DNA cleavage further support this view.

Size distribution analysis of IESs has revealed than 90% are shorter than 150 bp, which is the persistence length of B-form DNA. We thus hypothesize that a DNA bending factor might be involved in the looping of IES DNA necessary for the assembly of the Pgm-synaptic complex.

High Mobility Group (HMG) proteins are eukaryotic architectural factors that organize chromatin by bending and plasticizing DNA. The HMGB subfamily is characterized by an 80 amino acid box that binds to the minor groove of DNA with no sequence specificity. Members of this family are involved in V(D)J recombination.

We present here our strategy to test the possible involvement of HMGB proteins in *Paramecium* IES excision. *In silico* search of *Paramecium* DB allowed us to identify 18 families of ohnologous *HMGB* genes, each bearing one to seven members. Nine families were found to have members specifically induced during sexual processes. We have undertaken a knockdown approach by RNA interference for two of these families. Our phenotypical characterization of the knockdown cells is presented.

Keywords : Paramecium, IES excision, Pgm-synaptic complex, High Mobility Group proteins, DNA bending

IG45

Canonical P element in polytene chromosomes of *Drosophila willistoni*

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The presence and the local insertion of the canonical P transposable element (2.9 kb) were screened by *in situ* hybridization in the polytene chromosomes of isolines of different natural populations of *Drosophila willistoni*, collected from across a vast territory in South America. *Drosophila willistoni* is part of a bigger scenario of evolutionary studies on *Drosophila* and over the last three decades our group has worked toward producing a good photomap of *D. willistoni* polytene chromosomes. The scope of this report was to enlarge the knowledge about the chromosomal location of transposable elements and to carry out an intra and inter-population analysis of these insertion sites. Some natural populations studied have high variability of P insertion sites, with 8 to 13 different sites varying between isolines of same population. We presented pictures of these insertion sites, including some in heterozygous state, and we plotted all P sites over the polytene chromosomal photomap of the *D. willistoni*. We are trying to identify functional elements in the insect´s genomes, describing how evolution could have shaped the chromosomes of this important model organism.

Keywords : In situ hybridization, *D. willistoni*, variability, P transposable element

IG46

Transpositional landscape of the rice genome revealed by paired-end mapping of high-throughput re-sequencing data

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Transposable elements (TEs) are mobile entities that densely populate most eukaryotic genomes and contribute to both their structural and functional dynamics. However, most TE-related sequences in both plant and animal genomes correspond to inactive, degenerated elements, due to the combined effect of silencing pathways and elimination through deletions. One of the major difficulties in fully characterizing the molecular basis of genetic diversity of a given species lies in establishing its genome-wide transpositional activity.

Here, we provide an extensive survey of the transpositional landscape of a plant genome using a deep sequencing strategy. This was achieved through paired-end mapping of a four-fold coverage of the genome of Asian rice mutant line derived from an *in vitro* callus culture using *Illumina* technology. Our study shows that at least 13 TE families are active in this genotype, causing 34 new insertions. This next-generation sequencing-based strategy provides new opportunities to quantify the impact of TEs on the genome dynamics of the species.

Keywords : rice, transposable elements, next-generation sequencing, genomics, mutant line, paired-end mapping

IG47

Changes in small RNAs accumulation associated with loss of DNA methylation over transposable element sequences in *Arabidopsis thaliana*

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Two major classes of small RNAs accumulate in the flowering plant *Arabidopsis thaliana*. These are mainly composed of a small number of highly abundant 21-nt microRNAs (miRNAs), and of a large number of low abundance 24-nt small interfering RNAs (siRNAs), respectively. Whereas microRNAs are important regulators of gene expression, siRNAs correspond almost exclusively to transposable element and other repeat sequences and play key roles in guiding DNA methylation to these sequences. We and others have shown previously that loss of DNA methylation induced by mutations in the gene *DECREASE IN DNA METHYLATION 1* (DDM1) leads to TE reactivation as well as to changes in siRNA accumulation, most notably the appearance of an abundant class of 21-nt long siRNA corresponding to Athila retro-transposons.

We have used Illumina sequencing to explore further these changes and the involvement of the RNA dependent RNA polymerases RDR2 and RDR6 in the differential accumulation of siRNAs in *ddm1* compared to wt. Our analysis indicates that approximately one third of the ~18 000 annotated TE sequences with matching 24-nt siRNAs in wt accumulate less of these in *ddm1*. This decrease is not compensated by a gain of 21-nt siRNAs, which concerns instead another ~2 000 annotated TE sequences with matching 24-nt siRNAs in wt. We further show that accumulation of many 24-nt siRNAs as well as most 21-nt siRNAs corresponding to these sequences requires the RNA-dependent RNA polymerase RDR6, which is classically involved in antiviral defense as well as the production of 21-nt tasiRNAs. Remarkably, genome-wide DNA methylation analysis indicates further reduction of DNA methylation at these loci in *ddm1rdr6* but not in *ddm1rdr2* double mutant plants, thus revealing the existence of an RDR6-dependent DNA methylation pathway in addition to the well-characterized and predominant RDR2-dependent DNA methylation pathway. These and other findings will be discussed.

Keywords : Arabidopsis, DNA methylation, siRNAs, RDR2, RDR6

IG48

Reconstruction of the evolutionary dynamics of LTR retrotransposons in complex genomes

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Being highly mutagenic and repressed by overlapping epigenetic mechanisms, transposable elements (TE) are postulated to trigger structural and epigenetic modifications, thus playing a central role in fuelling genome reorganization and reproductive isolation. Allopolyploidy is a fascinating model to investigate genome reorganization under the influence of TEs because the merging of divergent genomes is postulated to reveal conflicts between quantitatively and qualitatively different TEs, leading to their activation. Using wild wheats as a model system, our goal is to better understand how hybridization induces TE activity and genome restructuring, and whether they influence hybrid viability and stability. As a first step, we used 454 massive sequencing of shotguned DNA from tetraploids *Aegilops cylindrica* and *Ae.geniculata* to identify candidates TEs, mostly LTRs, for activation at hybridization. Bioinformatic and molecular population genetics approaches on low coverage genome (~2.5%) reconstructed the evolutionary history of TEs and revealed specific divergence suggesting recently active TEs. The second step was to confirm restructuring in candidate TE fractions in tetraploids through molecular analyses (AFLP, marking random sequences vs. SSAP, marking insertions of specific TEs), by comparing profiles between diploids and derived tetraploids. Although the evolutionary consequences of TE activity remain to be assessed, this study highlights a suitable approach to investigate the long-term dynamics of TEs in association with hybridization.

Keywords : genome evolution, hybridization and polyploidy, LTR, NGS, wild wheat

IG49

Role of LINE-1 Retrotansposition in HCC initiation and progression

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The most common strategy of a cell to silence L1 is methylation of its promoter, thus hypomethylation could lead to increased incidence of retrotransposition and genomic instability. Global hypomethylation including L1 promoter is commonly observed in liver cancer. In the present study we wish to investigate the role of active retrotransposition in development and progression of hepatocellular carcinoma (HCC). We have collected 8 hepatitis B virus (HBV)-induced HCC tumors and adjacent non-tumour liver tissue with a varying degree of HBV infection. L1 copy number variation has been analysed and somatic L1 insertions are detected using retrotransposon capture sequencing (RC-Seq). As liver inflammation is closely associated with HCC development we are currently studying the effect of an inflammatory environment on L1 activity. To these ends, we have performed cell culture based retrotransposition assays to assess the effects of various proinflammatory cytokines on activation of retrotransposition. Overall the study will give new insight towards mechanisms of L1 activation and its role in the context of liver cancer development and progression.

Keywords : Retrotransposons, RC-Seq, Hepatocellular carcinoma, inflammation

IG50

Single strand transposition and the IS*200*/IS*605* family: diversity, distribution and evolution

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Transposable elements (TE) are important in genome evolution and central to bacterial horizontal gene pool. Insertion sequences (IS) are the most compact autonomous and the most abundant TE in prokaryotes. There nearly 4000 different complete ISs classified in more than 25 families in the international IS register, ISfinder (www-is.biotoul.fr), a number which is growing continuously and rapidly. We have identified and characterised a widespread family of IS (IS*200*/IS*605* family) quite different from classical TEs. Studies on two members IS*608* and IS*Dra2* have shown that they use obligatory single strand DNA intermediates and have ends with subterminal palindromes (SP) which are recognised and bound by their Tpases.

These Tpases, with a catalytic site containing a single Tyr and a His-u-His amino acid triad, are members of a larger "HUH" endonuclease superfamily including RCR Rep proteins (Rolling Circle Replication), relaxases (conjugal plasmid transfer) and Tpases of the IS*91* family. All create a covalent 5'-phosphotyrosine enzyme-substrate intermediate. Interestingly, cleavage sites are not recognised directly by the protein but by short "guide" sequences 5' to the SP foot. Recognition involves a network of base interactions similar to those found in RNA structures.

We have demonstrated the importance of the lagging strand template for activity of IS*608* and IS*Dra2* and our *in silico* genomic analysis suggests that other members have also evolved a mode of transposition that exploits ssDNA at the replication fork.

We present here an analysis of the diversity of this family of ISs, its distribution and evolution.

IG51

Methylation-unrestricted, tissue-specific and dynamic RNA-expression of a polymorphic LINE-retrotransposon in rat tissues

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*L*ong *I*nterspersed *N*uclear *E*lements (LINEs) are abundantly present in the mammalian genomes. LINEs (L1s) are methylated and epigenetically modified to suppress their expression into RNAs in order to prevent genomic-DNA rearrangement by retrotransposition. Germ line expression of L1 is a normal phenomenon in the early stage of life, but its somatic expression is usually observed in cancer cells or tissues. Recent advances in LINE-studies showed that it is involved in several cellular processes like splicing, gene expression, exonization, somatic mosaicism, neurogenesis, X-chromosome inactivation, etc instead of just acting as a mutagenic and parasitic genetic element. We have cloned a 2.8 kb genomic LINE DNA (P1-LINE, Accession GQ244317) from the rat brain, *in-silico* analysis showed its wide-spread presence and abundance in the rat genome as well as in the LINE-SINE cluster. We report the somatic as well as germ line expression of the P1-LINE. It is strongly expressed from multiple genomic loci into heterogeneous RNAs, ranging from 4.0 to 0.15 kb in the brain, liver, lungs, heart, kidney, testis, spleen and thymus in a tissue-specific manner. Msp I/Hpa II DNA-methylation assay showed that the P1-LINE is heavily methylated at the CpG-sites in most tissues, and hypomethylated to a much lesser extent in few tissues. The tissue-specific, heterogeneous and dynamic RNA-expression pattern of the P1-LINE is variable in individual rats and is unrestricted by its methylation-state in the genome under *in vivo* conditions. Study of the P1-LINE expression in three age groups (young-4 week, adult-16 week and old-70 week) of the rat showed strong RNA-expression in all the age groups with tissue-specific and age-related variations. In case of the brain, there was significant increase in the RNA-expression level, which correlated with the concept of the role of L1 in adult neurogenesis. The P1-LINE showed nucleotide sequence polymorphisms in different tissues in the three age groups. This could be a reflection of the P1- LINE expansion in the genome during the course of evolution. The high endogenous RNA-expression of the P1-LINE in the somatic tissues of the rat is in contrast to the previous studies, where L1-expression in somatic tissues was considered as a rare event. We hypothesize that the presence of abundant LINE-sequences in the mammalian genomes must have some function(s) in the somatic tissues under normal conditions. Moreover, expression of this LINE may be regulated by some mechanism(s) in addition to methylation. The tissue-specific, wide-spread LINE RNA-expression may define functional chromosomal domains, a novel role for the LINE in the mammalian genome.

IG52

Transposable Elements as the structural component of meiotic chromosomes in human and mouse

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Synaptonemal complexes (SCs) are the intranuclear structures which are assembled between homologous chromosomes in the course of meiotic prophase I and facilitate synapsis the process of crossing over. The idea of current investigation is to detect the distribution of three typses of repeated sequences in the meiotic chromosomes structure. Combined FISH and immunochemical analysis were performed on human spermatocytes microspreads. Using FISH we had visualized and examined the arrangement of repeated DNA sequences in relation to pachytene SCs stained with fluorescent SYCP3 antibodies. It was found that probes containing Alu and L1 repeats formed “cases” (multiple signals) around SCs, while simple repeat (GT)22 signals were spreaded over the chromatin. Alu- and L1-repeats were mainly located nearby SCs however intensity and width of these regions were non homogeneous along the chromosomes. This possibly can be explained by different chromatin compaction and its genetic characteristics. In our previous investigation on mouse spermatocytes we had found out the B1(Alu)-repeats were colocalizated with the SCs the similar way as in human. We assume that these DNA repeats are very close to the “anchoring” segments of DNA in both mammalian species and may be involved in the attaching chromatin to SC.

The work was supported by RBRF grant 10-04-00666-a.

IG53

LTRsift: A graphical tool for semi-automatic classification and postprocessing of *de novo* detected LTR retrotransposons

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LTR retrotransposons are a class of mobile elements in eukaryotes well suited for computational identification. Current software allows for a comprehensive genome-wide *de novo* detection of such elements [1]. For example, we have developed the *LTRharvest* [2] and *LTRdigest* [3] tools which use a combination of index and similarity based as well as probabilistic approaches to detect and annotate LTR retrotransposon candidates from genomic sequences in a very flexible way. Classification of newly detected candidates resulting in (super-)families is the obvious next step [4]. A *de novo* classification approach based on sequence-based clustering of transposon features has been proposed before [3], resulting in a preliminary assignment of candidates to families as a basis for subsequent manual refinement. However, such a classification workflow is typically split across a heterogeneous set of scripts and generic software (e.g. spreadsheets), making it tedious for a human expert to inspect, curate and export the putative families produced by the scripts.

We present *LTRsift*, a graphical software tool for semi-automatic postprocessing of *de novo* predicted LTR retrotransposon annotations. Its user-friendly interface offers customizable filtering and classification functionality, displaying the putative candidate groups, their members and their internal structure in a hierarchical fashion. To ease manual work, it also supports GUI-driven reassignment, splitting and further annotation of candidates. Export of grouped candidate sets is possible in standard formats. In exemplary case studies, we demonstrate how *LTRsift* can be employed in the context of a genome-wide LTR retrotransposon survey effort, based on the output of the *LTRharvest* and *LTRdigest* software.

LTRsift is a useful and convenient tool for semi-automated classification of newly detected LTR retrotransposons by their internal features. It is intended for life scientists to aid them in postprocessing and refinement of their raw prediction software output up to the stage of preparing repeat libraries.

Keywords : LTR, retrotransposon, prediction, classification, software

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IG54

Whole genome sequencing to investigate genome size variation and transposable element content within and among species in *Zea*

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Eukaryote genome sizes vary widely both within and among species, due in part to genome expansion and contraction caused by Transposable Elements (TEs). TE content is in turn determined by transposition, removal, and host responses, and the efficacy of these forces are ultimately governed by genetic drift and natural selection. The genome of maize consists mostly of transposable elements (TEs) and varies in size among lines but this variation also extends to other species of the genus *Zea*. Using high-throughput sequencing reads in two *Zea* species, *Zea mays* ssp. *mays* (maize) and *Zea luxurians* (*luxurians*), mapped against both a database of TEs and to annotated maize genes, we found that TE copy number from Class 1 and Class 2 accounts for 70% of the 50% genome size difference between the two species. In addition, the relative abundance of TE families was conserved suggesting genome-wide control of TE content rather than family-specific effects. We discuss three non-exclusive hypotheses to explain this pattern: selection for genome shrinkage, differential efficiency of epigenetic control, and a purely stochastic process of genome size evolution. The same methodology was employed at the *Zea* intraspecific level on 38 lines (27 maize and 11 teosintes). TE abundance correlated here negatively with genome size but positively with abundance of heterochromatic knob repeats. These results suggest interspecific variation in genome size is largely determined by TE proliferation while much of the variation within species is due to segregation of large heterochromatic repeats.

Keywords : maize, genome shrinkage, epigenetic control, knobs

IG55

A mutator-like transposable element is differentially expressed in *Phalaenopsis* Hsiang Fei cv. H. F. somaclonal variant

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Somaclonal variation occurs during plant tissue culture and introduces changes that can result in the development of desirable traits. Using cDNA-amplified restriction fragment length polymorphism (cDNA-AFLP) analysis, we compared gene expression patterns between flower buds from wild type (donor) plants of Phalaenopsis Hsiang Fei cv. H. F., whose flowers are bronze in color, and from its somaclonal variants, whose flowers have a mosaic yellowish color, and sometimes an aberrant shape. Using 128 fluorescently labeled primer sets, a total of 2269 transcript-derived fragments (TDFs) were analyzed. Among them, 25 TDFs were differentially expressed between the wild type plant and its variant. After cloning and sequencing these differentially expressed TDFs, we found that they contained 27 distinct sequences. Further confirmation of the differential expression of these sequences was carried out by using semi-quantitative RT-PCR. We found that five sequences showed higher expression levels in the wild type plant compared to those in the variant plant. These corresponded to sequences that encoded casein kinase, isocitrate dehydrogenase, cytochrome P450, EMF2, and a no hits found protein. In contrast, two other sequences were expressed to a higher level in the variant plant compared to those in the wild type plant. One of these sequences is mutator-like transposable element. The ORF of the mutator-like transposable element was cloned by rapid-amplification of cDNA ends (RACE) and showed 1887 bp encoding 628 a.a. The differential expression of these genes may lead to the mosaic color patterns as well as the aberrant flower shapes in the somaclonal variants of *Phalaenopsis* Hsiang Fei cv. H. F.

Keywords : mutator, differentially expressed, *Phalaenopsis*, somaclonal variation, transcript-derived fragments

IG56

Tnt1 superfamily expression profile using NGS based approach

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Transposable elements are considered quiescent components of eukaryotic genomes. Usually they are referred as part of the non-coding fraction of the genomes. To challenge this view we have performed RNA-seq using tobacco leaves and tomato cotyledons and demonstrate that both Tnt1 and Retrolyc1 are expressed in those plant tissues. Differences are observed among the previously described subfamilies (tobacco Tnt1A, Tnt1B, Tnt1C and tomato Retrolyc1A, Retrolyc1B) and between plant varieties that support a close interplay between a particular subfamily and the genome they inhabit. Two tobacco lineages and two tomato lineages were contrasted and compared. SOLID reads were mapped to a full copy of the model element and LTR-U3 region specific reads were carefully analyzed. Sense and antisense transcripts were counted. Based on the results, we demonstrate that these LTR-retrotransposons are expressed in photosynthetic tissues under normal conditions. Further, we postulate these elements are constituents of plant regulatory networks based on mutant and transgenic plant lines.

IG57

Exploring the landscape of RNAs bound to the L1 retrotransposon ORF1p protein *in vivo*

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Long interspersed nuclear element-1 (LINE-1 or L1) sequences comprise 17% of human DNA and are the only active and autonomous retrotransposons in our genome. L1 retrotransposition requires the formation of a ribonucleoprotein particle (RNP) containing at least the L1 RNA and the two L1-encoded proteins, ORF1p and ORF2p, which have been proposed to be the core components of the L1 retrotransposition machinery. However the presence of other cellular factors within the L1 RNP or more transient interactions between the L1 machinery and cellular factors have been poorly studied.
Here we have applied the *in vivo* cross-linking and immunoprecipitation (CLIP) method to the ORF1p protein, which is endogenously expressed in human embryonal carcinoma cells. Using this approach, we aim at: (i) exploring the landscape of RNAs bound to the L1 ORF1p protein *in vivo*, and (ii) mapping the binding sites of ORF1p to these RNAs.
Using low-throughput sequencing we identified the expected L1 RNA as a partner of the ORF1p protein. Other non-L1 RNAs have been identified and will be presented.

Work in the laboratory of G.C. is supported by INSERM and INCa (Avenir program) and and the European Research Council (ERC Starting Grant 'Retrogenomics').

IG58

Re-annotation of transposable elements in the onion and asparagus genomes reveals previously unobserved nested structures of complete and young elements

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Although the Monocotyledons comprise one of the two major groups of angiosperms and include over 65,000 species, comprehensive genome analyses like full genome sequencing have been focused mainly on the Poaceae (grass) family. In Monocotyledons, description of transposable elements content and dynamics is therefore biased towards a small group of plants, and needs to be completed by the analysis of a larger species sample.

The order Asparagales, which includes nearly 5000 species, is the second most important order for agriculture after the Poales. It provides us with important crops (e.g. aloe, agave, asparagus, garlic, leek, onion and vanilla) as well as ornamental plants such as daffodils, irises and orchids, and is the third most cultivated group for worldwide vegetable production after the Solanales and the Cucurbitales. Despite their economic importance, genomic resources for the Asparagales are scarce, mainly due to their huge nuclear genome size.

Several BAC sequences have been produced in the past few years for onion (*Allium cepa*) and garden asparagus (*Asparagus officinalis*), and provide a good starting point to investigate transposable elements features of the order Asparagales. A first annotation of these BAC sequences has revealed that most of the transposable elements found were degenerated (fragmented), suggesting that these genomes may have encountered different transposable element dynamics than this observed in the Poaceae.

Using a more detailed annotation methodology, we could characterize several new LTR retrotransposons from both onion and garden asparagus, most of which are complete, *i.e.* with two LTRs. We show that these elements make up over 50% of the regions analyzed, have inserted within the past six million years and are inserted within each others in nested structures, a pattern that is similar to this observed in the Poaceae.

Our results highlight the importance of annotation quality to provide a clear description of transposable elements in plant genomes and suggest that the transposable elements dynamics observed in the Asparagales is similar to this observed in the Poaceae.

Keywords : LTR retrotransposons, Genome evolution, BAC sequence annotation, onion, asparagus

IG59

Developing bioinformatic resources for LINE-1 dependent retrotransposons

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Alus are short interspersed LINE-1 dependent retrotransposons, that are both very common and known to be active in humans. SVA elements are less numerous but much larger LINE-1 dependent retrotransposons. However there are few genome-scale centralised resources that represent the diversity of these elements in assembled human genomes and their variation between individuals. To address this limitation, and to enable researchers to analyse L1 dependent retrotransposon diversity both within genomic regions and between sequenced individuals, an automated annotation pipeline has been developed. This pipeline isolates Alus and SVAs, as well as their flanking sequence and maps them to a reference genome sequence. In the case of Alus it also annotates their predicted activity according to promoter activity, A tail quality, and divergence in conserved activity-associated nucleotides. The pipeline has been tested on three "reference" personal genomes to generate individual genome-wide "Alu genotypes". The data is stored in a database which will ultimately be visualised in a publicly accessible resource. The pipeline and database are modular in design and in principle can accept and analyse sequence data generated by a wide variety of sequencing technologies. Current work involves adapting the pipeline to deal with high throughput sequencing data.

IG60

Transcriptional regulation of human-specific SVA_{F1} (CpG-SVA) retrotransposons by cis-regulatory *MAST2* sequences

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SVA elements represent the youngest family of hominid non-LTR retrotransposons and stand out due to their organization as composite transposable elements. Recently, a human-specific SVA subfamily was discovered whose members derived from an ancient retrotransposition event that led to the fusion of the CpG island-containing exon 1 of the *MAST2* gene and a 5'-truncated SVA (termed SVA_{F1}, or CpG-SVA, or MAST2-SVA). This subfamily includes at least 84 members, which suggests exceptionally high retrotransposition level compared to the overall SVA proliferation rate. We aimed to investigate if the acquirement of the *MAST2* CpG-island played a role in the success of the SVA_{F1} subfamily, and if transcriptional activity of the *MAST2* gene correlates with the expression of functional mammalian non-LTR retrotransposons.

In order to investigate any potential correlation between the transcriptional regulation of *MAST2* and the expression of functional mammalian non-LTR retrotransposons, we performed quantitative reverse transcription PCR (qRT-PCR) assays on total RNA preparations from various human, mouse and rat tissues. We could confirm that human *MAST2* transcription peaks in testicular and heart tissues. We found that in human testicles, *MAST2* transcription correlates with the transcription pattern of non-LTR retrotransposon families L1, *Alu* and SVA, and with the expression of L1 ORF1 protein. Similarly, the non-LTR retrotransposons L1Rn and B2Y in rat as well as L1Md and B2 in mouse were cotranscribed with the respective rodent *MAST2* gene. We show that the 324 bp-long *MAST2* sequences that are part of SVA_{F1} subfamily members act as positive transcriptional regulators in the human testicular germ cell tumor cell line Tera-1. Our data demonstrate that the methylation status of the *MAST2*-derived sequences of SVA_{F1} subfamily members reversely correlates with the transcriptional activities of the respective SVA elements in testicular tissues. Therefore, we conclude that the acquisition of the *MAST2*-derived CpG islet was beneficial to the expression of the SVA_{F1} subfamily that is characterized by an exceptionally efficient amplification process during the recent evolution of the human genome.

Keywords : SVA-retrotransposons, CpG-SVA, *MAST2,* non-LTR elements

IG61

Novel L1 and *Alu* Retrotransposon Insertions in Cancer Related Gene Loci in the NCI-60 Cancer Cell Lines

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Introduction
Long Interspersed Elements (L1 LINE) and *Alu* family Short Interspersed Element (SINE) are active Transposable Elements (TEs) in the human genome which have been implicated in oncogenesis. The functional roles of TEs remain under-explored due to the inability of current methods to locate these high copy number repeats in the genome-a barier recently overcome. Using a new genomic method, this study aims to discover cancer-specific L1 LINE and *Alu* SINEs in the NCI-60 panel.
Materials and Methods
Using transposon insertion profiling microarray (TIP-chip) on genomic DNA from the NCI-60 tumor cell lines, vectorette PCR selectively amplified the 3' end of TEs to yield unique DNA fragments. These unique PCR amplicons were fluorescently labeled and hybridized to a custom genomic tiling microarray. Contiguous positive probes were detected as peaks. Data analysis included filtering peaks for 'true' insertions and annotating their locations with respect to known TEs and cancer-related genes.
Results
We report 853 candidate L1 insertions (199 known and 654 novel) and 2,889 candidate *Alu* insertions (1,249 known and 1,640 novel) across the NCI-60 panel.
Of the L1 insertions, 289 were present in more than a single cell line while the number of insertions unique to a cell line ranged from 0-65. There were 506 cancer genes within 5kb of the predicted L1 insertion intervals and 224 putative L1s within 500 bps of an exon. Relevant genes included *RB1, ALK, IGF1R* and *BCL2*.
2,224 of the 2,889 candidate *Alu* insertions were present in multiple cell lines. The number of unique *Alu* insertions ranged from 1-79 per cell line. 1,027 cancer genes including *BRAF*, *KRAS, BRCA2* and *EGF* were identified within 5kb of the predicted *Alu* insertions. 4 of the candidate *Alu* insertions were within 500 bps of an exon and 377 *Alu* insertion intervals mapped to the first intron of a gene.
Conclusions
This is the first study to use a high through-put method to discover novel TE insertions in the NCI-60 tumor cell lines. L1 and *Alu* TEs may affect gene expression, alter genomic stability or act as insertional mutagens in neoplastic cells. Discovering L1 and *Alu* locations within the genome is the first step to understanding these TEs and their roles in oncogenesis. This study affirms that TIP-chip provides an effective high through-put method by which novel L1 and *Alu* insertions may be discovered. The discovery of these novel L1 and *Alu* insertions in proximity to known cancer genes provides specific targets to help elucidate the relationship between TEs and the development of different neoplasias. On-going work will evaluate the functional significance of these insertions.

Keywords : Transposon, NCI-60

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CT1

Drosophila endogenous retrovirus regulation in natural populations

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Natural populations of *Drosophila* display a wide variability in the patterns of insertions and copy numbers of TEs. They, therefore, are the ideal material to decipher the mechanisms underlying TE dynamics and regulation in a genome. In addition, piRNA-related genes also present a high level of polymorphism in natural populations. By putting these data side by side, and using the example of an endogenous retrovirus of *Drosophila*, which displays sequence variability in natural populations, I will show how we can take another look at intra-species variation in TE silencing.

CT2

Mos1 transposition regulation by transposase phosphorylation

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Transposases are phosphatidyl transferases encoded by class II transposable elements. They mediate most steps involved in the cut and paste mechanism of transposition by which DNA transposons move from one genomic location to another. The relative non-specificity of the transposition process makes it potentially genotoxic for the host, implying a tight control of transposase activity. The study of the relationships between transposable elements and their host genome has highlighted several regulatory mechanisms, such as the role of transposase/DNA complexes organization in the promotion of catalysis, topological constraints, transposons silencing, sub-optimal activity of transposases, or overproduction of transposase that poison transposition reactions. We have been interested in the fact that the transposition of the *Mos1* element does not happen in mammalian cells, suggesting that MOS1 retains some inbuilt regulatory elements.

Mos1 is a member of the widespread *mariner* / Tc1 super-family of eukaryotic transposable elements. The *Mos1* transposase (MOS1) is a 345-aa protein that contains two functional domains. The N-terminal domain promotes the assembly of MOS1 dimers and their subsequent binding to *Mos1* inverted terminal repeats (ITR). The C-terminal domain exerts the catalytic functions of DNA strand cleavage and transfer. The cut-and-paste transposition mechanism of *mariner* elements does not appear to require additional cellular host factors since it occurs *in vitro* with the purified enzyme.

The regulation of the transposase activity itself (*e.g.* by post-translational modifications) has not been the matter of great attention, except in the case of the *P*-element transposase, and the HIV-1 integrase that could be regulated by phosphorylation. More recently, the possible regulation of the *Mos1*-element transposase by phosphorylation has been suggested by *in silico* prediction and alanine scanning [1], but not demonstrated.

We have thus investigated whether phosphorylation could be involved in the regulation of MOS1 activity.

Using mass spectroscopy, we found that MOS1 is phosphorylated *in vivo* at two residues: pS2 and pS170. The kinase responsible for S2 phosphorylation has not been identified yet, whereas S170 is phosphorylated by the cyclic AMP dependent protein kinase (PKA). This phosphorylation results in a dramatic decrease in MOS1 activity, which becomes unable to promote transposition of a pseudo-*Mos1* element. We will present the mechanism by which pS170 controls MOS1 activity that we have identified, and we will discuss this regulation in the context of cell genome integrity.

1. Germon S, Bouchet N, Casteret S, Carpentier G, Adet J, et al. (2009) *Mariner Mos1* transposase optimization by rational mutagenesis. Genetica 137: 265–276.

CT3

Implication of the AC40 subunit of RNA Polymerase III in Ty1 integration

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LTR-retrotransposons (LTR-RT) are widespread transposable elements in eukaryotic genomes and share analogies with retroviruses in their structure and mode of replication. They propagate by reverse transcription of their RNA into a cDNA copy that is next integrated by the action of the LTR-RT-encoded integrase (IN). The site of integration is critical for both cell survival as it can induce deleterious mutations, and LTR-RT replication as it dictates whether the element is transcriptionnally expressed or silenced.
Integration does not occur randomly throughout the host-cell genome but presents a pattern of preferred sites that is specific to each element. Studies on yeast LTR-RT (Ty3 and Ty5 of *Saccharomyces cerevisiae* and Tf1 of *Schizosaccharomyces pombe*) have uncovered the essential role of cellular factors in controlling the specificity of integration. They converge on a common targeting mechanism, based on the tethering of pre-integration complexes (PIC) to host-cell chromosome, through interactions between IN and cellular factors bound at preferential insertion sites.
Ty1, the most active LTR-RT in *S. cerevisiae,* integrates in a window of 750 base pairs upstream of RNA polymerase III (Pol III)-transcribed genes, where it targets a specific nucleosomal surface. The molecular bases of Ty1 integration selectivity remain unknown. We discovered an interaction between Ty1 IN and AC40, a subunit of Pol III in a two-hybrid screen and confirmed the interaction by co-IP experiments. The AC40 protein is the first Ty1 IN cellular cofactor identified to date.
Since *RPC40*, which encodes AC40 is essential in yeast, we could not analyze the impact of its deletion on Ty1 retrotransposition and/or integration. We found out that AC40 of *S. Pombe* does not interact with Ty1 IN in a two hybrid-assay, although it could suppress the lethality of an *rpc40*Δ strain. Replacing *RPC40* by its *S. Pombe* ortholog did not affect the frequency of Ty1 retrotransposition. This result suggests that the interaction between Ty1 IN and AC40 is not required for Ty1 retrotransposition but does not exclude a role of AC40 in Ty1 preferential integration by anchoring Ty1-PIC at Pol III-transcribed genes. Experiments are in progress to answer this question.

Keywords : Ty1 retrotransposon, Integration targeting, Pol III transcription

CT4

COPIA78, a copia-type LTR retrotransposon is activated by prolonged heat stress in *Arabidopsis*

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With over 100 families consisting of more than 1500 copies, LTR/COPIA elements form one of the most diverse superfamilies of retrotransposons in the compact Arabidopsis genome. In order to limit the highly mutagenic potential of retrotransposons, epigenetic mechanisms act through transcriptional gene silencing (TGS) such as DNA methylation, posttranslational histone modifications and small RNA regulation to generate a repressive chromatin environment. This can be reversed in a developmentally regulated or environmentally induced manner. We show that several *Arabidopsis* sequences, including one LTR retrotransposon, transcriptionally silenced at ambient temperatures, are differentially activated by prolonged heat stress in several *Arabidopsis* accessions. Activation can occur without loss of DNA methylation and with only minor changes of histone modifications, but is accompanied by loss of nucleosomes and heterochromatin decondensation. The results provide evidence that environmental conditions can transiently override epigenetic control, but might open a window also for longer lasting epigenetic switches. We address the role of *cis*- and *trans*-acting factors that control the transcriptional response to heat stress.

Keywords : heat stress, retrotransposon, COPIA78

CT5

Novel sRNA patterns mapped to several plant LTR-Retrotransposons families

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Background
Long Terminal Repeat retrotransposons (LTR-RTs) are the single largest components of most plant genomes and can substantially impact the genome in many ways. It is therefore crucial to understand their contribution to the genome and transcriptome. Despite sugarcane being an important crop worldwide for sugar production and as a renewable energy source, a detailed study of LTR-RTs in sugarcane has not been previously carried out. A large number of sugarcane ESTs were sequenced in 2001, and previously studies suggest that 3% of the sugarcane transcriptome is composed of TEs. In plant somatic cells two main classes of siRNAs are generated, the 21-nt class regulates post-transcriptionally related mRNAs while the 24-nt class is involved in RNA-dependent DNA methylation (RdDM) and heterochromatin maintenance and therefore suppresses gene expression at transcriptional level. Previous studies mapping sRNAs to LTR-RTs in wheat and maize genomes, reported pattern of 24nt sRNA concentrated in the LTRs ('24nt LTR' pattern).
Results
Transcriptional activity based on ESTs counts revealed tissue patterns and for the first time, family patterns. Moreover, individual LTR-RT elements lineages and families are distinctively targeted by 21 and 24 nt sRNA. The '24nt LTR' pattern described for maize and wheat LTR-RTs was observed for all *Maximus* families, and for *Del1* and *Tat3*. For all other LRT-RT families different types of patterns were observed. For almost half of the families (18 out of 33) very few sRNAs (< 2000 counts) mapped to the reference copy. Two other patterns were observed, one in which high numbers of 21nt sRNAs mapped along the coding region, represented by *Ivana6* and *Reina3*, and one in which a very large number of 24nt sRNAs mapped within the coding region, seen only in *Ale1*. The results presented support the conclusion that distinct small RNA-regulated pathways in sugarcane target different lineages of LTR-RT elements.
Conclusions
Individual LTR-RT sugarcane families have distinct transcriptional and regulatory signatures. Our results indicate that in sugarcane individual LTR-RT families have distinct behaviors and can potentially impact the genome in diverse ways. For instance, these transposable elements may affect nearby genes by generating a diverse set of small RNAs that trigger gene silencing mechanisms.

Keywords : LTR-Retrotransposon, Regulation, Expression, small RNA

CT6

Alu escapes nucleotide excision repair surveillance

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The mobile elements known as Long INterspersed and Short INterspersed Elements (LINEs and SINEs) are major sources of insertional mutagenesis in the human genome. The elements, LINE-1 (L1) and *Alu,* belong to the retrotransposons, the only class of mobile elements known to be currently active in the human genome. Because, both L1 and *Alu* contribute to human genetic instability, cells have developed multiple mechanisms to control these elements. Our previous work demonstrated that the ERCC1/XPF heterodimer known as "flap endonuclease" from the nucleotide excision repair (NER) pathway limits L1 retrotransposition. XPA is a key protein considered central to the two branches of the NER pathway and recruits ERCC1/XPF to the site of damage by directly binding to ERCC1. We present data demonstrating that these NER proteins specifically suppress L1 activity but not Alu activity. Both *ercc1*- deficient cells (Chinese hamster ovary) and *xpa* deficient cells (human) support higher L1 retrotransposition rates relative to their isogenic wildtype counterpart. In contrast, *Alu* retrotransposition is marginally affected by the lack of *ercc1*$^{-}$ cells and undetectable in *xpa*$^{-}$ cells. Complementation of the ERCC1 or XPA deficiency reduces L1 rates to wildtype level, while recovering *Alu* retrotransposition in an inefficient manner. Evaluation of the effect of *ercc1* deficiency on the retrotransposition rates of SVA, B2 and the pseudogene like ORF1mneo construct demonstrate that the ability of NER proteins to limit retrotransposition correlates with the use of L1 ORF1p for insertion and/or transcription by RNA polymerase II. We present a model highlighting the steps during the insertion L1 process that may function as the potential recruitment signal of the NER response.

Keywords : Alu, ORF1, NER, retrotransposition, SVA

CT7

Loss of transcriptional control over endogenous retroelements during reprogramming to pluripotency

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Endogenous retroelements (ERs) are a driving force in shaping the genome architecture of higher organisms, yet they remain a threat to genomic integrity owing to their ability to generate potentially mutagenic novel integrants and to induce transcriptional perturbation. Consequently, ERs are tightly controlled by epigenetic silencing mostly established in pluripotent cells, during early embryogenesis.
The reprogramming of a somatic cell to an induced pluripotent stem cell (iPSC) involves profound epigenetic remodelling. We thus asked whether the transcriptional control over ERs is appropriety maintained during this transition and reset in iPSC. We reprogrammed mouse embryonic fibroblasts (MEFs) to iPSC with a standard set of trancription factors and performed transcriptome analyses at several time-points of the transition as well as in the parent MEFs and resulting iPSCs. We observed increased expression of all endogenous retroelements (ERs) tested (MusD, L1 and IAP). The timing of their upregulation was variable between experiments but was generally initiated shortly after expression of the reprogramming transcripts, with two distinct patterns amongst ERs. Class II ERVs such as IAPs were strongly induced during reprogramming, but returned to a repressed state in fully reprogrammed cells. In contrast, MusD and LINE1, the transcription of which also increased from early times of the procedure, remained highly expressed in iPSCs, correlating with their known absence of silencing in ES cells. A similar phenomenon was recorded during the reprogramming of human hematopoietic stem cells to iPSCs. The transient upregulation during reprogramming of a subset of ERVs, which then return to a repressed state in pluripotent cells, makes this class of elements particularly intriguing. Future experiments should address the full impact of ERs deregulation on the efficiency of reprogramming and on the suitability of iPSC-derived cells in the clinic.

Keywords : iPS, reprogramming, epigenetic control, endogenous retroelement

CT8

piRNA-mediated transgenerational inheritance of an acquired trait

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The maintenance of genome integrity is an essential trait to the successful transmission of genetic information. In animal germ cells, piRNAs guide PIWI proteins to silence transposable elements (TEs) in order to maintain genome integrity. In insects, most of TE silencing in the germline is achieved by secondary piRNAs that are produced by a feed-forward loop (the ping-pong cycle), which requires the piRNA-directed cleavages of two types of RNAs: mRNAs of functional euchromatic TEs and heterochromatic transcripts that contain defective TE sequences. The first cleavage which initiates such amplification loop remains poorly understood.
Taking advantage of the existence of strains that are devoid of functional copies of the LINE-like *I-element,* we report that in such *Drosophila* ovaries, the initiation of a ping-pong cycle is achieved only by secondary *I-element* piRNAs that are produced in the ovary and deposited in the embryonic germline. Once acquired, for instance after ancestors' aging, this capacity to produce secondary piRNAs is partially transmitted through generations *via* maternal piRNAs. Furthermore, such piRNAs acting as ping-pong initiators in a chromatin-independent manner confer to the progeny a high capacity to repress the *I-element* mobility. Our study explains at the molecular level the basis for epigenetic memory of maternal immunity that protects females from hybrid dysgenesis caused by transposition of paternally inherited functional *I-elements.*

Keywords : Drosophila, *I-element* retrotransposon, piRNAs, epigenetics, hybrid dysgenesis

CT9

Methylation of SIRE1 retroelement family members in *Glycine max*

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SIRE1 is a young, relatively high copy-number retroelement that is among the most prevalent transposon families in the soybean genome, with between 1,000 and 2,000 full-length copies. It is a member of the Maximus lineage in the Ty1/Copia superfamily and is characterized by an intact, *env*-like ORF immediately downstream of *pol*. Such elements have been designated Sireviruses and are widely dispersed with moderate to high copy-numbers in *Lotus, Trifolium, Medicago*, *Arabidopsis*, *Brassica*, *Mimulus*, rice, maize, and other taxa. In *G. max*, most members are found in pericentromeric regions, having inserted into the morass of other transposons. Despite the observations that the ORFs of dozens of SIRE1 copies contain no stop codons or frameshifts and that the 5' and 3' LTRs of these copies are identical, full-length transcripts have not been detected. Transcripts of unknown biological function have been detected by RT-PCR of leaf and root tissue.

Cytosine methylation is known to epigenetically silence plant transposons, as has been experimentally demonstrated in the case of several plant elements. But detecting the collective and specific methylation of a large family of elements like SIRE1 may be less straightforward. Knowing the sequences of flanking DNAs, in a preliminary, small-scale analysis we designed primers to amplify the 5' LTRs of specific, recent SIRE1 insertions (LTRs 100% identical) with primers flanking a single MspI/HpaII site (CCGG) in the LTR. In all cases, digestion of genomic DNA with the methyl-insensitive restriction enzyme MspI, as opposed to its methyl-sensitive isoschizomer, HpaII, prior to PCR amplification resulted in the absence of amplicon(s) corresponding to the flanking fragments. Thus even the newest insertions are apparently silenced by DNA methylation.

Keywords : Retrotransposon, silencing, epigenetics, DNA methylation

CT10

Cellular partners of the telomeric retrotransposons in *Drosophila melanogaster*

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Telomere length is closely involved in processes as important as aging, tumorogenesis and genome stability and needs to be tightly regulated. While most eukaryotes use the telomerase enzyme for maintaining their telomeres, *Drosophila* uses three non-LTR retrotransposons, *HeT-A*, *TART* and *TAHRE*, which transpose exclusively to telomeres. In *Drosophila*, telomere biology is based on a complex equilibrium between the transposition of the telomeric retrotransposons whenever telomere replication is needed and tight control of their activity in order to preserve genome stability. In order to understand the basis of this equilibrium, it is necessary to know which cellular components interact, regulate and are involved in the life cycle of the telomeric retrotransposons. We have isolated different proteins that directly interact with the main component of the telomeres in *D.melanogaster*, the HeT-A Gag protein, or that co-purify with the telomeric RNP. Z4 and Nap1 are two proteins that directly interact with HeT-A Gag, the telomeric protein in charge of telomere targeting. Both Z4 and Nap1 also interact with different components of chromatin remodeling complexes like NURF and DREF/TRF2. The direct interaction of these proteins with HeT-A Gag reveals a mechanism to target these chromatin remodeling complexes to the array of the telomeric retrotransposons, the HTT array. We have already shown that Z4 regulates the activity of the HeT-A transposon by changing the chromatin environment around the HeT-A promoter. We are currently investigating the role of Nap1 in the epigenetic regulation of the HTT array.
On the other hand we have also identified the proteins Lost and Tral as partners of the telomeric RNP. Lost and Tral are important players in the protection, processing, transport and localization of different mRNAs involved in development in the Drosophila germ line. We are currently investigating if the telomeric RNP uses these proteins to be protected from degradation at the nuage by the piRNA machinery and /or to be transported to a specific location in the early embryo.

Keywords : Drosophila Telomeric transposons, Z4, Nap1, Lost and Tral

CT11

Maize LTR retrotransposons: all zombies?

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Many ongoing studies are addressing the importance of environmentally induced epigenetic variation and its eventual role in plant adaptation and important ongoing efforts are aiming at including the destabilisation of the epigenome in plant breeding programs. However recent studies, notably in the model plant *Arabidopsis*, have shown that transposable elements (TEs) are under epigenetic control and that the RNAi pathway plays a role in their transgenerational mobilisation (Mirouze et al., 2009; Tsukahara et al., 2009; Ito et al, 2011). As a consequence, destabilising the epigenome and especially the DNA methylome inevitably lead to the mobilisation of endogenous transposons, either immediately or after several generations of inbreeding in *Arabidopsis* (Reinders et al., 2009; Johannes et al., 2009). However, in this small-genome species, the phenotypic impact was rather limited as relatively few families of LTR retrotransposons, for example, were still intact in their sequence and capable of being mobilised in RNAi or DNA methylation mutants. In crops with a much larger genome (e.g. 3 Gb for maize) and displaying a larger TE compartment (e.g. 85% for maize, Schnable et al., 2009) it could be expected that the number of intact LTR retrotransposons would be higher, but the impact of their epigenetic misregulation on their mobility is not elucidated. We are focusing our analysis on candidate LTR retrotransposons in maize. We will present our preliminary data on the characterisation of the putative mobility of candidate retroelements in diverse maize epigenetic mutant and natural lines.

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CT12

Regulation of the I-factor a drosophila retrotransposon LINE-like element

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Most genomes from plants to mammals are highly repetitive. They contain several families of transposable elements (TEs). They are classified according to their DNA topology and the mechanism by which they transpose. The Drosophila *melanogaster* 'I factor' belongs to the LINE family which represents the major class of TEs in mammals (20%) that move via an RNA intermediate. Drosophila *melanogaster* falls into two categories of strains, Inducer strains 'I' contain several functional copies of I factors, while Reactive strains'R' lack such elements. However, both possess non-coding sequences called I-Related elements (I-RE), which are homologous to the current I-factor but they have lost their ability to transpose. We use the Hybrid-Dysgenesis of Drosophila *melanogaster* as a model to study the regulation of I factor. Indeed, a cross between an inducer male and a reactive female induces a high level of transposition of I factors in the germline of the F1 female progeny. This system allows the understanding of I-factor mobilization. Previously we showed that we can inhibit the activity of I factors by introducing fragments of the I factor itself. This mechanism could be compared to a "genetic vaccination". Later, the RNA interference has been shown to be involved in the defense against the harmful effects of TEs. In drosophila, the Piwi-interacting RNAs (piRNAs) pathway is the principal mechanism that silences TEs. This defense acts in germline and gonadal somatic tissues by two different mechanism. The Piwi, Aubergine (Aub), Argonaute3 (Ago3), Armitage (Armi), Zucchini (zuc) and Spindle-E (spnE) proteins are expressed in both tissues and some of them are known to silence the I factor. In 2007, we showed a correlation between the increased rate of I-RE transcripts, which reside in the pericentromeric heterochromatin with the decreased I factor activity. Later, molecular analysis showed that the drosophila Piwi protein interact directly with Heterochromatin protein alpha 1(HP1a). The aim of my PhD is to understand the molecular mechanism which regulate the I factors and my project is divided into two parts: The first part tends to elucidate the impact of the chromatin structure on the I-RE and I factors transcripts by using several approaches: allelic mutations of the gene *Su(Var)2-5* encoding HP1, the RNAi knock-down against HP1 and CHiP analysis. The second part is to understand the fate of I-RE and I factors transcripts in several mutants affecting the piRNA pathway, and to ask whether proteins of I factors can be detected in the ovaries in these different contexts. This analysis could reveal a hypothetical regulation of I-factors at the translation level.

Keywords : drosophila, retrotransposon, HP1a, piRNA, heterochromatin

CT13

Immediate resilencing of an active LTR retrotransposon in Arabidopsis F1 epigenome hybrids

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DNA methylation is an epigenetic modification that is widely used to repress the transcription of transposable elements (TEs) in plant and other genomes. Consistently, loss of CG methylation in Arabidopsis *met1* mutants leads to the transcriptional activation of a large set of TEs. When these active TEs are reintroduced in a wild-type background in met1 x WT F1 hybrids, they either remain hypomethylated and transcribed and some of those are progressively remethylated and silenced in successive generations[1-3].

In *met1* mutants, the LTR retrotransposon *Evadé* (*EVD*) is not only transcribed but it also transposes, thereby threatening genome integrity[4]. Similar to *Athila* TEs, EVD transcriptional activation is associated to the production of 21 nt-small RNAs (sRNAs) in addition to the 24 nt-sRNAs that already accumulate in wild-type plants[4,5].

In stark contrast with other TEs, we found that surprisingly, the EVD is efficiently and immediately resilenced in *met1* x WT F1 hybrids. We further found that the silent state established at *EVD* in F1 plants is stably transmitted to the progeny and is independent of DNA methylation, thus differing from the silent state initially present in the wild-type parent plant. The establishment of an alternative silent state at *EVD* prevents the creation of active *EVD* epialleles and thus protects the genome from a burst of transposition. We are currently dissecting the genetic requirements of this silencing mechanism as well as its genome-wide extent.

Keywords : DNA methylation, LTR retrotransposon, resilencing, F1 hybrids

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CT14

Endogenous LINE-1 retrotransposons are mobilized in human pluripotent stem cells and affect genome stability

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The ability of pluripotent stem cells to self-renew and differentiate into cells of the three germ layers makes them an invaluable tool for regenerative medicine. Human induced pluripotent **s**tem **c**ells (hiPSCs) represent a source of autologous cells compatible with the immune system and avoid ethical issues associated with human embryonic stem cells (hESCs). However, recent surveys show that reprogramming and subsequent cultivation of hiPSCs *in vitro* can induce genomic abnormalities in these cells. It is unclear if hiPSCs or their derivatives are safe for administration since it is questionable if the integrity of the genome of these stem cells remains stable during generation, expansion and differentiation. The appearance of genomic mutations could undermine stem cell therapies. Such mutations can be induced by human endogenous non-LTR retrotransposon families **LINE-1 (L1)**, *Alu*, and SVA which are currently mobilized in the genome. About 35% of our genome is the consequence of retrotransposon mobilization that is executed by the L1-encoded protein machinery. L1 activity can cause various forms of genetic instability including deletions, and chromosomal translocations, and can affect gene expression. 70 cases of genetic disorders and/or tumorigenic diseases were reported to be the consequence of L1 activity so far. Thus, it is important to understand if L1 activity can impact genomic integrity in hiPSCs and hESCs.

To investigate if endogenous L1 activity can affect genome stability of human pluripotent stem cells, we analyzed the expression of functional endogenous L1 elements in hESC and hiPSC lines by qRT-PCR and immunoblot analyses. We found that reprogramming of somatic cells into iPSCs activates L1 mRNA and protein expression, and differentiation into embryoid bodies reduces L1 mRNA levels. Furthermore, individually transcribed functional L1 elements in hESC and hiPSCs lines could be identified. *High-Throughput* sequencing of genomic L1Hs libraries generated from genomic DNA of hiPSC and parental cell lines uncovered L1 *de novo* transposition events that occurred after reprogramming. Applying R-banding, mFISH, FISH, and array-CGH, we are currently characterizing karyotypic abnormalities and local genomic destabilization events caused by L1 mobilization in hiPSC lines. Taken together, our data demonstrate that reprogramming of somatic cells into iPSCs and the pluripotent status of hESCs causes the mobilization of endogenous L1 elements which can affect genome stability.

Keywords : LINE-1, pluripotent stem cells, human non-LTR retrotransposons, genomic destabilization

CT15

Programming group II intron RmInt1 to recognize target sites other than its wild-type site

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Group II introns are catalytic RNAs and mobile retroelements initially identified in the mitochondrial and chloroplast genomes of lower eukaryotes and plants, and later found in bacteria and archaea. They have recently been identified in the mitochondrial genomes of the annelid *Nephtys* sp. and basal placozoan *Trichoplax adherens*. It is thought that both nuclear spliceosomal introns and non-long terminal repeat (LTR) retrotransposons evolved from mobile group II introns. A typical group II intron consists of a highly structured RNA that fold into a conserved three-dimensional structure consisting of six distinct double-helical domains, DI to DVI. Most bacterial group II introns have a multifunctional intron-encoded protein (IEP) ORF within DIV. Group II IEPs have an N-terminal RT domain homologous to retroviral RT sequences, followed by a putative RNA-binding domain with RNA splicing or maturase activity (domain X), and a C-terminal DNA-binding (D)/ DNA endonuclease (En) region.The group IIA intron Ll.LtrB from *Lactococcus lactis* and the group IIB intron EcI5 from *Escherichia coli* have intron-encoded proteins (IEP) with a DNA-binding domain (D) and an endonuclease domain (En). Both have been successfully retargeted to invade target DNAs other than their wild-type target sites. RmInt1, a subclass IIB3/D intron with an IEP lacking D and En domains, is highly active in retrohoming in its host, *Sinorhizobium meliloti*. We are currently programming group II intron RmInt1 to recognize target sites other than its wild-type site. Our results extend the range of group II introns available for gene targeting.

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CT16

Unraveling the relationship between transposons and spermatogenesis

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Genome-wide remodeling of epigenetic profiles occur in primordial germ cells in mammals, including a loss of methylation at various transposable elements (TEs). This opportunistic window for TE activity is rapidly counteracted by a cooperative effort between the RNA interference pathway and the DNA methylation machinery to ensure the stable suppression of TEs in developing male germ cells. With the number of studies increasing rapidly in the field, there are now 14 genes known to be involved in this pathway. *Dnmt3L*, one of the key genes in this pathway is a DNA-methyltransferase co-factor and is required for TE methylation during spermatogenesis. A germline-specific class of small RNAs, the piRNAs (PIWI associated small RNAs), promote the degradation of TE transcripts and also help establish TE methylation. Male mice carrying mutations for genes in the piRNA/DNA methylation pathway show massive TE reactivation that leads to complete sterility.
Despite increasing studies on the piRNA/DNA methylation pathway, very little is currently known about the nature and the chronology of the interactions that exist between the male germline and TEs. We have characterized the dynamics of activity and silencing of various classes of TEs (LTR and non-LTR) in pre-natal and post-natal spermatogenesis, along with the kinetics of expression of TE repressors. In wildtype mice, TEs are globally repressed throughout spermatogenesis, as assessed by the lack of transcript of these elements. Interestingly, using single and double-mutants of the piRNA (*Miwi2*) and DNA methylation (*Dnmt3L*) pathway, we find that mRNAs of most derepressed TEs accumulate briefly at 18.5dpc, and then burst much later at the time of meiosis entry. Concurring with our mRNA data, LINE1 ORF1 protein is up-regulated in meiotic mutant cells, which are eventually lost by apoptosis. Our studies have produced a clear time line of TE activity throughout male germ cell development in wildtype, methylation deficient and piRNA deficient mice. We highlighted specificities and common themes in the regulation of these different TEs, and the interplay between transcriptional and post-transcriptional regulation throughout spermatogenesis.

Keywords : retrotransposon, germline, DNA methylation, piRNA

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EV1

Structural evolution of Alu ribonucleoprotein particles

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Alu elements are small non-LTR retrotransposons that are only found in higher primates. They originated ~65 million years ago from the gene encoding 7SL RNA, the structural scaffold of the mammalian signal recognition particle (SRP). Early in primate evolution, a tandem duplication resulted in the left and right arm monomers of Alu elements. This was followed by several rounds of massive expansion, which gave rise to successive and distinct Alu families (Alu J, followed by Alu S, and Alu Y families). As a result, the human genome presently harbors approximately 1.1 million Alu elements (10 % of its mass).

Alu elements are also quite unusual because retrotransposition seems to depend on the threedimensional structure of the RNA and on its ability to interact with the SRP9/14 protein heterodimer encoded by the 'host' genome (1). However, despite of a crystal structure for the Alu domain of the human SRP (2), little is known about the structural requirements and molecular mechanisms that promote or control Alu retrotransposition in human cells.

Here we investigate the structural evolution of Alu RNA and its ability to interact with the SRP9/14 protein heterodimer. An in vitro competition assay reveals a significant drop in SRP9/14 affinities at the transition from the J to the S families. This is particularly true for the Alu right arm monomer that allows an alternative base-pairing scheme within an asymmetric loop of the Alu RNA 3'domain. Nevertheless, both Alu left arm and right arm monomers still form compact and highly specific complexes with SPR9/14 that can be purified as Alu RNP particles. This shows that the ability to bind SRP9/14 has been conserved for both monomers among Alu family consensus sequences and therefore seems to be a requirement for an efficient retrotransposition. Ultimately, we seek to obtain a high-resolution structure to reveal the molecular details of the structural changes and their functional significance; e.g. for interactions of Alu RNPs with the ribosome that lead to a recruitment of the LINE-1 reverse transcriptase.

Keywords : biochemistry, evolution, non-LTR retrotransposon, RNA-binding, structure

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EV2

Cohorts of highly active L1 retrotransposons in human populations that share distinctive 3' sequence transductions

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Long INterspersed Element-1 (LINE-1 or L1) retrotransposons are the only autonomously active retrotransposons in the human genome. The average human genome contains ~80-100 active L1s[1], but only a subset of these are highly active (or 'hot'), as judged by cell culture based retrotransposition assays. The lineage succession pattern of primate L1 evolution[2] leads to the proliferation of human-specific L1s that are very closely related in sequence. As a result it is often difficult to unequivocally establish progenitor/offspring relationships using phylogenetic methods. Fortuitously, bypass of the L1 polyadenylation signal often leads to the mobilization of genomic sequences flanking the L1 3' end, a process known as 3' transduction[3]. This process results in the production of lineage specific sequence "tags" that mark the descendants of active L1 progenitors. Here we report the development of a method (Transduction Specific Amplification Typing of L1 Active Subfamilies or TS-ATLAS) that exploits L1 3' transductions to identify active L1 lineages in a genome-wide context. TS-ATLAS enabled the characterisation of a putative active progenitor of the L1 lineage that includes a disease causing L1 insertion ($L1_{RP}$)[4] and identified new retrotransposition events within two other 'hot' L1 lineages. Intriguingly, the analysis of newly discovered transduction lineage members suggests that L1 polyadenylation, even within a lineage, is highly stochastic. TS-ATLAS thus provides a new tool to explore the dynamics of L1 lineage evolution and retrotransposon biology.

Keywords : human, retrotransposon, transduction, polyadenylation, evolution

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EV3

Use of Trasposable Elements Polymorphism to Study the Divergence and the Diversity of Locus *S1* Region in African Rice

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Hybrid sterility is a mechanism that prevents the hybridization between different species. The *O. sativa* (Asian rice) X *O. glaberrima* (African rice) crosses are mainly controlled by the *S1* locus. This locus is in epistasis with two adjacent loci. We studied the divergence and diversity of this region in *O. glaberrima* and its wild ancestor *O. barthii.* We have established a physical map and sequenced 508kb in the *S1* region in *O. glaberrima* cv. TOG5681, a cultivated rice of lowland ecology. We compared this sequence with the ortholog region in CG14, a cultivated rice of upland ecology. The sequences are highly conserved and the divergence between the two varieties of *O. glaberrima* is limited to insertions of transposable elements and some SNPs found in an interval of LRR genes. Transposable elements insertion polymorphisms were used to analyze the diversity of the locus using a collection of African wild and cultivated rice varieties. While a very low diversity was observed for the majority of the accessions, some varieties of *O. glaberrima* revealed a haplotype for the *S1* region. These results indicate that these *O. glaberrima* cultivars come from an ancient hybridization with Asian rice and suggest that they may be more fertile when crossed with *O. sativa.*

Keywords : *O. glaberrima, O. barthii, S1* locus, RBIP markers, diversity, interspecific hybridation

EV4

Expression of the transposable elements in cactophilic *Drosophila*

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There are some evidences that transposable elements (TEs) may be associated with interspecific sterility and incompatibility that could drive to speciation, but very few data describe the dynamics of TEs in this type of crosses. *Drosophila mojavensis* and its sibling species, *Drosophila arizonae*, evolved recently, with partially overlapping distributions in the nature. Sympatric populations can be observed in part of the Sonoran Desert (Mexico) and southern Arizonae-USA, and allopatric populations are described in Baja peninsula and southern California. In the crossing *ex situ*, these species exhibit differences in the reproductive isolation (pre- and poszygotic), depending on the geographic origins. In the present study we built hybrids from two different strains of each species (one sympatric and one allopatric) and followed in the interspecific crosses the expression several TEs in the parental lines and in their offspring hybrids. Our results indicate that TEs are differentially expressed in the parental lines and in somatic and germline tissues. For some of the TEs analyzed, expression seems to be release in the F1 hybrids. These results indicate that TEs may be implicated in the post zygotic sterility observed in some interspecific crosses.

Keywords : transposable elements, hybrids, gene expression, speciation

EV5

Characterization and expression of *Mariner*-Like Transposons in the marine microalga *Amphora acutiuscula* (Bacillariophyceae)

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Transposons of the *Mariner*-Like Element (MLE) superfamily were investigated in the genome of the marine microalga *Amphora acutiuscula*. Detection of MLE was performed by PCR using degenerate primers MLE5A and MLE3A [1]. These primers allowed to amplify fragments of the transposase gene in various plant species. Using this strategy, six 380 bp fragments of MLE transposons have been isolated in the genome of *A. acutiuscula*. The translated protein sequences revealed the presence of putative ORF for all the sequences detected.
In the literature, the expression of transposable elements under various stress conditions is well documented. To detect a potential expression of MLE, a thermal stress of short duration (5 h) was applied to this microalga usually cultivated under controlled conditions at 24°C. To stress this species, the temperatures 4, 8, 16°C (low temperatures) and 32°C (high temperature) were applied to 8 day-old cultures. The expression of MLE has been revealed by RT – PCR and all the fragments obtained have been cloned and sequenced. The transcribed sequences obtained and the 380 bp MLE fragments detected in genomic DNA possess a relatively high similarity. This observation suggests the expression of a MLE transposase gene in *A. acutiuscula.*The use of the primers MLE5A and MLE3A provides a partial sequence of the transposase gene; then, to characterize a full-length element a strategy using the inverse PCR method has been performed. The iPCR method allowed to extend a sequence up to 4000 bp. The *in silico* analysis of translated sequence in protein helped to identify the characteristic structural motifs of the MLE transposase. In plants, the MLE transposase is a sequence from 412 to 520 amino acids with only an open reading frame. The patterns corresponding to the primers MLE5A and MLE3A are present and slight modified from the initial features IDEKWF and IQQDNA. In *A. acutiuscula*, these motifs are respectively VDEEKWF and IQQDGA. The third aspartic acid (D) of the catalytic triad was found in the pattern SPDTNINDLAFF instead of SPD(L/M)N-LDLGFF motif highly conserved among plants. The distance between the two last aspartic acids is 43 residues instead of 39 in the classic pattern (DD39D) found in terrestrial plants. It is now necessary to extend the region of the 5'-end to identify the ITRs (Inverted Terminal Repeats) to reconstruct a complete transposon element in *A. acutiuscula*. By applying the UPGM-VM procedure [2], we have shown that this nucleotide sequence belongs to the DDTChlDia class (DNA transposons, Chlorophyllis, Diatomis).

Keywords *:* MLE transposon, marine diatom, thermal stress, inverse PCR

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EV6

Molecular characterization of mariner-like elements in *Oryctes agamemnon* and its host plant Date palm tree, *Phoenix dactylifera*

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Mariner-like elements (MLEs) are members from class II transposable elements also known as DNA transposons. These elements have a wide distribution among different groups of organisms, including insects. In addition to their propensity for intragenomic spread, MLEs have the ability to cross species barriers and spread to the genome of a new species by horizontal transfer.
Oryctes agamemnon is a Coleoptera pest originating from the Middle East, and a new invasive species in Tunisia. Several tens of partial transposon sequences were obtained from both the insect and its host plant *Phoenix dactylifera,* by using degenerate primers previously designed by Robertson for *Mariner*, Feschotte for *Plant mariner*, and Avancini for *Tc1*. Although the intimacy of the relationship between the insect and its host plant, sequences analyses revealed low homology of MLEs sequences, that reject any potential horizontal transfer events between the two species.
By using the inverted terminal repeat (ITR) of *Mauritiana* MLEs, a complete sequence was designed in the genome of the host plant. Phylogenetic analysis showed that this element belongs to the *Dipteris* Tribe of the *Mauritiana* SubFamily, and is likely to be inactive, based on the presence of 8 stop codons and 7 frameshifts mutations. However this *Phoenix dactylifera* MLE sequence revealed a high homology with the *Mos1* element present in insects (*Drosophila, Zaprionus*, *Musca*, *Blatella*, *Mamestra)*. This sequence, the first *mariner* found in a plant, is very alterated, suggesting a very ancient horizontal transfer, probably between a palm and an insect. These results have to be confirmed in other species of the genus *Phoenix* such as *P. canariensis.*

EV7

Large scale comparative genomics of transposable elements in fish

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For a long time, transposable elements (TEs) have been considered as selfish junk DNA and hostile invaders of genomes. Nowadays, thanks to multiple sequencing projects, whole genome analyses have revealed the important contribution of TEs to the structure and evolution of genomes. TEs have been found in almost all eukaryotic genomes, with major variations noticed in copy number and diversity of elements. More and more data accumulate, especially for vertebrate genomes. It has been shown that at least 45% of the human genome, 38% of the mouse genome and 37% of the clawed frog genome are composed of TEs. However, fewer data are available for fish genomes. With almost 28,000 species, fish represent the largest vertebrate group (about half of all extant vertebrate species), making them an interesting model to perform comparative genomic analyses. Indeed, such analyses will help to reveal major insights into the roles of TEs during genome evolution.
Fish genomes can drastically vary in size, from 0.32 to 133 billion base pairs. In this study, we performed a large scale comparative genomic TE analyses using already sequenced fish genomes (zebrafish, medaka, stickleback, tetraodon and torafugu), data from ongoing fish (rainbow trout, platyfish, tongue sole and spotted gar) and coelacanth genome sequencing projects in which we are implied. We manually and automatically annotate and compare TEs from different species to assess their evolution and genomic impact in fish. One of the main results of this work is that, in contrast to mammalian genomes, teleost fish genomes exhibit a huge diversity of TEs and many of them are still active. Almost all families of eukaryotic TEs have been found in teleost fish genomes. Interestingly, we observe an apparent positive correlation between genome size and TE content.
The contribution of TEs to the pool of host genes has been well characterized in mammals, TEs can be a source of new coding and regulatory sequences. One process to acquire new coding potential is molecular domestication. Several examples of host genes derived from TE have been identified in fish and vertebrate genomes. Most of them are only present in some group of vertebrate, they might therefore contribute to lineage-specific genetic innovation. The function of those genes is currently analyzed in different fish models.

Keywords : comparative genomics, fish genomes, molecular domestication

EV8

Mar, a MITE family of *hAT* transposons in *Drosophila*

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Miniature inverted-repeat transposable elements (MITEs) are short non-autonomous DNA elements, flanked by subterminal or terminal inverted repeats (TIRs). MITEs were recognized as important components of plant genomes where they can attain extremely high copy numbers. They are found also in several animal genomes, including mosquitoes, fish and humans. In *Drosophila*, few families were described up to now. In this work, we investigated the distribution and evolution of *Mar*, a MITE family, in Drosophilidae species. The *Mar* distribution is restrict to the *willistoni* subgroup of *Drosophila* and the phylogeny supports the view that the origin of this element might have occurred prior to diversification of these species. In the *D. willistoni*, *D. paulistorum, D. equinoxialis* and *D. insularis* the fragment amplified length varies from roughly 270 bp to 450 bp. On the other hand, the fragment amplified in *D. tropicalis* was larger than expected (around 3,000 bp). Analysis of the *D. tropicalis* sequences revealed putative complete element. In spite of these sequences contain stop codons, a well conserved exon and a *hAT* family dimerization domain were present. Considering that MITEs require autonomous elements for transposition, we analyzed the *D. willistoni* genome for the presence of putative autonomous elements based on *D. tropicalis* sequences. An homologous sequence was found in the *D. willistoni* genome. Alignment of this sequence with the *D. tropicalis Mar* showed that the consensus sequence can be a putative *Mar* transposase. The relationships between *Mar* consensus transposase and the *hAT* superfamily elements revealed that the placement of the *Mar* consensus transposase was within the recently determined *Buster* family of the transposons. Additionally, it forms a clade with *Buster* transposase sequences from bat, mosquito, sea urchin (*Strongylocentrotus purpuratus*), zebrafish (*Danio rerio*) and freshwater planarian (*Schmidtea mediterranea*). By searching for *Mar* copies in *D. willistoni* genome we observed that several copies presented conserved TSDs (target site duplication) and TIRs (terminal inverted repeats), indicating recent mobilization of these sequences. The presence of the *Mar* relic transposase can be evidence of the origin of their MITEs by internal deletions and suggest that the full-length transposon was recently functional in the *D. willistoni* genome promoting the *Mar* MITEs mobilization.

Keywords : Miniature inverted-repeat transposable elements, MITE, *Mar* element, transposon, *Drosophila*

EV9

Inter- and intra-specific variability in the piRNA pathway controlling transposable elements

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Post-transcriptional regulation of transposable elements *via* small interfering RNAs has recently been demonstrated and the deciphering of the fine mechanisms is currently in the heart of many studies. We believe that considering this topic with an evolutionary point of view allows to understand the dynamics of transposable elements, illustrated by the variability in their amounts and activities observed in different genomes for all kinds of organisms.
We have performed a population study of the variability of the genes governing these regulatory pathways *(ago3*, *aub*, *armi*, *piwi*, *spnE*, *squ* and *zuc)* in *Drosophila simulans*, a species known to display elevated populational variability of the copy numbers and activities of its transposable elements. We observe significant polymorphism for these genes in natural populations, as well as high variability in the expression levels of genes and transposable elements, such that each population displays a unique combination of alleles and expression levels. Our data reveal associations between the abundancy of certain classes of elements and particular genes in the regulatory pathways.

EV10

Bacterial IS607-like sequences in eukaryotes

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Horizontal transfer of transposable elements (HTT) plays a key role in prokaryotic evolution and mounting evidence suggests that it has also had an important impact on eukaryotic evolution. While many prokaryote-to-prokaryote and eukaryote-to-eukaryote HTTs have been characterized, only few HTTs have been reported between prokaryotes and eukaryotes. Here we provide a detailed analysis of sequences similar to bacterial insertion sequences (IS) previously reported in three yeast genomes (*Eremothecium gossypii*, *Saccharomyces kluyveri* and *S. cerevisiae*) and characterize new ones in two strains of *S. cerevisiae* and in various other eukaryotic lineages (brown algae, oomycetes, Amoebozoa). We show that all of them derive from IS607, an IS family composed of two open reading frames (orfA and orfB). Among these eukaryotic IS607-like sequences, a subset shows homology to the full-length IS607 sequence (up to 51% amino acid similarity), but most sequences match either orfA only (up to 59% similarity), orfB only (up to 48% similarity), or the C-terminal DNA binding domain of orfB only (up to 51% similarity). Phylogenetic inferences reveal that the presence of these sequences in eukaryotic genomes is the result of several horizontal transfer events. Furthermore, selection analyses indicate that in most eukaryotic taxa in which they were found, one or more IS607-like sequences are evolving under strong purifying selection. Together, these results suggest that prokaryote-to-eukaryote HTT followed by multiple, independent IS domestication events, has contributed to the birth of new eukaryotic genes.

Keywords : insertion sequences, horizontal transfer, molecular domestication

EV11

Accretion by site-specific recombination and conjugative mobilization: major mechanisms of evolution and transfer of streptococcal genomic islands?

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Transfer of genomic islands (GIs) between bacteria plays a key role in their evolution whereas its mechanism is rarely known. At least some GIs belong to poorly known classes of mobile elements, the Integrative and Conjugative Elements (ICEs) and the Integrative and Mobilizable Elements (IMEs). The ICEs encode their own excision, transfer by conjugation and integration. For most ICEs, the site-specific recombination between identical sequences carried by the attL and attR flanking sites leads to the excision of a circular form harboring an attI site and to a chromosome carrying an *attB* site. After transfer, these ICEs integrate by recombination between the *attI* site and the *attB* site. The IMEs are non autonomous elements that encode their excision/integration, harbour their own origin transfer and frequently encode some proteins involved in transfer but use the conjugation pore encoded by a conjugative element.

Various strains of the lactic acid bacterium *Streptococcus thermophilus*, a bacterium used in dairy fermentation, were found to harbor related GIs. One of them, ICE*St3* is an ICE whereas most ICE*St3*-related GIs ("CIMEs") derive from ICEs by deletion of recombination and conjugation modules, but have retained *att* sites. Using an engineered CIME, composed of *attL* and *attR* sites flanking a chloramphenicol resistance gene, CIMEL_3catR_3, we show that ICE*St3* transfers to a recipient harboring this GI and integrates by site-specific recombination into either one or the other CIME *att* sites, leading to their accretion. The resulting composite island can excise showing that ICE*St3* mobilizes CIMEL_3catR_3 in *cis*. The transfer of ICE*St3* alone, of CIMEL_3catR_3 alone and of both elements was observed using the strain harboring the composite structure as a donor. The ICE*St3* transfer to a recipient bearing CIMEL_3catR_3 can lead to retromobilization, i.e. capture of CIMEL_3catR_3 by the donor strain.

CIMEL_3catR_3 would be the prototype of a novel class of non autonomous mobile elements, the CIMEs (CIs Mobilizable Elements), which would be devoid of mobility genes but hijack the recombination and conjugation machinery of a related ICE to excise, transfer, and integrate. This novel class could include the numerous GIs that are flanked by direct repeats but do not harbor any gene that could be involved in their transfer. Genome analyses of streptococci show that ICEs, IMEs and CIMEs are very widespread. These data, joined to the presence of internal repeats that could result from site-specific accretions in many of these elements, suggests that accretion and *cis*-mobilization play a key role in GI evolution and transfer.

Keywords : Conjugation, Mobilization, Site-specific recombination, Genomic islands

EV12

The LINE landscape in genomes of higher plants

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A major fraction of the repeated DNA in eukaryotic genomes is made up of long interspersed nuclear elements (LINEs). Due to their capability to create copies of themselves in an error-prone reverse transcription process, they can generate multiple families and reach high copy numbers. Up to date, 28 LINE clades and a plethora of LINE families have been described in plants, animals and fungi. Since LINEs are highly active in human, a large body of knowledge about mammalian LINEs has been accumulated. Plant LINEs, though ubiquitously present, are less abundant and occur in much higher diversity. Although clear differences between plant and mammalian LINEs have been stated, findings of mammalian LINE research are widely applied to their plant counterparts.
In order to close this knowledge gap, we present a systematic cross-species survey of the LINE content within plant genomes. Based on a Hidden Markov Model analysis, 7663 unfragmented LINE reverse transcriptases (RTs) have been extracted from the genomic sequences of thirteen flowering plants, including three monocots and ten dicots. Key finding is the presence of only two out of 28 LINE clades (L1 and RTE) in the genomes analyzed. Whereas plant RTE LINEs are highly homogenous and most likely constitute only a single family per genome, plant L1 LINEs are extremely diverse and form numerous families. We distinguish seven L1 subclades encompassing all identified members across the analyzed plant species. Finally, we exemplarily focus on the genome of *Beta vulgaris* to show that the subclade classification level does not only reflect RT sequence similarity, but also mirrors the structural aspects of complete LINE retrotransposons, like element size, position and type of enzymatic domains.
Taken together, we established a comprehensive catalogue of the plant LINE population and provide a classification system for highly diverse plant L1 LINEs.

EV13

piggyBac elements and associated MITEs in the genome of the phytopathogenic fungus *Fusarium oxysporum*

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Fusarium oxysporum is an asexual ascomycetous fungus, pathogenic on a large number of cultivated plants. It is known to harbor a large diversity of transposable elements, including Class I (retroelements transposing through a RNA intermediate) and Class II elements (DNA transposons) (Ma et al. 2010). In *F. oxysporum*, as in most fungal genomes, DNA transposons belong to few different superfamilies, such as *Tc1-mariner-pogo*, *hAT,* or *Mutator*. Several MITE (Miniature Inverted-repeat Transposable Element) families have also been detected, fortuitously or using homology search based strategy, and leading to the identification of 13 MITE families/subfamilies, related to *pogo, Tc1-mariner* or *hAT* elements. The release of the genome sequence of the strain FOL4287, pathogenic on tomato, has revealed that in this strain, transposable elements (TEs) are mainly concentrated on dispensable chromosomes. These chromosomes are also enriched with segmental duplications and pathogenicity genes, and appear strain-specific (Ma et al., 2010).

We used different strategies of *de novo* detection of MITEs in this genome. This approach provided several new putative MITE families. We focused on three of them, present in moderate to low copy number, and that share TIR sequences. Their characteristics did not permit to identify the superfamily to which they belong, and we searched for autonomous/longer partners using MITE TIRs as query. We recovered 3 longer putative elements. Although probably inactive, the conceptual translation of the open reading frames of one of them indicated strong homology with the *pep1* gene from the related fungus *Nectria haematoccoca*, described as involved in pathogenicity on pea. Weak homology was also detected with some metazoan *piggyBac* transposases. The search for related sequences uncovered a large and divergent TE family. This superfamily has never been described in fungi previously.

A search within the junk of the *de novo* MITE initial search revealed the existence of numerous *piggyBac* MITE-like sequences sharing related TIRs but that are present in less than 3 copies in the genome. The total number of *piggyBac*-associated MITE subfamilies is then estimated to more than 20 in *Fusarium oxysporum*. Finally, the recent availability of 10 different genomes of *F. oxysporum* has permitted (i) to confirm that most of these new subfamilies are indeed mobile, (ii) to compare the distribution of these different MITE subfamilies, including at the insertion site level, and (iii) to shed light into the evolutionary history of this old but newly discovered superfamily in fungi.

EV14

Improving ISs and MITEs identification using *de novo* methods : a case study with archaeal genomes

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Transposable elements as ISs and their non-autonomous derivatives (MITEs) are essential components of prokaryotic genomes. Traditionally, these elements are identified using similarity search with a reference data bank compiling all known ISs sequences (the IS Finder database).
Here, we have developed two pipelines that use *de novo* methods of ISs and MITEs identification :
- a method based on the identification of the inverted repeat (IR) of the elements
- a method that recognize the repeated sequences
We have tested these two pipelines against a reference dataset composed of 30 archaeal genomes. Compared to similarity search methods, *de novo* methods increased significantly the number of identified ISs and MITEs (+8% and +56% respectively) and appears faster and easier to implement for non-specialist user. In order to increase the efficiency of ISs and MITEs identification, we suggest that future studies in the field may used a combination of similarity and *de novo* methods.

EV15

The impact of retrotransposon integration on gene expression

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Because of their similarities to retroviruses, long terminal repeat (LTR)-retrotransposons are important models for retrovirus replication. In the yeast *Schizosaccharomyces pombe*, integration of the LTR-retrotransposon Tf1 has a strong preference for pol II promoters. This choice of target sites is similar to the preferences of HIV-1 and MLV for integration into pol II transcription units. To identify patterns of integration throughout the genome we used ligation mediated PCR and ultra high throughput sequencing. These methods identified 73,125 independent Tf1 integrations in *S. pombe*. The insertions exhibited the same strong preference for promoter regions observed in small-scale experiments. Although the number of insertions in each promoter varied widely, four independent integration experiments demonstrated that the levels of integration activity of each intergenic region were highly reproducible. These target activities of the promoters did not correlate with overall transcription activity. An analysis of microarray data from cells grown under a variety of conditions revealed Tf1 integration has a strong preference for promoters that are induced by specific conditions of stress.
The biological impact of transposons on the physiology of the host cells depends greatly on the position of integration and the effect of integration on the expression of adjacent genes. The integration of Tf1 in promoters induced by stress raised the possibility that some insertions could significantly alter the physiology of the host. To determine the biological impact of Tf1 integration, we measured the effect of Tf1 integration on the expression of adjacent genes. We studied integration next to 27 genes that are among the most common targets of insertion. Surprisingly, we found that Tf1 integration did not reduce gene expression. RNA blot and RACE experiments revealed that Tf1 insertion did induce the expression of some adjacent genes by enhancing levels of the native transcripts. Intriguingly, the induction of some genes by Tf1 was stress dependent and this correlated with the stress activation of the Tf1 promoter. Our findings indicate that Tf1 insertion can induce the expression of genes, particularly stress response genes, and this suggests that the pattern of Tf1 integration may have evolved to improve the survival of cells in conditions of environmental stress.

Keywords : integration, transposon, gene expression, Schizosaccharomyes pombe

EV16

Long-TIR transposons, could a disadvantage become useful? *Galileo* long TIRs explored

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Galileo is a transposable element discovered because of its implication in the generation of natural chromosomal inversions in *Drosophila buzzatii*. This long-TIR element (up to 1.2 Kb) is widespread in the *Drosophila* genus and it has been classified as a member of the *P* superfamily of DNA transposons. In order to track possible effects of *Galileo* long TIRs in the transposon mobilization, we tested the DNA binding activity, which is the first step of the transposition reaction. We inferred consensus and ancestor sequences encoding for the DNA binding (THAP) domain of *Galileo* transposase from three different species. We expressed *in vitro* these sequences and tested their binding activity to the endmost part (150 bp) of the *Galileo* TIR. Specific DNA binding activity was observed in each case. We also determined the sequence of the DNA binding site, which shows similarities with other THAP domains binding sequences. Furthermore, putative secondary binding sites were found bioinformatically in the internal tandem repeats of the TIRs, which might help to explain their remarkable length of the *Galileo* TIRs.

In addition, we carried out a thorough search of *Galileo* copies present in the *D. mojavensis* genome. We found and annotated a set of 170 *Galileo* copies, revealing a huge variability in length and structure, ranging from nearly-complete copies to copies mainly composed by the two TIR or even solo-TIR elements. We classified sequences in five subfamilies according to sequence divergence and phylogenetic reconstruction in five subfamilies: C, D, E, F, and X, four of them harbouring transposase-coding sequence and a fifth one which presents a putative chimeric origin. Furthermore, the phylogeny reconstructed by means of Bayesian Inference methods using a relaxed molecular clock, showed that *Galileo* has been active until very recently and suggests that it might be currently active. Finally, we explored the structure and length variation of the *Galileo* copies, which points out to relatively frequent rearrangements within and between *Galileo* elements. Different mechanisms such as recombination and gene conversion might be responsible of these rearrangements and sequence dynamism.

Conclusions: Although long TIR may affect transposition efficiency, long TIR could harbour secondary binding sites that would be helpful for transpososome assembly. Furthermore, long TIR are more prone to recombine and to be affected by gene conversion, which provides TIR sequence and structure dynamism that could be crucial for transposon evolution.

Keywords : binding site, transposase, long TIR, evolution, DNA transposon

EV17

Sugarcane LTR-RT retrotranspons; lineages, structure, age and genomic localisation

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Introduction
Long Terminal Repeat retrotransposons (LTR-RTs) are the single largest component of most plant genomes and can substantially impact the genome in many ways. Sixty complete LTR-RTs were extracted from sugarcane BACs and classified into 35 families within four *Copia* and three *Gypsy* lineages. Structurally, within lineages elements were similar, but between lineages there were large size differences. Differences in size were chiefly due to differences in LTR size, and the presence and size of spacer regions between the internal coding domain and the LTRs.
Chromosomal distribution of LTR-RTs in sugarcane
Two *Gypsy* and two *Copia* elements were localised to metaphase chromosomes using fluorescence *in situ* hybridation (FISH). One of the *Gypsy* and one of the *Copia* elements had 'classic' distributions, the *Gypsy* element concentrated in heterochromatic regions, the *Copia* in euchromatic regions. The other 2 elements (*scTat* and *scAle*) had patterns of clustered localisation. We compared the distribution of related elements in sorghum and rice by creating *in silico* heat maps. The *Tat* and *Ale* lineages in sorghum and rice did not have clusters of localisation like that seen in sugarcane. Modern sugarcane cultivars have polyploid, large complex genomes, with highly unequal contributions from ancestral genomes. The clusters of the localisation of *Tat* and *Ale* elements in sugarcane may be the outcome of larger copy numbers of these elements in one parental type or preferential loss from one parental genome.
Estimated age of insertion for sugarcane LTR-RTs
We also estimated the time of insertion for all 60 LTR-RT sugarcane elements and for related elements in sorghum and rice. Our estimates indicate most of the LTR-RT elements are two million years old in rice, sorghum as well as in sugarcane. This is consistent with previous estimates for rice and sorghum and other grasses and indicates a similar high turnover of most LTR-RTs in the modern hybrid sugarcane genome, independant of recent hybrid events in modern sugarcane cultivars.
Conclusions
In summary, in general, sugarcane LTR-RTs have similar structures, chromosomal distributions and high turnover rates to LTR-RTs from other grass genomes. Clusters of localisation in metaphase chromosomes for two families of LTR-RTs suggest that recent hybridisation events have impacted the genome in terms of differential contributions from ancestral genomes.

Keywords : LTR Retrotransposons, Sugarcane, FISH

EV18

On the track of horizontally transferred sequences between two closely related species *Drosophila melanogaster* and *Drosophila simulans*

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Transposable elements (TEs) are DNA sequences that can move around their host genome. Long been viewed as junk or selfish DNA, they are nowadays recognized as having a significant part in the evolution of genomes. The genome of the model species *D. melanogaster* contains around 15% of TEs, whereas its sibling species *D. simulans* genomes consists of only 5% of these sequences. However, recent studies have shown that *D. simulans* contains more TE copies than *D. melanogaster*, but that these copies are more damaged and shorter in *D. simulans* than in *D. melanogaster*. Moreover, the high degree of sequence identity of some TEs between *D. simulans* and *D. melanogaster* and the fact that *D. melanogaster* displays more active copies of these TEs can be explained by massive horizontal transfers (HT) of TEs from *D. simulans* to *D. melanogaster,* followed by bursts of transposition. In order to explore this hypothesis, we have developed a method to search for HT between these two species.

Horizontal transfers correspond to the transfer of genetic material between species. There are different ways of detecting HT events. Here we have developed a bioinformatics pipeline to detect horizontally transferred regions between two species based on the nucleotide identity between sequences. This approach consists mainly in four steps. 1) The definition of an identity threshold for each chromosomes under study. 2) The pruning of all n x n pairs of sequences identifiable between the two species in order to obtain 1 x 1 pairs of sequences. 3) The test of the identity of these pairs according to the threshold obtained in the first step. 4) The correction of the p-values obtained in the previous step for multiple testing, accounting for the spacial dependency between the pairs of sequences.

We have detected sequences likely to have been horizontally transferred between the two species that can be analyzed to identify CDS, TEs, and intergenic sequences. The coding sequences found were analyzed in order to determine if their high identity between the two species is due to particular evolutionary pressures or reflect true HT. Our approach allowed us to detect all the TEs suspected or proved to have been horizontally transferred between *D. simulans* and *D. melanogaster* in the literature, but also new ones. Globally this method, that can be applied to any pair of genomes, allows the accurate identification of horizontally transferred sequences without any *a priori* concerning their type.

Keywords : Horizontal Transfer, Transposable Elements, bioinformatics pipeline, Statistical methods, Multiple testing under dependence

EV19

Characterization of Domesticated Transposable Elements Related to the SChAT (hAT-like sugarcane) Family

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Molecular Domestication is the phenomenon in which repetitive sequences, as the transposable elements, are recruited by the host genome for the formation of new genes. These domesticated sequences play important roles as donors of functional protein domains, creators of regulation networks and promoters of genetic variability in all living organisms. The hAT transposase superfamily is defined as elements that share dimerization and zinc-finger domains with the previously described; hobo (*Drosophilla melanogaster*), Activator (*Zea mays*) and Tam3 (*Antirrhinum majus*) transposable elements. Transcriptional activity and genomic distribution of transposons related to hAT superfamily in sugarcane genome (called SChAT family) were evidenced from large EST sequencing projects and subsequent genome hybridization studies (de Jesus *et al*., 2011). Related to the SChAT family was found a transposase group (EST), which could belong to a domesticated element. These elements cluster significantly with domesticated transposases from Arabidopsis, rice and sorghum. The aim of this work is to characterize genomic versions of these putative domesticated transposases using BAC sequencing and expressions assays, in order to understand the genomic context where these elements were inserted and possibly domesticated. Were identified ten sugarcane BACs carrying a genome version of the putative domesticated transposase, which was characterized in detail and used as a query to scan other genomes. These BACs corresponds to one of the two expressed *loci* in sugar cane genome, this element showed low copy number and presence along the grasses in genome hybridization studies. Since this transposase is related to the DAYSLEEPER transposase, which is a single copy gene in Arabidopsis, this gene could have suffered duplication in the sugarcane genome, it is known that duplication events have occurred during the *Poaceae* evolution. Moreover, there were found related copies in *Sorghum bicolor*, *Oryza sativa*, *Zea mays* and *Brachypodium* genomes. Were recovered seven alleles of the *loci* and showed that this element have being fixed in this region at least since 12MYA, when occurred the division between *Zea Mays* and sorghum/sugarcane. The *in vivo* assays showed expression of the putative domesticated genes on different tissues of sugarcane and on the same tissue between different varieties of sugarcane along plant development. The expression patterns showed are similar to the ones observed for genes. These results encourage the domestication candidature of these transposase, hypothesis that could be further sustained with functional assays.
Supported by CNPq and FAPESP.

Keywords : Transposable elements, Syntenic regions, Sugarcane, Molecular Domestication

EV20

Diversity, dynamic and mobility of integrative and conjugative elements (ICE) of *Streptococcus agalactiae*

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Integrative and Conjugative Elements (ICEs), also called conjugative transposons, and related genomic islands are widespread in bacteria and play a key role in horizontal gene transfer. ICEs excise by site-specific recombination as circular intermediate, promote their own transfer by conjugation and then integrate into one replicon of the recipient cell. In addition to conjugative functions, ICEs encode adaptative functions that may cause drastic changes in the ecological and pathogenic properties of bacterial species, leading to microbial diversification and speciation.

Streptococcus agalactiae is a commensal organism and an opportunistic pathogen that causes severe invasive neonatal infections as well as mastitis in various ruminants. *In silico* analysis of eight sequenced genomes of *S. agalactiae* identified twelve putative ICEs and twenty three related elements. These eight strains harbor a different element integrated in a tRNA Lysine gene (four of them are putative ICEs). Furthermore, despite a large screening of isolates, no strain lacking an element integrated in this locus could be found.

A study of the mobility and conjugative transfer of the four ICEs integrated in the 3' end of the $tRNA^{Lys}$ gene was performed. Circular form and chromosomal empty site resulting from excision were detected only for two ICEs. In order to test the conjugative transfer by filter mating experiments, an erythromycin resistance gene was inserted in the two ICEs. Since no strain devoid of element was available, various strains harboring different elements (an ICE or a related element) were used as recipient. Transconjugants were obtained with one ICE (ICE_*515_tRNA*^{Lys}) with transfer frequencies dependent of the recipient strain.

Since the recipient cell harbors an element, the incoming ICE can integrate either in one of the recombination sites flanking the resident element leading to their accretion or in a secondary integration site. We showed that accretion occurred in the right end (*att*R site) of the resident element. The incoming element never integrated in another site although *S. agalactiae* harbor three copies of $tRNA^{Lys}$ gene.

ICE transfer to other species than *S. agalactiae* was tested but until now no transfer was observed.

We demonstrated that the ICE carried by strain 515 of *S. agalactiae* is able to transfer to other strains of *S. agalactiae* but not to other species. It can integrate in a site flanking the resident element leading to a composite genomic island. It remains to examine if this whole element can then transfer to other cells (process called mobilization).

Keywords : *Streptococcus agalactiae*, ICE, conjugative transfer, mobility

EV21

The Drosophila inversion-inducing transposon *BuT5*is an active MITE family related to the P element

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MITEs are small non-autonomous transposable elements that can reach high copy numbers and have been found in a wide range of organisms. Although some of them are related to autonomous copies of TIR transposons and are seemingly mobilized by them, others have no known autonomous partners and its transposition mechanism is uncertain. *BuT5* is an unclassified transposon discovered in *Drosophila buzzatii*, a member of the *repleta* species group, which has recently been shown to be responsible for the generation of two chromosomal inversions fixed in *D. mojavensis* and *D. uniseta*, two other species of the *repleta* group. The described *BuT5* copies are ~1-kb long, have very short TIRs (3 bp), imperfect sub-TIRs and do not harbor any significant ORF. In order to investigate the distribution and evolution of *BuT5* we carried out a bioinformatic search in > 2000 available sequenced genomes, including 20 Drosophila genomes, using *Dbuz\BuT5* as query. Positive results were only found for *D. mojavensis*. We also used PCR and Dot Blot for an experimental search with samples of 43 Drosophila species and found that *BuT5* is present in 38 out of 41 species of the *repleta* group suggesting that it was already present in the group ancestor ~16 mya. These features indicate that *BuT5* is an active non-autonomous MITE widespread within the *repleta* group. In order to search for the autonomous master element that mobilizes *BuT5*, we carried out a thorough characterization of the transposon copies found in *D. mojavensis*. We found a copy 3221-bp long that harbors a transposase-coding segment with significant similarity to the *D. bifasciata P-transposon*. This element shares the two ends with typical *BuT5* copies (90% identity over 267 bp at one end and 99% over 98 bp at the other end) but not the middle region. Two other partial copies of this element are present in the *D. mojavensis* genome. We suggest that the *Dmoj\P-element* is the master copy that promotes *BuT5* mobility. *D. mojavensis* belongs to the *Drosophila* subgenus. The *P-element* is widespread within the *Sophophora* subgenus but scarce within the *Drosophila* subgenus. Thus, this is likely to represent another example of horizontal transfer.

Keywords : MITE, Drosophila, P-element, inversions, horizontal transfer

EV22

The UPGM-VM procedure for the classification of Transposable Elements

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Many TEs identified in genomes are truncated, present frameshifts or indels, and most sequences available in databases are partial. Consequently, the analyses of this heterogeneous data set are not easy. Therefore, it is necessary to develop a procedure able to compare all TEs, to propose a classification and to perform automatic sorts, on the basis of incomplete nucleotide sequences.
The **Unweighted Pair Group Method – Variation of the Metric** (Rouault et al, 2009) was specially designed for this purpose. As UPGMA, it uses a hierarchical ascending clustering algorithm. However, there are major differences. The main one is the variation of the gap weight in the clustering process: at the beginning, a gap is considered as a true difference (weight =1), and progressively the weight vanishes. This method of metric variation allows us to mix sequences with very different lengths. Moreover, there are no multiple alignments, but only pairwise alignments. There is no arithmetic mean or consensus, but a concept from the categorization theory and the fuzzy logic: a central item and inner and outer radii. This approach allows defining an automatic sorting for identifying unknown sequences.
Is this new procedure better or worse than the classical procedures, such as Neighbor joining, Parsimony, Bayesian inference, … ? A first response is that all these methods are based on a multiple alignment of all the sequences, reduced to an identical length (the Procustes paradox) and are unable to work at the same time with complete sequences as truncated ones or MITEs. Today, if the UPGM-VM is the unique method able to deal with such heterogeneous data, it can also be compared to the classical procedures for sequences of same lengths.
What are the objective criteria able to validate the capacity of a classification procedure to reconstruct an evolutionary tree? To answer this question, we have built an artificial evolutionary tree of 32 final branches, based upon a substitution (mutation) mechanism without indels, interacting with a bifurcation process. In this context, the best method is the one able to re-built a correct tree (from a topological point of view, i.e. a tree with the right nodes and branches) including the highest number of mutations. With an original sequence of 1000 nucleotides, the UPGM-VM process is able to restore a correct tree with 205 true substitutions between two bifurcations, with 5 bifurcations. Comparisons with classical phylogenetic procedures will be presented.

Keywords : Transposable Elements, Automatic sorting, Phylogenetic analyses, Validation

Rouault JD, Casse N, Chénais B, Hua-Van A, Filée J, Capy P, 2009. Automatic classification within families of transposable elements: Application to the mariner Family. Gene 448:227-232.

EV23

Deletions in Transposable Elements

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Several mechanisms can limit the activity of Transposable Element (TE) like silencing, substitutions, and deletions. In genomes, many truncated (deletions including one or both extremities) and deleted (internal deletions) copies can be observed; these types of copies being more frequent than full-length copies. In the present work, we only focus on internal deletions, with the underlying question: do internal deletions occur at random?
Lemi (Feschotte and Mouchès, 2002) and *Emigrant* elements (Casacuberta *et al.* 1998) are two groups of TE belonging to the *Tc1-mariner-IS630* SuperFamily and to the *Lemis* Family. About 350 sequences can be found in dicotyledonous plants (*Arabidopsis*, *Medicago*, *Glycine*, *Lotus*, *Solanum*, *Lycopersicum*, *Gossypium*,...), with a very high number of internal deletions. The Family *Lemis* includes two SubFamilies, with 2 and 10 Tribes respectively. The Tribe *Papilonis* is defined by a set of 196 sequences, mainly found in *Glycine max*, *Lotus japonicus* and *Medicago truncatula*. In this Tribe, the reference element is 2128 bp long, with a potentially functional ORF of 541 aa. A total of 187 internal deletions can be detected in 135 sequences of this Tribe; 62 of these deletions have micro-homologies (SDRs) exactly at the breaking points (BPEE), 38 close to these points (BPNN) and 87 with a SDR exactly at one breaking point and a second SDR close to the other breaking point (BPEN). For the whole SubFamily *Lemis*, the corresponding numbers are 168 sequences with deletions with frequent SDRs at the BP.
In the *mauritiana* SubFamily, 375 sequences can be identified mainly from *Drosophila* and *Zaprionus* species. Similar observations can be done. More precisely, 12 sequences present 21 internal deletions, with 7 BPEE, 11 BPNN, and 3 BPEN confirming the previous results of Brunet *et al.* (2002). Similar feature have also been described for other elements like *Gypsy* and *Copia*.
We present the first statistics on internal deletions from a large set of data. This includes the distribution of the lengths of the sequences, the list of SDRs and the distribution of their lengths. We also compare these experimental distributions with theoretical distributions simulated with null-models (deletions at random). The mechanisms involved in the internal deletions are probably not specific to transposable elements but can be associated to the transposition, like for instance, the gap-repair mechanisms that occurs after the excision of *Class II* elements.

Keywords : *Lemi*, *mariner*, *Gypsy*, *Copia*, deletions, micro-homologies, Transposable elements

EV24

An overview of the *Tc1-mariner-IS630* SuperFamily

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The *Tc1-mariner-IS630* SuperFamily is one of the 30 SuperFamilies described by Wickers et al (2007). This SuperFamily is a set of Families previously defined like *Tc1, mariner, maT, Pogo-Fot, plant-mariner, IS630, Gambol*, … However, the definition of theses Families is somewhat fuzzy.
Today, we set up a database including about 5000 nucleotidic sequences of this SuperFamily. Some of them are complete, others are incomplete but most of them are not functional. These transposable elements (TEs) were previously described in Genbank and RepBase. They can also be found in genomes project by data mining, or experimentally identified. These TEs were found in bacteria, protists, fungi, plants, animals or viruses,
Using the UPGM-VM procedure (Rouault et al 2009) to these sequences, we are able to classify this set of data and to define Families, SubFamilies and Tribes. We confirm most of the Families and SubFamilies previously defined from phylogenetic approaches. However, some the previous groups considered as Families can be subdivided into two or more new Families. New Families can be also described. Thus today, the *Tc1-mariner-IS630* SuperFamily represents about 25 Families.
More precisely, in the *mariner* group the most common Families are: *MareNostrum* (15 SubFamilies : *Briggsae, Capitata, Cecropia, Elegans, Lineata, Mauritiana, Mellifera*, *Rosa*,…) and *Atlantis* (12 SubFamilies : *Irritans,* the sequences from marine invertebrates, the sequence *Hsmar2* and related, ...) and probably two other new ones. The *Tc1* Family is confirmed, with 14 SubFamilies, but the marginal *Tc3* and the *Impala+Crawler* groups are clearly two new Families. In the *maT* group, there are the *Matelotis* Family (11 SubFamilies), the *Ludens* Family (the *Rosa* subgroup) and the *Pomonis* Family with *TCp3.7*. The *Chlorophillis* Family (The plant-mariner group) is homogeneous, and includes at least 22 SubFamilies. The *Ant1* and *Hupfer* elements form the *Fongis* Family. The *Pogo-Fot* group is subdivided into the three Families *Fotis* (2 SubFamilies), *Pogis* and *Lemis* (2 Subfamilies with 2 and 10 tribes respectively). The *Gambol* Family is confirmed and the *ITmD37E* group becomes the *Mosquitis* Family. The *IS630* group defines at least 2 Families: *Melilitis* (ISRn sequences) and *Solfatis* (IS630, IS870, …).
Now, we are characterizing each new Family by specific traits (synapomorphies) at the protein level. In this respect, the indels observed from the aligned sequences are very useful since they seem very well conserved between groups.

Keywords : Classification, Gaps, Nucleotidic sequences, *Tc1-mariner-IS630* SuperFamily, Transposable éléments

EV25

The *mos1* transposable element specific expression pattern in *Drosophila*

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Mos1 was first discovered in 1985, in *D. mauritiana*, a sister species of *D. melanogaster*. It belongs to the large *mariner* Class II family, widespread in metazoa. *mos1* is mainly present in most of the 9 species of the *melanogaster* subgroup, with the notable exception of *D. melanogaster*. Phenotypic excision of a non-autonomous copy inserted in the *white* gene were observed in several species, both in germinal (w^+ reversion in progeny) or somatic (mosaic eye phenotype) cells, demonstrating that this is an active element.

The phenotypic excision test has been widely used for evaluating somatic activity level of *mos1* copies found in natural populations. Variations

of the activity were observed between populations and species and have raised the question of *mos1* regulation. Is *mos1* activity controlled by specific cellular processes and how populational parameters influence regulation?

Our laboratory has set up quantitative measurements (quantitative PCR and RT-qPCR) in order to estimate copy number within the genome, and transcriptional activity.

Using several natural populations of *D. simulans*, a sister species of *D. melanogaster*, we showed a correlation between the number of copies, the transcriptional level and the somatic excision level. We also observed a variation of the transcriptional level during development, with an over expression of *mos1* at the metamorphosis stage, maintained in adults. Interestingly this over expression was particularly strong in males only, suggesting a sex-specific regulation. Furthermore we could show that the differences are significantly stronger in the male genital organs, compared to the carcass. Finally, preliminary results obtained in the *D. simulans* strain Fukuaka (Japan) show a transcriptional activity decrease in old male flies. Those results could be quite interesting in the context of the origin of the multiplication of the element.

When different species of the *melanogaster* subgoup were tested, a male over expression of *mos1* was always observed but was significant only in most populations of *D. simulans,* as well as in transgenic *D. melanogaster* flies, two species characterized by a wide geographical distribution (cosmopolitan). Interestingly, in *D. simulans*, this male over expression is not significant in ancestral (African, not invasive) populations, which suggests that constraints encountered during invasion of others ecological niches may have shaped sex-specific regulation of *mos1*.

These first results will be completed by (i) a more individual-centered approach to precisely determine the cells concerned by the over-expression, (ii) by a genetical approach to evaluate the involvement of epigenetic processes/sex determination factors and (iii) a populational approach using other cosmopolitan species containing *mos1*.

EV26

From wildness to domestication : *Hsmar1* transposon in the light of evolution

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Hsmar1, a non-replicative DNA transposon from the Tc1/mariner superfamily of transposable elements, entered the primate genome lineage 50 millions years ago. The human genome now contains 200 copies of the full-length element, together with 4500 copies of a short deletion-derivative. Nearly all of these copies are now inactive but a fraction of these leftovers now encode for microRNAs. Amidst these remnants, a fusion event between a pre-existing histone methyltransferase gene and one copy of the *Hsmar1* transposase has given birth to the *SETMAR* gene. SETMAR, a member of the SUV39 family, dimethylates the histone H3 at position K36 (H3K36me2). The methylation of H3K36 is associated with active chromatin but is also implicated in alternative splicing, transcriptional repression, dosage compensation, and DNA replication, recombination, and repair. The biological role of SETMAR remains elusive, although there is some evidence for a role in non-homologous end joining (NHEJ). SETMAR has also preserved some of the activities of the Hsmar1 transposase, such as site-specific DNA binding, assembly of the pair-ends complex, and a weak nicking activity.
The region of SETMAR responsible for sequence specific binding of the transposon ends is under purifying selection and must therefore perform a function. This project aim is to investigate whether the ~ 7500 potential binding sites in the human genome provided by the *Hsmar1* remnants have a role in targeting the protein to specific sites where it might function as an epigenetic transcriptional regulator. In cultured cells either over-expressing or depleted for SETMAR, RNAseq and ChIP-Seq are used to assess the distribution of binding sites and their potential role in gene regulation. Bioinformatics analysis will determine whether changes in gene expression can be correlated with the locations of SETMAR binding sites and the potential binding sites provided by the *Hsmar1* remnants.

Keywords : DNA Transposon, Evolution, Histone Methyltransferase, Molecular Domestication

EV27

Evolution of the transposon *Galileo* in the Drosophila *willistoni* species group

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Galileo is one of the three members of the *P* superfamily of DNA transposons. It was originally discovered in *Drosophila buzzatii* where three natural segregating chromosomal inversions have been generated by ectopic recombination between *Galileo* copies. Subsequently, the finding of *Galileo* in six of the 12 sequenced *Drosophila* genomes indicated a widespread distribution within this genus. *Galileo* was found to be strikingly abundant in *D. willistoni*, a Neotropical species highly polymorphic for chromosomal inversions, suggesting a role of *Galileo* in the genome evolution of this species. Here we (1) characterized in detail all the *Galileo* copies present in the *D. willistoni* genome and (2) searched experimentally for *Galileo* copies in nine species of the *willistoni* species group. Different bioinformatic searches were carried out using as queries the nearly-complete copy of *Galileo* detected in the *D. willistoni* genome in previous work, the terminal inverted repeats (TIRs) and segments of the transposase-encoding ORF. A total of 196 copies were characterized, including 134 copies with two TIRs. Copies vary considerably in length and structure. No copies with an intact ORF encoding a functional putatively transposase were found, i.e. all characterized copies are non-autonomous. Target site duplications are 7- or 8-bp long. Two Galileo subfamilies with a substantial nucleotide divergence were observed by phylogenetic analysis of the transposase-encoding segments. The experimental searches of *Galileo* were done by PCR with two primer pairs amplifying segments 530- and 470-bp long of the transposase-encoding ORF. We investigated the following species: *D. willistoni* (three strains other that the sequenced stock), *D. tropicalis*, *D. equinoxialis*, *D. insularis* and *D. paulistorum* (four semispecies: Amazonica, Andean-Brazilian, Interior and Orinocan) of the *willistoni* subgroup, and *D. succinea*, *D. nebulosa*, *D. capricorni* and *D. fumipennis* of the *bocainensis* subgroup. *Galileo* copies were isolated from all nine *willistoni* group species. Fifty copies from seven species were cloned and sequences and a phylogenetic tree was built with the element sequences. The tree shows two highly divergent clades. Each clade contains representative species of the two subgroups and at least five species (*D. willistoni, D. tropicalis, D. paulistorum*, *D. nebulosa* and *D. capricorni*) contain representatives of the two clades. Our data show that two *Galileo* subfamilies coexist within the *willistoni* species group and vertical transmission and horizontal transfer are likely to contribute to the present element distribution.

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MT1

Synthetic Biology with a Retrotransposon - Transposition Mechanism and Transposon Tagging

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Retrotransposons are ancient components of eukaryotic genomes. They can multiply more often than their host genome by conversion of element mRNA into a DNA copy, and insertion of the new copy into the host DNA by element-encoded enzymes. However, for all known plant retroelements, transposition is rare and usually linked to stress conditions. We are using a synthetic biology approach to analyze and improve the life cycle of tobacco retrotransposon Tto1 in the model plant *Arabidopsis thaliana*. Under control of a chemically inducible promoter, we can trigger transposition in whole plants, by-passing the requirement for tissue culture stress as an activation condition (1). We are analyzing cis and trans acting sequences for their role in the retrotransposon life cycle, and use insights to improve transposition efficiency. For instance, the Tto1 ORF contains several ATGs that might serve as start codons. While all of the ensuing proteins can form virus-like particles, only the largest protein can support reverse transcription (2). Furthermore, we have analyzed the consequence of deletions in the 3´ long terminal repeat on the formation of reverse transcripts (3). We also compare different natural variants of Tto1 for their transposition efficiency, to relate natural variation with transposition competence. In the future, the element shall be adapted for expression in other plant species, to explore its potential for gene tagging in those plants where methods of genetic manipulation are not as well developed as in Arabidopsis.

1) Böhmdorfer et al., 2010, Syst Synth Biol **4**, 133-138.
2) Böhmdorfer et al., 2008, Virology **373**, 437-446.
3) Tramontano et al., 2011, Virology **412**, 75-82.

MT2

Endo III stabilizes the pseudo-Holliday junction integration intermediate of the cholera toxin phage

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Most strains of *Vibrio cholerae* are not pathogenic or cause only local outbreaks of gastroenteritis. Acquisition of the capacity to produce the cholera toxin results from a lysogenic conversion event by a filamentous bacteriophage, CTXφ. In contrast to most other lysogenic phages, such as λ phage, CTXφ does not encode its own integration machinery. Instead, two host encoded tyrosine recombinases that normally serve to resolve chromosome dimers, XerC and XerD, promote CTXφ integration by directly recombining the ssDNA genome of the phage with the dimer resolution site *dif* of either or both *V. cholerae* chromosomes. Following synapsis of the phage attachment site *att*P$^{CTX\varphi}$(+) and *dif*, one pair of strand exchange is catalyzed by XerC to form a pseudo-Holliday Junction. The integration is efficient if the pseudo-HJ is stable enough to be processed by host replication and/or DNA repair machineries.

We will show how Endonuclease III (EndoIII), a protein of the Base Excision Repair system encoded by the *nth* gene, identified in a genetic screen, is involved in the CTXφ integration. Although deletion of endogenous EndoIII reduces CTXφ integration, a catalytically inactive mutant of EndoIII still promotes integration suggesting that its DNA glycosylase and associated AP lyase activity are not required. Recombination assays *in vitro* indicate that EndoIII stimulates integration of the phage by stabilizing the pseudo-Holliday junction intermediate. This is the first evidence for a non-catalytic function of EndoIII.

MT3

Co-translational control of transposition : a mechanism for transient protein activation

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An intriguing observation shows that the frequency of transposition of IS*911* is much higher if the proteins are expressed spatially near to the ends (in "*cis*"). This occurs either if the transposase gene are located between the ends in a natural configuration, or if they are expressed simply on the same plasmid carrying the ends and artificial IS*911* derivative. On the contrary, expression of proteins from another replicon (in "*trans*") results in an important decrease of transposition efficiency (a factor of about 240 depending on constructions), even if the proteins are expressed from strong promoters. A second observation showed that the full length transposase OrfAB was almost unable to bind *in vitro* to IS*911* ends, whereas truncated forms including the first 149 amino-acids (of 382 for OrfAB) binds efficiently.

To explain these observations, we propose that the C-terminal end of OrfAB inhibits sequence-specific binding to the IS*911* ends by the N-terminal domain containing DNA recognition motives (HTH domain and "M" domain implicated in specific recognition of ends), possibly by sterically masking it. We propose that the N-ter domain of OrfAB is able to fold correctly before translation of the C-ter domain has taken place. We also shown that this nascent protein is capable of binding the IS end(s) before the synthesis of the C-ter domain. If the transposase has not bound during this window of opportunity, the C-ter would inhibit the interaction of the N-ter to the end. Therefore, this explain the strong decrease of transposase activity of the protein and the absence of efficient binding *in vitro*, equivalent to a "trans" condition. This mechanism would also imply that once transposase has accomplished its task, its reutilisation in a second transposition event would be quite inefficient. We discuss the fact that this new mechanism of structural auto-inhibition intended to prevent damages to the host by the presence of constantly active transposase, which could induce a "chain reaction" due to the replicative mode of IS*911* transposition.

MT4

A real-time monitoring by quartz crystal microbalance and an AFM visualization of the interaction between *Mos1* transposase and dsDNA

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Mos1 is a member of the widespread *mariner* / Tc1 super-family of eukaryotic transposable elements. The *Mos1* transposase (MOS1) is a 345-aa protein that contains two functional domains. The N-terminal domain promotes the assembly of MOS1 dimers and their subsequent binding to *Mos1* inverted terminal repeats (ITR). The C-terminal domain exerts the catalytic functions of DNA strand cleavage and transfer. The cut-and-paste transposition mechanism of *mariner* elements does not appear to require additional cellular host factors since it occurs *in vitro* with the purified enzyme.
With an aim of studying the particular interaction between simple or double DNA strands with proteins, we had developed a specific surface to fix DNA on conducting base surfaces. With this type of modification, we had fixed a dsDNA (2100 bp) which contained specific ITRs of the *Mos1* transposase on a quartz crystal microbalance (QCM). The QCM is a piezoelectric resonator that measures the variation of the frequency at the surface, applicable to a variation of mass by the Sauerbrey equation. To the dsDNA fixed on the quartz crystal, if we inject a protein able to interact with it, then a response in frequency (and so in mass) corresponding to the fixation of the protein with dsDNA will be observed. In literature, we can note the use of QCM that measure the activity step by step of a KF DNA polymerase during the construction of a complete dsDNA. In this consideration, we had studied the interactions between a Mos1 transposase with its specific ITRs by a report of the variation of mass during the time of the reaction. The capacity of the QCM to record most points in a few times (one point every the 0.1 s), permits to monitor the initial rate of the reaction and reported it for different concentrations in starting protein (with all the time, the same concentration in fixed ITRs). Different applications of mathematical calculations operate on the mass variation enable to obtain the kinetics rates, constants of the association and dissociation of the MOS1 protein with its ITRs. To complete the kinetic study of the interaction, a surface modification was operated on a low roughness surface to fix dsDNA. This surface was used to visualize by Atomic Force Microscopy (AFM) after we applied a MOS1 protein solution. We had observed with this technique the formation of the SEC2 complex confirmed the parallel QCM kinetic study.

MT5

Functional study of *mariner*-like elements (MLE) from marine organisms

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Our laboratory is studying *mariner*-like elements MLE in different marine organisms, including microalgae, coastal and hydrothermal invertebrates (especially crabs and annelids). Beside phylogenetic study of these DNA transposons, the present work aims to look at the functionality of these MLEs which are different from those derived from terrestrial animals. Two full length sequences from two crabs were characterized previously: *i.e. bytmar* from the hydrothermal crab *Bythograea thermydron* (Halaimia-Toumi *et al.* 2004) and *pacmar* from the costal crab *Pachygrapsus marmoratus* (Bui *et al.* 2007). Transposition assay was performed for each transposase using the human HeLa cell line and specific donor and helper plasmids. The donor plasmid derived from the pHsmar1RA-Neo (Miskey *et al.* 2007) and contains the neomycin resistance marker flanked by MLE's ITR and UTR. The helper plasmid is based on the pCMV expression vector and produces the transposase. After transfection, cleavage sites were determined by linker mediated PCR and cloned before sequencing. Sequencing results showed imperfect excision sites with variable cleavage position (for example with pPacmmar 1 donor plasmid, the cleavage sites oscillate from +17 to -113 for the 5' end and +58 to +182 for the 3' end). To conclude, first results obtained for MLE from *B. thermydron* and *P. marmoratus* seem to present an illegitimate cut with a deviation in direction of the 3' end. However the transposase of these MLE present some differences for their cleavage sites. Beside the analysis of the cleavage sites, the insertion sites in the genome are currently analyzed. Finally this approach will be extended to other MLE from marine organisms, including annelids and microalgae, in order to make a comparison with already know MLE, and, hopefully, to find new active elements.

MT6

snRNA pseudogene formation mediated by LINE retrotransposons

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More than 45% of the human genome is made of transposable elements, grouping DNA transposons and retrotransposons. Long Interspersed Nuclear Element-1 (LINE-1 or L1), a retrotransposon, is the only remaining active autonomous mobile element of our genome. Since its discovery, in early eighties, the possibility that L1 contributed to the mobilization and amplification of cellular RNA was proposed. In the last decade, experimental demonstrations were made for many transcripts such as SINE (Short Interspersed Nuclear Element), like Alu, messenger RNA or small non coding RNA. Interestingly, the mobilization of small nuclear RNA revealed distinct pathway by which they could be recruited during retrotransposition; template choice or template switching. Here, by analyzing the sequence structure of genomic snRNA copies of the human genome, we observed four distinct groups of sequences. Three of them derive from L1 mediated amplification, and could reflect distinct mechanisms of RNA recruitment by the L1 machinery during the retrotransposition process. Our models propose that cellular RNA can be recruited by L1 retrotransposition complexes in the nucleus.
We also used the U6 snRNA to compare LINE-1 retrotransposition dynamics in all sequenced mammalian genomes (36 genomes). As expected, retrotransposition activity varies between genomes and could reflect L1 divergence after speciation or host-transposon interaction variability. Finally, by comparing vertebrate genomes, we were able to identify LINE elements, outside the L1 clade, that share similar properties in term of cellular RNA recruitment pathways to form processed pseudogenes.

Keywords : retrotransposition, LINE-1, pseudogenes, mammals

MT7

Is IS*608* targeted to host replication forks by direct transposase interaction?

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Insertion sequences (IS) are small ubiquitous transposable elements which impact on genome stability and evolution. IS*608*, a 1787 bp IS isolated from *Helicobacter pylori* but functional in *Escherichia coli*, is a member of a widely distributed IS family whose members transpose using an unusual mechanism. This involves obligatory single stranded DNA intermediates and tyrosine mediated chemistry. Transposition is strand specific and occurs exclusively by excision of the "top" strand of the IS in the form of a circle and integration into a single strand target 3' to a specific tetra- or penta-nucleotide sequence.

We have recently demonstrated a link between transposition and host replication: both excision and insertion of IS*608* and of a relative from *Deinococcus radiodurans*, IS*Dra2*, occur preferentially on the lagging strand template of the replication fork. In particular, we observe that IS*608* insertion occurs preferentially into the lagging strand template of highly transcribed *rrn* genes, which may constitute collision spots between replication and transcription machineries, resulting in fork arrest.

Here, we have addressed how IS*608* might be targeted to a replication fork and what host factors might be involved. We have used an *in vivo* approach with stalled replication forks on chromosome. These were obtained either by binding of the TetR/LacI repressor to an array of *tetO/lacO* operators inserted at a known location in the *E. coli* chromosome. IS*608* insertions were found to increase significantly in the vicinity of the arrays when replication was stopped by operator-bound repressor compared to conditions in which replication proceeds normally.

Using fluorescence microscopy, we have also asked whether a catalytically active mCherry-tagged TnpA derivative is targeted to these stalled forks in the absence of the IS. In this case, we observe specific co-localization of TnpA on the blocked forks, consistent with our previous data on insertion specificity. This suggests that IS*608* may be targeted to replication forks via its transposase.

We initiated an *in vitro* approach to determine the length of single-stranded DNA necessary at the target site for TnpA binding and cleavage activity in view of determining the features of the replication fork important for IS*608* activity.

In addition, we also investigated whether host proteins associated with replication forks interact and influence TnpA localization and targeting of insertions. However, Tandem Affinity Purification did not reveal any significant relevant partner, suggesting that TnpA alone is sufficient to target forks.

MT8

Mutational analysis of the conserved "linker region" of the mariner transposase yields numerous hyper-active proteins

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The Tc1-mariner-IS630 superfamily of DNA transposons is found in almost all branches of the tree of life. The mariner sub-group is particularly widespread in higher animals and plants. These elements are highly divergent, with many sharing less than 30% amino acid identity. Apart from the DDD triad of catalytic residues, the most highly conserved region is located between the DNA binding and the catalytic domains of the protein. This motif, which has the sequence WVPHEL, has become known as "the linker region." The crystal structure of the Mos1 transpososome subsequently revealed that the linker region did indeed connect the two domains of the protein, but also that it was an important part of the dimer interface.

The linker region is perfectly conserved in most of the family members, raising a question about its function, which one might expect to be important for the activity of the protein. However, contrary to expectations, alanine scanning in the insect Himar1 transposase revealed that most of the mutations in the linker region were hyperactive. This was surprising because mutations are usually detrimental, and it is rare that they enhance the activity of a protein.

The insect Himar1 transposase is difficult to work with because of a non-specific nuclease activity. We therefore investigated the function of the linker region using the human Hsmar1 transposase, which has a high fidelity reaction in vitro and in vivo. Using degenerate oligonucleotides we generated a library of mutation for each of the six amino acid residues in the linker. Each library contained all of the 19 possible changes to the respective amino acid. We screened the libraries first with a high-through-put papillation assay. The activities of interesting candidates were then quantified using a bacterial mating-out assay. We found that almost all mutations in the WV and the EL residues were hyperreactive. In contrast, none of the mutations in the PH residues improved activity and most were highly detrimental. The activity of the best mutants in the WV and EL residues was more than 20-fold up in the bacterial assay. This translated to improvements of about 6-fold in cultured human HeLa cells.

MT9

Atypic site-specific recombination in integrons

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Integrons are bacterial recombination systems constituted by genetic platforms able to capture, stockpile and rearrange gene cassettes through a site-specific recombination mechanism. They are responsible for the gathering of antibiotic resistance genes in mobile elements. Recombination catalyzed by tyrosine recombinases follows a common pathway with two consecutive strand exchanges. In integrons, since *attC* sites recombine as a folded single strand, the second exchange of the Holliday junction is presumably prevented, necessitating a resolution by an uncharacterized mechanism. By using a double strand *attC* site carried on a plasmid with each strand specifically tagged (“mismatches test”), we demonstrated that, during recombination, only one strand, the one carrying the bottom *attC* site, is exchanged. Indeed, the final product contains the entire bottom *attC* strand and its *de novo* synthesized copy, whereas the top strand of the substrate is not found in the products of the recombination. We thus demonstrated the involvement of host replicative process in gene cassette insertion.
Contrarily to the *attI* x *attC* recombination reaction, we postulate that recombination between both *attI* canonical sites (*attI* x *attI*) could follow the "classical" tyrosine-recombinase recombination with two consecutive strand exchanges. To demonstrate this, first, we will test the *attI* x *attI* recombination using the "mismatches test" described above. We expect here to see the implication of both top and bottom strands, by obtaining products having the signature mismatches corresponding to the two strands instead of exclusively those of the bottom strand, as seen for the *attI x attC* reaction.
After this first attempt to directly demonstrate the second strand cleavage and definitively exclude any role of replication, we will perform the *attI* x *attI* recombination by introducing in the chromosome both sites in direct and inverse orientations. If there is a single strand exchange, the reaction with inverted *attI* sites should be lethal, as it linearizes the chromosome, whereas the double cleavage and strand transfer should be viable and lead to the fruitful inversion of the complete *attI* fragment. This inversion will be followed by the reconstitution of a functional *dapA* gene.
We will also test the ability of that this second strand cleavage could potentially be generated either by the RecG and/or RuvABC host systems implicated in HJ resolution by testing the *attI* x *attI* recombination efficiency in the corresponding deficient strains.

Keywords : Integrons, site specific recombination, Holliday junction, semi-conservative mechanism

MT10

MRSA on the move: SCCmec element transfer by Ccr recombinases

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Staphylococcal cassette chromosome mec (SCCmec) element is, so far, the only vector identified for the *mecA* gene encoding methicillin resistance in staphylococci. Transposition is mediated by site-specific recombinases called Ccr (chromosome cassette recombinases) that belong to the large serine recombinase family. Most SCCmec elements encode two adjacent Ccr proteins, CcrA and CcrB, while some variants encode a single copy of a third allotype, CcrC. Chromosomal integration of SCCmec by Ccrs turns a broad variety of *Staphylococcus aureus* strains into MRSA (methicillin-resistant *S. aureus*), the most prevalent pathogen causing hospital infections. The molecular mechanisms underlying transposition of methicillin resistance are largely unknown, and are topics of great interest in the Rice laboratory. We wish to understand the regulation, structure and function of the Ccr proteins. Serine recombinases use a serine residue as a nucleophile to generate double-strand breaks in cognate DNA and then realign and reseal the broken ends. We have characterized the DNA-binding activity of purified Ccr proteins to their cognate DNA sites using electrophoretic mobility shift assays. Furthermore, we have studied *in vivo* recombinational activity in *E. coli* and found that the catalytic potential of CcrA and CcrB is very promiscuous, which is unusual among serine recombinases. They do not require any accessory DNA-binding sites or additional host-specific factors for the reaction. CcrA and CcrB co-expressed together in *E. coli* conduct both integrative and excessive recombination, but intriguingly they also act on many non-canonical pairs of recombination sites. Moreover, CcrB is always required, but CcrA is only required if certain sites are present. Understanding the mechanism and regulation of the Ccr proteins at the molecular level can lay the foundation to identify ways to stop the spread of the methicillin-resistance gene into new *S. aureus* strains or to trigger its excision and loss from the MRSA genome.

Keywords : site-specific recombination, methicillin resistance

MT11

Mariner Mos1 transposase inhibitors selection

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Transposase inhibitors might be useful tools to get a better understanding of *Mariner* transposition mechanism and might also provide a better way of controlling transposition in the context of transposon-tools. In our lab, a screening of compounds has been achieved against *Mos1* transposase (MOS1). This work highlighted inhibitors displaying IC_{50} around 10 mM[1,2]. To get more efficient MOS1 inhibitors, pharmacomodulations based on the model of structure/activity of highlighted molecules are achieved and pharmacomodulated molecules are tested.
In this poster, we outline our inhibitor selection strategy. Compounds designed by pharmacomodulation are first screened by a test mimicking the whole MOS1 transposition cycle. Then, the effects of inhibitory molecules are tested, using biochemical assays, for each transposition steps: ITR/transposase complexes assembly, excision, target capture and integration. The development of such assays allowed to better understand MOS1 transposition mechanism, especially the target capture step (Poster S. Renault, ICTE 2012). Our studies have demonstrated that addition of photo-activatable benzophenone increases the inhibitory activity of tested molecules. This addition allows us to covalently photo-crosslink the inhibitory molecules to MOS1 and therefore to identify MOS1 amino acids involved in the MOS1/inhibitor interactions by mass spectroscopy. This work is done in collaboration with M. Cadène lab (CNRS UPR4301, Orléans, France).

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2. Brevet CNRS/Université de Tours WO200909206.

MT12

How to capture a target : the case of the eucaryotic mariner element, *Mos1*

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The eukaryotic transposon element, Mos1, belongs to the widely represented IS630-Tc1-mariner super-family. They transpose by a cut-and paste mechanism. The transposase encoded by the element forms a dimer which will specifically first binds one ITR (inverted terminal repeats), assembles in a Paired End Complex (PEC) on the two ITR which cut first the non-transferred strand (NTS) on each side of the element, cuts the second strand, the transferred strand (TS), capture the target and integrate the transposon by strand transfer in a new target [1, 2, 3]. Recently, the crystallography of the MOS1 PIC (pre-integration complex, constituted of two cleaved ITR and two transposases) has been obtained [3]. MOS1 is a member of the widely represented family of DDD/E enzymes, which contained a catalytic domain similar to RNAseH domain, common of a lot of transposases (Tn5, Tn10, Mu) and integrases of retroviruses (HIV, PFV Prototype foamy virus) [4, 5]. Target capture and integration are common steps for transposases and integrases. Some inhibitors have been proved to cross-react with these enzymes [6] (Poster A Pflieger). Nevertheless, the target capture of eukaryotic transposases has been poorly studied. In this poster, we proposed the development of an assay for MOS1 target capture, in order to analyse biochemically the composition of the target capture complex, the timing at which the target is captured during the transposition cycle and the influence of the structure of the target on the target capture efficiency. We then proposed a model for the structure of the MOS1 target capture complex.

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MT13

Assembly and control of the XerCD-*dif* site specific recombination machine

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Tyrosine recombinases are well known to catalyse site-specific DNA recombination in bacteria, archeae and eukaryotes. In bacteria, these recombinases are extensively used for programmed integration, excision and inversion of DNA segments. XerC and XerD form together a highly conserved tyrosine recombinase devoted to recombine *dif* sites, located in the terminal domain of circular bacterial chromosome. The XerCD-*dif* recombination system is highly conserved in bacteria and is often hitch-hiked by EGMs like phages, ICEs or genomic islands. However, XerCD-*dif* function is to resolve chromosome dimers to monomers before segregation and is thus required for the faithful segregation of sister chromosomes during cell division. To do so, its activity is precisely tuned and controlled during the bacterial cell cycle. In *E. coli*, XerCD/*dif* activity is controlled at two levels: i) the direction of the recombination reaction (from dimer to monomer and not the inverse) and ii) the timing of the reaction (coupled to septation). Both controls necessitate the cell division protein FtsK. The way FtsK acts on XerCD/*dif* recombination is not completely understood but involves the control of the assembly of the nucleoprotic complex where recombination takes place as a critical step of the reaction. To understand XerCD-*dif* recombination and its FtsK-mediated control, we study the assembly of the recombination complexes on single DNA molecules. We will present the experimental setup we are using (Tethered Particule Motion, TPM) and our findings.

MT14

Crystal structure of the esterase encoded by the unusual ORF1 of the ZfL2-1 non-LTR retrotransposon

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Non-LTR retrotransposons (NLRs) are a distinct class of mobile genetic elements that have significant impact on the evolution of eukaryotic genomes. However, the molecular details and functions of the NLR-encoded proteins are only partially understood. At least four phylogenetically unrelated domain compositions have been described for NLR-encoded ORF1 proteins (ORF1ps), and it is currently unclear whether these structurally distinct proteins have similar or distinct functions in the retrotransposition cycle. Presumably, the best-studied example is the ORF1p from the human LINE-1 element. It trimerizes via an N-terminal coiled coil domain and forms stable nucleic acid complexes via its central RRM and C-terminal CTD domains. Its crystal structure revealed a highly sophisticated yet flexible architecture that is essential for retrotransposition [(1)]. In contrast, the ORF1 protein of the ZfL2-1 retrotransposon from *Danio rerio* is completely unrelated. It is characterized by a putative N-terminal coiled coil sequence and a C-terminal esterase domain with sequence similarities to extracellular lipolytic acetylhydrolases and to the receptor-destroying esterases of certain viral membrane fusion proteins.
Here, we determined a 2.5 Å crystal structure of the esterase that reveals a Flavodoxin-like fold and a highly conserved active site. In comparison to the human LINE-1 ORF1p, the biochemical characterization of the ZfL2-1 ORF1p revealed i) a multimerization of the coiled coil domain, ii) a failure of the esterase to form stable complexes with nucleic acids in size-exclusion chromatography, and iii) enzymatic activity of the esterase to selectively hydrolyze carbon esters. These results indicate that a successful retrotransposition can eventually be accomplished by different strategies. Further analyses should reveal the precise role of the various NLR-encoded ORF1ps in the retrotransposition cycle in correlation with their structural properties.

Keywords : biochemistry, crystal structure, non-LTR retrotransposon, RNA-binding

1) Khazina *et al.* (2011). Trimeric structure and flexibility of the L1ORF1 protein in human L1 retrotransposition. *Nat. Struct. Mol. Biol*. 18, 1006-1014.

MT15

Trans-mobilization of human-specific SVA retrotransposons by the LINE-1 protein machinery and the enhancing effect of SVA-encoded MAST2 sequences

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SVA elements are non-autonomous, hominid-specific non-LTR retrotransposons and distinguished by their organization as composite mobile elements. They represent the evolutionarily youngest, currently active family of human non-LTR retrotransposons, and sporadically generate disease-causing insertions. To date, eight cases of single-gene diseases were reported to be the consequence of SVA insertions. Since the~ 2700 preexisting, genomic SVA insertions are characterized by structural hallmarks of LINE-1 (L1)-mediated retrotransposition, we hypothesized that SVA elements are mobilized by the L1 protein machinery in *trans*. To test this hypothesis, we developed an SVA retrotransposition reporter assay in cell culture using three different human-specific SVA elements which were known to have proliferated efficiently since the human-chimp divergence, as retrotransposition reporter elements.

We demonstrate that members of the human-specific SVA subfamilies E and F1 are mobilized in HeLa cells only in the presence of both overexpressed L1-encoded proteins, ORF1p and ORF2p. SVA *trans*-mobilization rates exceeded pseudogene formation frequencies by 12 to 300-fold in the HeLa-HA cell line indicating that SVA RNAs are preferred substrates for L1 proteins. Acquisition of an *Alu*Sp element increased the *trans*-mobilization frequency of the SVA reporter element by ~25-fold. Deletion of $(CCCTCT)_n$ repeats and *Alu*-like region of a canonical SVA reporter element caused significant attenuation of the SVA *trans*-mobilization rate. SVA de novo insertions were predominantly full-length, occurred preferentially in G+C-rich regions, and displayed all structural hallmarks of L1-mediated retrotransposition.

Subsequently, we tackled the question if transcriptional upregulation played an additional role in the exceptional success of the SVA_{F1} subfamily encompassing at least 84 members. Applying luciferase reporter assays, we investigated, if the acquirement of the 324-bp CpG island-containing exon 1 of the MAST2 gene which is typical of human-specific SVA_{F1} subfamily members and located at their 5' ends, had any effect on transcriptional regulation of SVA elements. We found that the MAST2 sequence acts as a positive transcriptional regulator of SVA_{F1} subfamily members in HeLa and germ cells and guarantees efficient co-expression of SVA_{F1} elements with the *trans*-mobilizing L1 protein machinery Taken together, our data show that efficient *trans*-mobilization of human-specific SVA_{F1} elements beyond pseudogene formation frequency is a consequence of both the structural features of particular SVA-encoded modules and transcriptional upregulation mediated by 5'-terminal MAST2 sequences.

Keywords : SVA, LINE-1, *trans*-mobilization, human non-LTR retrotransposons

MT16

Transposases as a tool for transgenesis in microalgae

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Microalgae are unicellular algae growing in marine or freshwater ecosystems. They are an important resource for the sustainable production of a large diversity of naturally-produced valuable compounds, like polyunsaturated fatty acids (PUFAs), carotenoids, medically active molecules, etc. Algae are therefore considered as very promising cell factories. Algae cultivation and industrial production of valuable products are in development. However, some genetic modifications are needed to make microalgae more suitable for industrial exploitation.

The GIAVAP consortium (Genetic Improvement of Algae for Value Added Products) aims to adapt genetic engineering techniques to various algal strains of economic interest. For a more efficient integration of some genes of interest into microalgae genomes, we are developing a method using transposable elements. In this method, two plasmids are transfected at the same time. The first one, the helper plasmid, contains a transposase-coding gene and a promoter adapted to the target microalga species. The other one, the donor plasmid, contains a gene of interest flanked by the ITRs recognized by the transposase. This method should enable a transient expression of the transposase by the microalgae and a stable integration by the transposase of the gene of interest into the microalgal gDNA.

We have prepared two plasmids for transfection in *Phaeodactylum tricornutum*, a diatom that produces particularly interesting lipids, notably PUFAs. The transposable element used is piggyBac, because of its flexibility, high activity and large cargo capacity. The helper plasmid was constructed with the piggyBac transposase-coding sequence and a promoter from *P. tricornutum*. The donor plasmid was constructed with an antibiotic resistance gene as a selectable marker and piggyBac ITRs.

After verification of the efficiency of the method, the selectable marker will be replaced by a gene of interest, for example a gene coding for a desaturase in order to enhance the PUFA production. The method is intended to be applied to different genes of interest and different microalgae species. Several transposable elements will be tested.

Keywords : Microalgae, helper plasmid, donor plasmid, transfection, cell factories

MT17

Reconstitution of active L1 ribonucleoprotein particles *in vitro*

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L1s are the most common mobile genetic elements in the human genome and drive genome dynamics in humans. L1s use a copy-and-paste mechanism to proliferate in our genome, via an RNA intermediate and a reverse transcription step. They code for two proteins: ORF1p, an RNA binding protein with RNA chaperone activity, and the ORF2p protein, which exhibits endonuclease and reverse transcriptase activities. These proteins assemble *in cis* with their own encoding mRNA to form a stable L1 ribonucleoprotein particle (L1 RNP). The L1 RNP forms the core of the retrotransposition machinery.
Endogenous L1 RNPs are expressed at low level in most cells, limiting detailed studies of their assembly and mechanism of action. Thus the L1 retrotransposition process remains poorly understood.
Here we present an approach to produce L1 RNPs in a cell-free system and their initial characterization. This method will enable us to study the assembly of the L1 retrotransposition machinery.

Work in the laboratory of G.C. is supported by INSERM and INCa (Avenir program) and by the European Research Council (ERC Starting Grant 'Retrogenomics'). Thanks are due to Claude Philippe for DNA constructs.

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