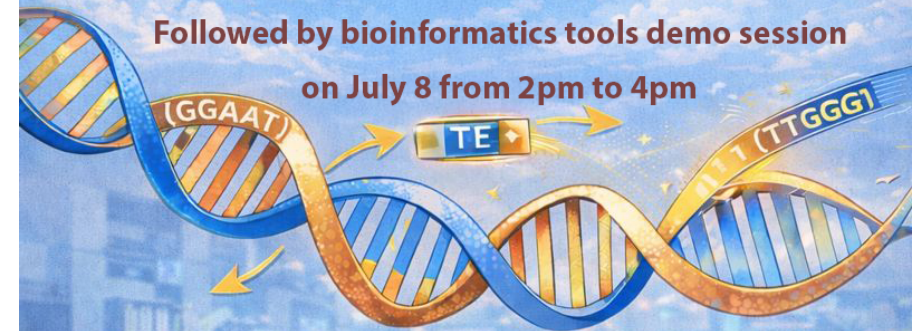


CNET 2026

26th National Congress on Transposable Elements & Repeated Sequences

From July 6th (2pm) to 8th (12am) 2026,
at Genopolys-Montpellier.

Followed by bioinformatics tools demo session
on July 8 from 2pm to 4pm



Invited speakers:

Josefa Gonzalez (IBB : Barcelona)

Didier Trono (EPFL: Lausanne)

Etienne Bucher (CGDG : Nyon)





Réseau Thématique
REPEATOME

CNET

Montpellier - Genopolys
July 6 - 8, 2026

July 6 (Monday)

13:00 - 14:00 Reception
14:00 - 14:15 Opening Remarks

14:15 - 14:55 Keynote 1 Josefa Gonzalez (Botanical Institute of Barcelona, CSIC, Barcelona)

Session 1: Functional impacts of transposable elements and repeated sequences

Chair Julie Delescluse

14:55 - 15:15 1 – PanTEon: a cross-kingdom framework to guide the design of transposable element classifiers (Simon Orozco-Arias)
15:15 - 15:35 2 – Cancer-specific LINE-1 reactivation signatures in cell-free DNA fragmentomics (Klaus von Grafenstein)
15:35 - 16:15 Coffee Break 1
16:15 - 16:35 3 – Harbor, a PIF/Harbinger MITE disseminating regulatory elements in the maize genome (Clémentine Vitte)
16:35 - 16:55 4 – Integrating Genetic and Epigenetic Information During Somatic Genome Development in *Tetrahymena* (Kazufumi Mochizuki)
16:55 – 19:00 Poster Session & Discussion

Evening with wine and cheese till 19:00
Free night

July 7 (Tuesday)

9:00 - 9:40 Keynote 2 Didier Trono (EPFL, Lausanne)

Session 2: Dynamics and Mechanisms of transposable elements and repeated sequences

Chair Tristan Maignan

9:40 - 10:00 1 – Uncovering retrotransposon-driven genome structure evolution in the moss *Physcomitrium patens* (Marc Pulido)
10:00 - 10:20 2 – Evolutionary history and climate-driven dynamics of transposable elements has shaped genome evolution in the *Coffea* genus (Romain Guyot)
10:25 - 10:35 Sponsor Presentation ONT
10:35 - 11:15 Coffee Break
11:15 - 11:35 3 – How do transposable elements integrate? Uncovering the mechanisms behind transposable element integration niche partitioning (Aina Ortiz Azorín)
11:35 - 11:55 4 – Epigenetic control and developmental timing of heritable transposable element mobilization in *Arabidopsis thaliana* (Pol Vendrell)
11:55 - 14:00 Lunch
14:00 - 15:30 Poster Session

Session 3: Regulations of transposable elements and repeated sequences

Chair Aina Ortiz Azorín

15:30 - 15:50 1 – Exploiting natural L1 retrotransposon sequence variation to identify internal features influencing their transcriptional activity (Misaki Matsuo)
15:50 - 16:10 2 – Targeted ablation of the large - LSD1 specific - CoREST isoform in *Drosophila* results in sterility and transposable elements derepression (Luisa Di Stefano)
16:10 - 16:30 3 – piRNA pathway characterization in the parasitoid wasp *Cotesia congregata* and impact on its endogenous bracovirus (Camille Heisserer)
16:30 - 17:00 Coffee Break
17:00 - 17:20 4 – Nuclear piRNA silencing couples heterochromatin formation with co-transcriptional RNA decay (Changwei Yu)
17:20 - 17:40 5 – Rad6 restricts Ty1 retrotransposon integration in *S. cerevisiae* through histone modification and post-replication repair (Nathan Lacombe)
17:40 - 18:00 6 – Unravel the Dynamics of the piRNA-Dependent Transcriptional Repression (Tristan Maignan)

Move to le Corum by tram

Dinner at le Corum from 19:00

July 8 (Wednesday)

9:00 - 9:40 Etienne Bucher (Crop Genome Dynamics Group, Agroscope, Nyon)

Session 4: Adaptive mechanisms of transposable elements and repeated sequences

Chair Ana Carolina Pires das Dores

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|---------------|---|
| 9:40 - 10:00 | 1 – Transposable Elements and Heat Stress Response in <i>Drosophila subobscura</i> (Vicente Chillida) |
| 10:00 - 10:20 | 2 – Impact of viral infections on transposition rates in <i>Drosophila melanogaster</i> (Chloé Garambois) |
| 10:20 - 11:00 | Coffee Break |
| 11:00 - 11:20 | 3 – Deciphering the interplay between transposable element control and antiviral immunity in <i>Drosophila</i> (Julie Delescluse) |
| 11:20 - 11:40 | 4 – Hypoxia activates adult neurogenesis through transposon-mediated epigenomic rewiring (Manvendra Singh) |
| 11:40 - 12:00 | 5 – Decoding endogenous retroelements contributions to heterogeneity in human immune-response (Federica Mantovani) |
| 12:00 - 12:10 | Closing remarks |

Extra Session: Bioinformatics Demo

14:00 - 16:00 Organized by Francois Sabot (IRD, Montpellier)

Session 1: Functional impacts of transposable elements and repeated sequences (July 6)

1 - PanTEon: a cross-kingdom framework to guide the design of transposable element classifiers

Simon Orozco-Arias¹, Iamíl Ferrer-Pomer², Fabiana Rodrigues de Goes³, Simon Gaviria-Orrego⁴, Juan Gómez-Fernández², Jordi Llatser-Torres², Alexandre R. Paschoal^{3, 5}, Romain Guyot⁶, Toni Gabaldón^{1, 7, 8, 9}

¹Life Science Department, Barcelona Supercomputing Center, BSC-CNS, 08032 Barcelona, Spain,

²Universitat Oberta de Catalunya, 08018 Barcelona, Spain,

³The Rosalind Franklin Institute, OX11 0QX Didcot, United Kingdom,

⁴Department of Computer Science, Universidad Autónoma de Manizales, 170001 Manizales, Colombia,

⁵Department of Computer Science (DACOM), Universidade Tecnológica Federal do Paraná (UTFPR), Campus Cornélio Procopio, 86300-000 Paraná, Brazil,

⁶UMR DIADE, IRD, CIRAD, Université de Montpellier, Montpellier, France,

⁷Institute for Research in Biomedicine (IRB Barcelona), 08028 Barcelona, Spain,

⁸Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain,

⁹CIBER de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain

Transposable elements (TEs) are fundamental drivers of genome evolution, yet their annotation and classification remain inconsistent, fragmented, and difficult to reproduce across species. This challenge arises from sequence divergence, lineage-specific innovations, and heterogeneous taxonomies across databases and computational tools, ultimately limiting large-scale comparative analyses. Here, we present PanTEon, a cross-kingdom deep learning framework designed to enable reproducible, scalable, and standardized TE classification. PanTEon integrates two key components: (i) the PanTEon Database, an automatically curated repository comprising ~240,000 structurally validated TE sequences from 2,790 species across animals, plants, and fungi, and (ii) a modular benchmarking and training platform that supports parallel evaluation and deployment of multiple ML and DL models. The PanTEon framework enables unified training, inference, and comparison across nine ML/DL architectures, while remaining fully extensible to user-defined models. Using this standardized environment, we benchmark seven state-of-the-art TE classifiers and demonstrate that classification performance is strongly influenced by taxonomic origin and TE superfamily, revealing significant biases and limitations in current approaches. Furthermore, we leverage the PanTEon training module to systematically retrain and compare nine architectures on a harmonized task involving 30 TE superfamilies. Our results show that ensemble strategies and taxon-specific models substantially improve predictive performance, while cross-kingdom generalization remains a key unresolved challenge. Additionally, we demonstrate the flexibility of the framework by addressing auxiliary tasks such as false positive detection in TE libraries. Overall, PanTEon establishes the first cross-kingdom, deep learning-driven ecosystem for TE classification, providing a robust foundation for benchmarking, model development, and large-scale genomic analyses.

2 - Cancer-specific LINE-1 reactivation signatures in cell-free DNA fragmentomics

Klaus von Grafenstein¹, Marine Gorse¹, Charline Bianchi¹, Florence Godey², Josselin Noreil³, Charlotte Proudhon¹

¹Univ Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail) - UMR_S 1085; Rennes, France,

²Centre de lutte contre le Cancer Eugène Marquis, Rennes, France.,

³Laboratoire GBCM (EA7528), Conservatoire national des arts et métiers, Paris, France

Circulating cell-free DNA (cfDNA) originates from genome fragmentation by endonucleases at the time of apoptosis during cell turnover, and is found in the bloodstream as DNA-nucleosome complexes (mainly mono-nucleosomes). In healthy context, cfDNA largely originates from hematopoietic cells, whereas in cancer patients a fraction of cfDNA derives from tumor cells; this is referred to as circulating tumor DNA (ctDNA). ctDNA carries tumor-specific alterations encompassing a broad range of molecular changes including mutations, copy number variations, epigenetic modifications and altered fragmentation profiles. The detection of those alterations in cfDNA enables non-invasive molecular profiling and longitudinal monitoring, complementing tissue biopsies and supporting precision oncology. However, detecting low ctDNA levels remains challenging. To improve ctDNA detection, we investigate and combine mutation-independent biomarkers such as cfDNA methylation and fragmentomics changes. Fragmentomics encompasses analyses of local fragment density and protection patterns across the genome, which informs on nucleosome positioning and transcriptional activity within the cell-of-origin but also fragment length distributions and end-motif composition, which were previously demonstrated to be altered in tumor-derived cfDNA. Hominoid LINE-1 retrotransposons (L1PA), constitute 4.8% of the genome and are generally silenced in healthy cells. In many cancers, including colorectal cancer, reactivation of evolutionary young L1PA occurs through DNA hypomethylation and transcriptional re-expression. We hypothesized that this reactivation could trigger specific cfDNA fragmentation patterns in cancer genome that could be exploited as a new biomarker. We developed DIAMOND-Capture, a hybridization-based targeted bisulfite sequencing method, that isolates cfDNA fragments covering L1PA genomic regions. Beyond L1PA hypomethylation and copy number alterations that we previously combined to detect 6 different types of cancers non-invasively, DIAMOND-Capture enables the analysis of fragmentomic signatures, including fragment size, end motifs and local protection patterns. In a proof-of-concept cohort (83 healthy donors and 16 colorectal cancer patients), I revealed cancer-specific reactivation signatures at full length human-specific L1 elements (L1HS) demonstrating its potential as a new biomarker for detecting cancer non-invasively.

3 - Harbor, a PIF/Harbinger MITE disseminating regulatory elements in the maize genome

Thomas Moreau¹, Klaus von Grafenstein¹, Abirami Soundiramourthy¹, Maud Fagny¹, Karine Alix¹, Johann Joets¹, Clémentine Vitte¹

¹GQE-Le Moulon

Due to their repetitive nature and their ability to contain regulatory elements, transposable elements (TEs) were early envisioned as drivers of the evolution of gene regulatory networks. First models have been recently refreshed by large-scale analyses of eukaryotic genetic regulatory landscapes, showing that TEs from various superfamilies can participate in the rewiring of different gene regulatory networks involved in key biological functions. Nevertheless, the molecular mechanisms underlying the generation of TE-driven networks have been characterized for a few cases only and remain to be investigated at a larger scale. Here, using a systems biology approach, we characterized enhancer-driven regulatory networks in two maize tissues - leaves at seedling stage and ear-covering leaves at flowering (husk) and showed that enhancers specifically activated in each tissue are enriched in different types of TEs and their target genes are enriched in different functions, thus suggesting that TEs may participate to tissue-specificity in plants (Fagny et al., 2021 - doi.org/10.3389/fgene.2020.606285). In particular, husk-specific enhancers are enriched in PIF/Harbinger MITEs, a subset of which contain a MYB-like Transcription-Factor Binding Site (TFBS) and target genes involved in late-leaf development. We therefore aimed at understanding the contribution of PIF/Harbinger MITEs to the dissemination of this MYB-like TFBS in the maize genome. We showed that only five PIF/Harbinger MITE families are enriched in this MYB-like TFBS, which all share the same structure, sequence and TSD, thus indicating that they correspond to a single family, that we named 'Harbor'. This family contains over 4600 copies and carries the MYB-like TFBS in its TIRs. Investigation of the genomic and epigenomic features of Harbor copies revealed that this family is mostly incapable of being methylated, shows high chromatin accessibility and separates euchromatin and heterochromatic regions, thus pointing to a possible role as an insulator.

4 - Integrating Genetic and Epigenetic Information During Somatic Genome Development in *Tetrahymena*

Kazufumi Mochizuki¹

¹Institute of Human Genetics (IGH), CNRS, University of Montpellier

Programmed DNA elimination (PDE) in the unicellular ciliate *Tetrahymena* is a striking example of transgenerational epigenetic inheritance. *Tetrahymena* cells contain a germline micronucleus (MIC) and a somatic macronucleus (MAC). During sexual reproduction, the parental MAC is replaced by a new MAC derived from the zygotic nucleus formed from meiotic products of the MIC. PDE occurs during MAC development, eliminating ~50% of the MIC genome, including most transposable elements.

PDE is directed by Piwi-associated ~29-nt RNAs (scnRNAs) via a genome subtraction mechanism. At the onset of sexual reproduction, scnRNAs are produced from the MIC genome and transported to the parental MAC, where those matching MAC sequences are selectively degraded. The remaining scnRNAs are then delivered to the developing MAC to guide elimination of complementary sequences.

We show that, in addition to this soma-to-soma pathway, a genetically defined mode of scnRNA production also contributes to PDE. Highly repetitive MIC-limited sequences (MLSs) at chromosome ends act as major scnRNA-producing clusters, reminiscent of animal piRNA clusters. Although MLSs comprise only ~2% of the MIC genome, they generate ~25% of scnRNAs and can directly target ~60% of eliminated sequences.

These findings support a two-layer model of scnRNA-mediated regulation: soma-to-soma epigenetic inheritance and germline-to-soma genetic control. I will discuss how these pathways shape the streamlined somatic genome in *Tetrahymena* and their evolutionary implications in relation to piRNA-mediated transposon silencing in animals.

Session 2: Dynamics and Mechanisms of transposable elements and repeated sequences (July 7)

1 - Uncovering retrotransposon-driven genome structure evolution in the moss *Physcomitrium patens*

Marc Pulido¹, Pol Vendrell-Mir², Pierre-François Perroud³, Alejandro Edera², Carles Borredá², Mame Seynabou-Fall², Fabien Nogué³, Leandro Quadrana², Josep M Casacuberta¹

¹*Centre for Research in Agricultural Genomics, CRAG (CSIC- IRTA-UAB-UB), Campus UAB, Cerdanyola del Vallès, Barcelona, Spain,*

²*Institute of Plant Sciences Paris-Saclay, Centre Nationale de la Recherche Scientifique, Institute National de la Recherche Agronomique, Université Evry, Université Paris-Saclay, Orsay, France, 3Université Paris-Saclay, INRAE, AgroParisTech, Institute Jean-Pierre Bourgin for Plant Sciences, 78000, Versailles, France*

In contrast to typical flowering plant genomes, which are characterized by gene-rich chromosomal arms, transposon-rich pericentromeric regions and centromeres mainly composed of satellite repeats, the genome of the moss *Physcomitrium patens* shows an even distribution of genes and transposons across chromosome arms. The main component of its TE fraction is a single Gypsy LTR-retrotransposon family, covering over a quarter of the genome. Also, centromeres are devoid of satellite repeats and are instead composed of a single Copia LTR-retrotransposon family. In this work, we leverage long-reads assemblies to investigate the contributions of LTR-retrotransposons to shaping the genome of 20 accessions of the genus *Physcomitrium* belonging to the two species *Physcomitrium patens* and *Physcomitrium readeri*. The comparison between species shows a remarkable conservation of genome structure in terms of number and size of chromosomes, centromere position, gene collinearity and even intergenic distances and position of TE-rich heterochromatin islands. However, despite this, an in-detail analysis of the TE content shows a complete replacement of TE sequences between species. This level of high structural conservation paired with a complete TE turnover suggests that there is an evolutionary constraint to maintain its particular genome architecture.

2 - Evolutionary history and climate-driven dynamics of transposable elements has shaped genome evolution in the *Coffea* genus

Mathilde Dupeyron, Laura Gonzalez-Garcia, Simon Orozco-Arias, Rickarlos Bezandry, Nathalie Raharimalala, Luiz Felipe Protasio Pereira, Dominique Crouzillat, Petra De Block, Coralie Fournier, Laurence Bellanger, Patrick Descombes, Perla Hamon, Douglas Silva Domingues & Romain Guyot¹

1UMR DIADE, IRD, CIRAD Université de Montpellier.

Genome size variation is a fundamental feature of plant genomes and plays an important role in phenotypic diversity, ecological adaptation, and plant evolution across angiosperms.

In the *Coffea* genus (*Rubiaceae*, 141 species/taxa), significant genome size variations have been observed. There has been nearly a twofold increase between species from East and West Africa and a notable increase from northwest to southeast Madagascar, resulting in geographic gradients. Previous studies suggest a role of Long Terminal Repeat (LTR) retrotransposons in these variations; however, the low resolution of the data to support this hypothesis did not allow for a clear understanding of LTR retrotransposons dynamics within the genus. Here, we present an analysis of the genomes of 22 *Coffea* species mainly from Africa and Madagascar and their genome size variations within a phylogenetic framework.

Our results show that genome size and Transposable Elements (TE) landscape are first structured by phylogenetic relationships, reflecting shared evolutionary history and lineage-specific LTR retrotransposon dynamics particularly involving the Tekay/Del, TAT, and SIRE lineages. These lineages contribute to the differentiation of phylogeographic groups, reflecting specific patterns of genomic divergence linked to species adaptation and speciation. We also detected significant association between specific TE families and environmental variables (such as isothermality and annual precipitation). These correlations suggest that environmental factors modulate repeatome evolution and a potential adaptive role of these TEs. These findings highlight the importance of TEs in genome dynamics at the intersection of evolutionary processes and environmental adaptations and open new perspectives on their adaptive role within the *Coffea* genus.

3 - How do transposable elements integrate? Uncovering the mechanisms behind transposable element integration niche partitioning

Aina Ortiz Azorín¹, Maëlys Lemoine¹, Bruno Mugat¹, Paul Lesbats², Mila Villatte², Delphine Lapailherie², Vincent Parissi², Alain Pélisson, Charlotte grimaud¹, Séverine Chambeyron¹,

¹Institute of Human Genetics (IGH), CNRS, University of Montpellier

²Fundamental Microbiology and Pathogenicity Lab (MFP), Mobility of pathogenic genomes and chromatin dynamics, mobilVIR team, UMR 5234 CNRS-University of Bordeaux, SBM department. Bordeaux, France.

Diverse transposable element families can coexist and transpose inside a single genome. For this to be possible, transposable elements undergo spatial and temporal niche partitioning by integrating into specific chromatin regions and during different embryonic stages. However, the mechanisms that drive these mobilization patterns are poorly understood. Previous research into the viral clade Retroviridae, a closely related taxon to class I TEs, has shown that integration specificity is mediated by the binding of co-factors to the inteasome, which is composed of a complex of four to sixteen integrases and the viral DNA. By investigating the two Long-Terminal Repeat RetroTransposable Elements (LTR-RTEs) Gtwin and gypsy, which are closely related but have different integration targets, we aim to uncover the factors that drive TE integration specificity. By using the yeast two hybrid system, we have tested the specificity of protein-protein interactions between viral integrases and their relative cofactors, and we aim to test these interactions in vivo using *Drosophila melanogaster* flies.

4 - Epigenetic control and developmental timing of heritable transposable element mobilization in *Arabidopsis thaliana*

Mireia Bueno Merino^{1, 2, 3}, Pol Vendrell Mir^{1, 2, 3}, Basile Leduque^{1, 2, 3}, Carles Borreda^{1, 2, 3}, Clotilde Descombes^{1, 2}, Elnaz Nouri^{1, 2, 4}, Céline Oury^{1, 2}, Nathalie Glab^{1, 2, 3}, Leandro Quadrana^{1, 2, 3}

¹IPS2,

²Université Paris Saclay,

³CNRS,

⁴Université Grenoble Alpes

Transposable elements (TEs) are mobile DNA sequences whose activity is tightly regulated by multiple epigenetic mechanisms, including DNA methylation and small interfering RNAs (siRNAs). While these pathways are known to repress TEs, the precise synergy between different silencing layers and the developmental windows where control is most critical for heritable mobilization remains unclear. Using *Arabidopsis thaliana* as a model, we performed a large-scale quantification of germline transposition across more than 160 TE families in various epigenetic backgrounds. By leveraging high-sensitivity sequencing capture and PCR enrichment, we resolved transposition trajectories from the whole plant to the increasingly restricted cellular niches of the gametogenic lineages. Our results reveal a hierarchical organization of epigenetic barriers, where transcriptional and post-transcriptional silencing pathways cooperate to limit mobilization upon TE reactivation. Furthermore, we show that distinct TE families exploit specific life-cycle stages to ensure transmission to the next generation. Notably, we identify cases where TE transcription and integration are uncoupled, suggesting sophisticated strategies to evade host repression during fertilization. Together, these findings provide a comprehensive map of the mechanistic and developmental factors that govern TE inheritance, highlighting how TEs exploits specific developmental transitions to ensure their evolutionary success.

Session 3: Regulations of transposable elements and repeated sequences (July 7)

1 - Exploiting natural L1 retrotransposon sequence variation to identify internal features influencing their transcriptional activity.

Misaki Matsuo^{1, 2}, Larisa Okorokova^{1, 2}, Emanuela Repetto¹, Anne Cucchiari³, Simona Saccani¹, Dominic Van Essen¹, Jean-Louis Mergny³, Gael Cristofari¹

¹University Cote d'Azur, Inserm, CNRS, Institute for Research on Cancer and Aging of Nice (IRCAN), Nice, France,

²These two authors contributed equally to this study.,

³Laboratoire d'Optique et Biosciences, Ecole Polytechnique, CNRS, Inserm, Institut Polytechnique de Paris, Palaiseau, France.

Long Interspersed Element-1 (L1) retrotransposons are mobile genetic elements that constitute 17% of the human genome and represent the only autonomously active family of retrotransposons in humans. L1 transcription is driven by an internal promoter located within its 5' UTR. This promoter exhibits both sense and antisense activities, which can give rise to chimeric transcripts with surrounding genes. Despite their abundance, only a small fraction of L1 elements is capable of expression and mobilization. L1 expression is influenced by epigenetic silencing mechanisms and integration sites. However, the extent to which variations in L1 internal sequences affect the sense and antisense promoter activities of individual copies remains unclear. To address this question, we developed nanoMPRA, a long-read nanopore-based massively parallel reporter assay, to comprehensively examine the promoter activities of approximately 7,000 recent primate-specific L1 elements (L1HS to L1PA5) in the human genome. We found that the majority of L1 5' UTRs are transcriptionally competent and that promoter activity declines with increasing evolutionary age. Interestingly, the balance between sense and antisense promoter activities varied between cell lines: in human embryonal carcinoma cells, sense activity was significantly stronger than antisense, a trend not observed in other cell lines. To identify the mechanisms underlying promoter activity, we assessed the importance of each nucleotide position to promoter activity and performed transcription factor (TF) enrichment analysis, comparing active and inactive promoter sequences. Based on these results, we identified G-quadruplexes and candidate regulatory TFs in the L1 5' UTR involved in regulating L1 promoter activity. Altogether, our results provide insights into the natural variation in retrotransposon activity and the mechanisms underlying their regulation. Work supported by ANR (ANR-15-IDEX-0001; ANR-21-CE12-0001), Fondation pour la Recherche Médicale (EQU202403018069), Région Sud (MobileGenomAI), Inserm (GOLD) and CNRS (GDR 3546). We thank IRCAN Genomics Core Facility for sequencing, the IRCAN Bioinformatics Service for computing resources, and Université Côte d'Azur's Center for High-Performance Computing (OPAL infrastructure) for resources and support.

2 - Targeted ablation of the large - LSD1 specific - CoREST isoform in Drosophila results in sterility and transposable elements derepression.

Haibin Mu¹, Denis Jullien¹, Clement Immarigeon¹, Rita Rebollo², Luisa Di Stefano¹

¹*Molecular Cellular and Developmental biology unit UMR5077, Center for Integrative Biology, CBI, University of Toulouse, France,*

²*Univ Lyon, INSA Lyon, INRAE, BF2I, UMR 203, 69621 Villeurbanne, France*

In eukaryotes, transposable element (TE) silencing relies in part on nuclear multiprotein complexes that act to change chromatin structure. Most of the machinery that modifies chromatin properties is evolutionarily conserved in metazoans, including the LSD1/CoREST complexes. Initially identified in mammals as a co-repressor of the RE1-Silencing Transcription factor (REST) in the context of neuronal gene transcriptional silencing, CoREST (also known as RCOR) has been subsequently characterized as a partner of the lysine demethylase KDM1A/LSD1. This LSD1/CoREST complex removes key transcriptionally active marks (mono & di-methyl H3K4) on the histone H3 N-terminal tail, thus contributing to gene silencing. However, CoREST's specific role in TE silencing in vivo remains to be elucidated. While mammals contain multiple copies of the LSD1 and CoREST genes, Drosophila contains a single copy of each, making Drosophila an ideal model system for studying the functional interplay between their protein products and their role in TE silencing. The single CoREST gene in flies produces four isoforms, of which only the largest one, CoREST-L, interacts biochemically with dLsd1. The team has generated a series of transgenic lines and mutants for the different CoREST isoforms in Drosophila using CRISPR/Cas9. Rescue experiments showed that only the CoREST-L isoform is essential for fertility. Analysis of flies generated via CRISPR/Cas9 to specifically prevent the expression of this isoform revealed that its absence leads to disrupted follicle cell integrity, defects in oocyte positioning, and abnormal egg chamber development, underscoring its critical role in maintaining ovarian architecture. RNAs-seq analysis showed that CoREST-L deletion leads to upregulation of muscle gene differentiation programs and upregulation of several TE families. Taken together, these data reveal an isoform-specific role for CoREST-L in fertility by ensuring proper ovary development, preventing aberrant lineage differentiation, and safeguarding the genome from TE invasion.

3 - piRNA pathway characterization in the parasitoid wasp Cotesia congregata and impact on its endogenous bracovirus

Camille Heisserer¹, Silke Jensen², Elisabeth Huguet¹, Johann Confais³, Karen Kester⁴, Emilie Brasset², Thibaut Josse¹, Jean-Michel Drezen¹

¹*Institut de Recherche sur la Biologie de l'Insecte (IRBI) UMR CNRS 7261, Université de Tours, France,*

²*Génétique, Reproduction et Développement - Institut National de la Santé et de la Recherche Médicale, Université Clermont Auvergne,*

³*Université Paris-Saclay, INRAE, BioinfOmics, URGI, 78026 Versailles, France,*

⁴*Department of Biology, Virginia Commonwealth University, Richmond, VA United States*

Braconid parasitoid wasps are associated with endogenous bracoviruses originating from the integration of a large dsDNA virus approximately 100 million years ago in the genome of an ancestor wasp. During oviposition, bracovirus particles are injected into the parasitized host, where viral genes encode virulence factors that enable wasp larvae development by suppressing host immune defenses. Previous studies reported no overexpression of immune genes during viral particle production in wasp ovaries, suggesting the virus is perceived as "self" by the wasp after 100 million years of association. However, antiviral regulation may still occur through small non-coding RNAs such as piRNAs, which were shown to mediate antiviral responses in some insects, such as mosquitoes. Here, we investigate the potential involvement of the piRNA pathway in the regulation of bracovirus activity during particle production in braconid wasps. In this study, we therefore characterized the piRNA pathway in the parasitoid wasp *Cotesia congregata* and examined whether piRNAs might contribute to bracovirus regulation. We detected piRNAs in the ovaries, testis, and head of both sexes involved in transposable elements targeting in all tissues, as indicated by ping-pong cycle signatures. In particular, a chromosome 8 short arm pericentromeric region of 850 kilobases containing piRNA-producing genomic loci was shown to concentrate over 40% of piRNA production in *C. congregata* ovaries. We also detected a piRNA response targeting bracovirus sequences. This piRNA-mediated regulation targets in particular virulence gene transcripts and could prevent the production of parasitism-related proteins that could be harmful if expressed in wasp ovaries.

4 - Nuclear piRNA silencing couples heterochromatin formation with co-transcriptional RNA decay

Changwei Yu¹, Toni Manolova², Laszlo Tirian¹, Dominik Handler¹, Ulrich Hohmann¹, Filip Nemčko³, Júlia Portell-Montserrat¹, Guillaume Giraud Collet⁴, Jakob Schnabl-Baumgartner¹, Ellie Blake⁵, Peter Duchek¹, Maria Novatchkova¹, Elisabeth Roitinger¹, Felipe Karam Teixeira⁵, Sebastian Falk², Julius Brennecke¹

¹Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Vienna BioCenter (VBC),

²Max Perutz Labs, Vienna Biocenter Campus (VBC),

³Research Institute of Molecular Pathology (IMP), Vienna BioCenter (VBC),

⁴Université Paris Cité, Magistère Européen de génétique; Paris,

⁵University of Cambridge, Department of Genetics

Transposon silencing by nuclear Argonaute proteins requires ongoing transcription for target recognition yet must prevent accumulation of transposon RNAs. We reveal that *Drosophila* Piwi resolves this paradox by integrating heterochromatin formation with co-transcriptional RNA decay. TurboID-based proximity proteomics at piRNA target sites identifies two paralogous proteins, TEsup-1 and TEsup-2, as critical cofactors for Piwi-mediated transposon silencing. Structural and biochemical analyses show that TEsup-1 directly binds the PAXT, TRAMP, and NEXT adaptor scaffolds via a conserved proline-recognition domain, thereby engaging the RNA exosome at piRNA-targeted sites. Exosome depletion causes up to 100-fold accumulation and nuclear export of piRNA-targeted transposon RNAs, without corresponding transcriptional increases. Strikingly, silencing of the P-element, a Piwi-targeted transposon that evades heterochromatin-based repression, depends on TEsup proteins and the nuclear exosome. These findings establish co-transcriptional RNA degradation as an essential arm of nuclear Piwi function, revealing how the piRNA pathway reconciles the requirement for target transcription with effective transposon suppression.

5 - Rad6 restricts Ty1 retrotransposon integration in the genome of *S. cerevisiae* through histone modification and post-replication repair

Nathan Lacombe¹, Beatrice Capuzzi¹, Pascale Lesage¹

¹Université Paris Cité, Inserm, CNRS, Institut de Recherche Saint Louis (IRSL), F-75010 Paris, France.

Transposable elements (TEs) are mobile DNA sequences capable of changing position within the genome. TEs play a critical role in genome evolution and regulation but can threaten genomic integrity if not properly controlled. The Ty1 retrotransposon of *Saccharomyces cerevisiae*, a well-studied model of LTR-retrotransposons, exhibits remarkably high integration specificity for the first three nucleosomes upstream of RNA Polymerase III (Pol III)-transcribed genes, a bias guided by the interaction between its integrase (IN1) and the AC40 subunit of Pol III [1,2]. When this interaction is disrupted or in certain chromatin mutants, Ty1 can also integrate in subtelomeres, a 20-kb region extending from the telomere to the first essential gene, or in coding regions [1,3]. The presence of hotspots in these regions suggests that additional host factors may contribute to Ty1 integration site selection beyond the IN1-AC40 interaction. RAD6 encodes an ubiquitin-conjugating enzyme involved in DNA repair, maintenance of genome stability and heterochromatin formation. Its deletion increases Ty1 insertions at both Pol II and Pol III-transcribed genes and at subtelomeres without affecting Ty1 RNA levels, indicating that Rad6 represses Ty1 retrotransposition post-transcriptionally. The altered Ty1 integration profile further shows that Rad6 may act at the integration step. By performing retrotransposition frequency and site-specific integration assays in different genetic contexts, we showed that Rad6 restricts Ty1 integration through two regulatory pathways, involving Bre1 and Rad18 ubiquitin ligases. On one side, Rad6-Bre1 represses Ty1 via H2B-K123 ubiquitination and Set1-mediated H3K4 methylation, which modulate the chromatin structure and gene expression. On the other side, Rad6-Rad18 inhibits Ty1 through the Rad5-dependent DNA damage tolerance pathway, which leads to fork regression and template switching in response to DNA damage during replication. Our findings reveal that Rad6-mediated histone modifications and DNA replication repair pathways act together to restrict Ty1 integration into the genome, which highlights a new function of Rad6 in maintaining genome integrity. 1. Bridier-Nahmias, A. et al. An RNA polymerase III subunit determines sites of retrotransposon integration. *Science* 348, 585-588 (2015). 2. Nguyen, P. Q. et al. Structural basis of Ty1 integrase tethering to RNA polymerase III for targeted retrotransposon integration. *Nat Commun* 14, 1729 (2023). 3. Asif-Laidin, A. et al. A small targeting domain in Ty1 integrase is sufficient to direct retrotransposon integration upstream of tRNA genes. *The EMBO Journal* 39, e104337 (2020).

6 - Unravel the Dynamics of the piRNA-Dependent Transcriptional Repression

Tristan Maignan¹, Florian Bondil¹, Bruno Mugat¹, Daniel Gourion², Antonio Trullo³, Mounia Lagha³, Alain Pelisson¹, Charlotte Grimaud¹, Séverine Chambeyron¹

¹*Institute of Human Genetics (IGH), UMR9002, CNRS and Univ. Montpellier, Montpellier, FRANCE*

²*University of Avignon, FRANCE*

³*Institut de Génétique Moléculaire de Montpellier, Univ Montpellier, CNRS, Montpellier, France.*

Transcriptional repression relies not only on transcription factor recruitment but also on chromatin dynamics. How chromatin-based mechanisms silence specific genomic loci remains poorly understood. Among the numerous factors that influence chromatin and transcription, PIWI-interacting RNAs (piRNAs), a class of 23-30 nucleotides small non-coding RNAs are particularly intriguing regulators. At the transcriptional level, piRNAs complexed with Piwi repress transposable elements (TEs) by recruiting several cofactors and chromatin-modifying enzymes to convert active chromatin into repressive heterochromatin. Although the core components of piRNA-mediated silencing are well known, how Piwi-piRNA targeting influences Pol II dynamics remains poorly understood. My PhD project aims to use live imaging and cutting-edge molecular tools to uncover how piRNAs and their associated cofactors, upon targeting nascent TE transcripts, influence RNA pol II transcriptional dynamics. To address these questions, we introduced a piRNA target sequence (derived from the I-element) into a MS2-tagged transcription reporter, enabling live imaging of the first steps of piRNA-mediated transcriptional repression in *Drosophila* embryos. Whereas a control reporter shows typical transcriptional bursts, the piRNA-target reporter displays oscillatory bursts interspersed with periods of inactivity. SmFISH experiments combined with Auxin-inducible degron system confirmed that maternally deposited PIWI pathway components (PIWI*) are necessary for the onset of this repression. Furthermore, using MS2/PP7 double labeled reporter constructs, we observed that piRNA targeting of nascent transcripts impairs RNA pol II transcriptional elongation. Altogether, our data suggest that interference with RNA Pol II dynamics is one of the primary steps in the establishment of piRNA-dependent transcriptional repression.

Session 4: Adaptive mechanisms of transposable elements and repeated sequences (July 8)

1 - Transposable Elements and Heat Stress Response in *Drosophila subobscura*

Vicente Chillida¹, Marta Coronado Zamora¹, Miriam Merenciano¹, Maria Pilar García Guerreiro¹

¹*Genomics, Bioinformatics and Evolutionary Biology Group, Departament de Genètica i de Microbiologia. Universitat Autònoma de Barcelona, Spain.*

Global warming is forcing insect populations to shift their geographical distributions, triggering adaptive genetic responses. While heat stress is known to alter both gene and transposable element (TE) expression, how these rapid transcriptional responses are regulated remains to be fully elucidated. Using *D. subobscura* as a climate change indicator species, we investigate sex-specific transcriptional remodeling of genes and TEs in the gonads by leveraging a high-quality de novo assembly together with RNA-seq and ChIP-seq data targeting the histone marks H3K9me3 and H3K27ac. We examine the genomic distribution of TEs near genes to evaluate their potential impact on gene expression under heat stress vs control conditions. We found that TEs harboring Heat Shock Elements (HSEs) may play a relevant role in modulating the heat stress response of nearby genes. Furthermore, our data suggest potential sex-specific differences, with TEs potentially having a greater impact on the ovarian response. Elucidating the extent to which TEs act as regulatory modulators driving the rapid deregulation of genes is of paramount importance, as it reveals the molecular engines of stress-induced plasticity. This research provides critical insights into the complex interplay between TEs and host genome regulation, enabling rapid adaptation to a changing environment.

2 - Impact of viral infections on transposition rates in *Drosophila melanogaster*.

Chloé Garambois¹, Philippe Veber¹, Julie Delescluse¹, William Vilas Boas Nunes¹, Agnès Vallier², Hélène Henri¹, Rita Rebollo², Séverine Chambeyron³, Matthieu Boulesteix^{1, 4}, Marie Fablet^{1, 4, 5}

¹Laboratoire de Biométrie et Biologie Evolutive; Université Lyon 1; CNRS; VAS; UMR 5558, Villeurbanne, France,

²Univ Lyon, INRAE, INSA-Lyon, BF2I, UMR 203, 69621 Villeurbanne, France,

³Institute of Human Genetics, UMR9002, CNRS and Université de Montpellier, Montpellier, France,

⁴equally contributed,

⁵Institut Universitaire de France

Transposable elements (TEs) are repeated and mobile portions of DNA that move along genomes, thus behaving like true molecular parasites. Their ability to transpose gives them strong mutagenic potential, which can lead to deleterious effects such as double-strand DNA breaks or the disruption of coding sequences. Most TE-induced mutations are deleterious for the host, and the emergence of epigenetic control mechanisms allows the maintenance of genome integrity against the potential negative effects of TEs. In insects, TE regulation and antiviral immunity are both mediated by RNA interference pathways. Recent results from our research group suggest that different viral infections change TE transcript levels by altering the small RNA repertoire in *Drosophila*. TE transcription is often the first step required for transposition, however, there is no strict correlation between TE transcript amounts and transposition rates. We sought to determine whether virus-induced changes in TE transcript abundance lead to altered transposition rates. To investigate this, we quantified transposition rates upon viral infection by either DCV (*Drosophila* C Virus) or IIV6 (Invertebrate Iridescent Virus 6) in a *D. melanogaster* host. Germline transposition refers to TE mobilization events in germ cells or in the zygote. These insertions are transmitted to the offspring and contribute to genome evolution. Somatic transposition refers to TE mobilization in one or a group of somatic cells. These insertions are not transmitted to the offspring and are involved in intra-individual diversity. Using Oxford Nanopore Technology for germline transposition rates, we show that viral infections increase the number of insertions passed on to the offspring, particularly for certain specific TE families. Using double-strand DNA breaks as an indirect measurement of somatic transposition rates in the guts, we show a significant positive effect of DCV. We subsequently carried out ONT sequencing on both guts and heads of flies infected with DCV or the corresponding mock condition, in order to detect which TE families change their somatic transposition rates upon viral infection.

3 - Deciphering the interplay between transposable element control and antiviral immunity in *Drosophila*

Julie Delescluse¹, Camille A. Mayeux¹, Anaïs Larue¹, Chloé Garambois¹, Zacharie Grillon¹, Agnès Vallier², Carole Monegat², Hélène Henri¹, Natacha Kremer¹, Rita Rebollo², Séverine Chambeyron³, Marie Fablet^{1, 4}

¹Laboratoire de Biométrie et Biologie Évolutive, Université Lyon 1, CNRS UMR 5558, Villeurbanne, France,

²INRAE, INSA-Lyon, BF2i, UMR 203, Villeurbanne, France,

³Institute of Human Genetics, UMR 9002, CNRS and Université de Montpellier, Montpellier, France, ⁴Institut Universitaire de France

Transposable elements (TEs) are repeated DNA sequences able to mobilize and proliferate within genomes. While they contribute to genome evolution, their activity poses a significant threat to the genome integrity. TE mobilization, referred to transposition, relies on various molecular mechanisms. Regulatory pathways have emerged in living organisms, including RNA interference (RNAi), which allow TE control, and limit the potentially harmful effects of TEs to their host. Two main RNAi pathways are involved in TE silencing, distinguished by their molecular effectors and RNA precursors : the siRNA and piRNA pathways. Furthermore, in *Drosophila*, the siRNA pathway is also involved in antiviral immunity. This close interplay between antiviral defense and TE control has led our research group to investigate the cross-talk between viral infections and TE control, and conversely, the impact of the TE load on viral infection dynamics. By using several viruses and two strains of *Drosophila* (from two different species *Drosophila melanogaster* and *Drosophila simulans*), and performing RNA-seq and small RNA-seq data analyses, our study demonstrates that viral infections modulate TE transcript amounts and small-RNA repertoires. The direction of the modulation is context-dependent, varying by the *Drosophila* host strain, viral species, and tissue analyzed. Specifically, infection with *Drosophila* C Virus (DCV) triggered derepression of the TE silencing, an effect potentially mediated by the Viral Suppressors of RNAi (VSRs) encoded by DCV. In addition, we demonstrated that *Drosophila* strain displaying high TE transcript levels have reduced DCV replication. These discoveries highlight a pivotal role for TEs in the long-term arms race between a virus and its host, and reveal potential protective antiviral effect of TEs

4 - Hypoxia activates adult neurogenesis through transposon-mediated epigenomic rewiring

Manvendra Singh^{1, 2, 3}

¹Centre national de la recherche scientifique (CNRS),

²Necker Institute Enfants Malades (INEM),

³Imagine Institute

Understanding how external signals trigger adult hippocampal neurogenesis has remained an enigma. Hypoxia-inducible factor 1 α (HIF1A) is the master regulator of the cellular response to low oxygen and, unexpectedly, triggered a robust neurogenic response. In parallel, endogenous retroviruses (ERVs), the remnants of ancient viral infections that make up ~8-10% of human and mouse genome and encode a vast repertoire of latent regulatory elements. We asked whether these two systems intersect to control adult neurogenesis. Here, we identify intermittent hypoxia as a potent extrinsic stimulus and uncover a surprising role for ERVs as key mediators of this response. We generated a single-nucleus multi-omic atlas of the adult mouse hippocampus under intermittent hypoxia by combining snATAC-seq and snRNA-seq to resolve cell-type-specific chromatin and transcriptional changes. We observed a striking ~200% increase in newly formed and immature excitatory neurons, accompanied by extensive lineage-specific chromatin remodeling and transcriptional activation. At the regulatory level, a large fraction of hypoxia-induced accessible regions mapped to transposable elements, including ERVs as well as ancient LINEs and SINEs. These elements harbor latent enhancer and promoter sequences and were highly enriched for binding motifs of key neurogenic transcription factors, including NEUROD1/2, NEUROG2, FOXG1, and ASCL1. Functionally, these transposon-derived regions were linked to genes controlling neuronal differentiation, maturation, and synaptic plasticity, suggesting that they act as regulatory hubs during neurogenesis. To extend these findings to humans, we found that HIF1A directly engages a primate-specific endogenous retroviral family, HERVH, whose regulatory sequences contain hypoxia-responsive elements. Activation of HERVH was associated with induction of neuronal differentiation programs, indicating that hypoxia-driven transposon regulation is conserved at the level of mechanism, while utilizing species-specific elements. Together, our findings reveal a previously unrecognized principle: intermittent hypoxia activates adult hippocampal neurogenesis by leveraging a transposon-derived regulatory reservoir. This work positions endogenous retroelements not as passive genomic relics, but as inducible regulatory modules that enable the adult brain to respond to environmental stress.

5 - Decoding endogenous retroelements contributions to heterogeneity in human immune-response

Federica Mantovani¹, Umut Cakir^{1, 2}, Manvendra Singh^{1, 3}

¹RETROGEN - RETROéléments et régulation du GENome INEM - Institut Necker Enfants Malades U1151 INSERM Faculté de médecine Necker - Université Paris Cité,

²Max Planck Institute for multidisciplinary sciences - Gottingen, Germany,

³IMAGINE Institute - Paris 75015, France

Buried within our DNA are the remnants of ancient viral infections - also known as Endogenous retroelements (EREs) - which continue to modulate several aspects of host physiology, including the human immune response through both cis- and trans-regulatory activities. Yet, how EREs regulate the immune response, and why it varies between individuals and cell types remains an enigma. To address this, we combined multiple layers of publicly available genomic data. Using ATAC-seq data, we identified specific ERE families that became more accessible upon stimulation across different immune cell types. We then layered publicly available ChIP-seq and RNA-seq datasets to investigate how key transcription factors HIF-1 α , a master regulator of the cellular response to low oxygen, and NF- κ B, a central player in inflammation interacts with these elements across five major cell types T-, B-, Macrophages, Monocytes and Dendritic cells. Our analysis uncovered a striking pattern over two families of EREs bound by HIF-1 α and NF- κ B, respectively. This binding activates their target ERVs and consequently, their neighbour genes. Among many, one intriguing example is the regulation of APOBEC3G expression - an antiviral enzyme known to restrict HIV replication. More intriguingly, this regulatory network appears especially robust in the so-called "elite controllers" - individuals that can naturally suppress HIV infection and associated with their lower viral load. Our findings reveal an unexpected functional link between hypoxia signalling and ERE-mediated regulation of immune genes, suggesting that these EREs may function as oxygen-sensitive switches to fine-tune our antiviral defences. To move from correlations to causations, we are now employing CRISPR-based perturbation to selectively disrupt ERE loci to evaluate their regulatory impact on nearby gene expression under stimulated versus unstimulated conditions, as well as normoxic and hypoxic environments. Overall, we show the transcriptional activity of ERVs may be regulated by the interplay between HIF-1 α and NF- κ B, which in turn promotes cellular resistance to the viral infection and varies between sexes, age and even individuals.

Posters

1 - How does GAG interact with its own mRNA in the Ty1 context ?

Pauline Magré¹, Chantal Pichon¹, Jérémie Buratto¹

¹*bYoRNA*

The interaction between the GAG protein of the *Saccharomyces cerevisiae* Ty1 and its own mRNA is a critical step in the retrotransposon cycle. We characterized this interaction using Microscale Thermophoresis (MST) and fluorescence microscopy to evaluate the binding affinity of various GAG protein truncations, based on established structural literature. To enhance the relevance of our assays, we employed an optimized gag mRNA sequence designed to maximize complementarity with the native GAG protein. We hypothesized that the binding mechanism is primarily driven by π - π stacking interactions between aromatic residues in the GAG protein and nucleobases within the mRNA. To validate this hypothesis and assess the contribution of aromatic residues to binding affinity, we performed site-directed mutagenesis followed by recombinant expression, purification, and quantitative affinity measurements of the resulting mutants. Our study will allow us to create computational docking models to elucidate the molecular determinants of the Ty1 GAG-mRNA interaction, providing a foundation for further mechanistic understanding of retrotransposon regulation.

2 - A PHD zinc finger protein promotes Spt6 SUMOylation and long non-coding RNA transcription during DNA elimination in Tetrahymena

Julie Saksook¹, Tomoko Noto¹, Kazufumi Mochizuki¹

¹*Institute of Human Genetics (IGH), Univ Montpellier, CNRS, Montpellier, France*

In the ciliated protozoa Tetrahymena, small RNA-directed DNA elimination depends on the transcription of long non-coding RNAs (lncRNAs) from the parental macronucleus (MAC), which facilitates target-directed small RNA degradation (TDSD). Previously, we demonstrated that the SUMO E3 ligase Ema2 promotes lncRNA transcription and the SUMOylation of the transcription regulator Spt6. In this study, we used LOV-TurboID, a light-activated proximity labeling technology, and identified Ema3 (TTHERM_00071060), a PHD-zinc-knuckle-PHD (PZP) domain protein, as a novel factor that interacts with Spt6 in an Ema2-dependent manner. Functional analysis revealed that Ema3 is essential for TDSD, the accumulation of lncRNAs in the MAC, and the completion of programmed DNA elimination. Notably, Ema3 facilitates the SUMOylation of Spt6, suggesting a cooperative role of Ema2 and Ema3 in promoting transcriptional events required for small RNA-genome communication. These findings establish LOV-TurboID as a powerful tool for mapping protein interactions in Tetrahymena and uncover Ema3 as a crucial effector in the Ema2-dependent SUMOylation pathway that links Spt6 activity to small RNA-mediated genome surveillance.

3 - Assessing the contribution of viruses to horizontal DNA transfer in insects

Annabelle Tiret¹, Clément Gilbert¹, Julien Varaldi²

¹*Université Paris-Saclay, CNRS, IRD, UMR Evolution, Génomes, Comportement et Ecologie, Gif-sur-Yvette 91198, France,*

²*Université Claude Bernard Lyon 1, LBBE, UMR 5558, CNRS, VetAgro Sup, Villeurbanne 69622, France*

Horizontal transfer (HT) of DNA is increasingly recognized as a potent source of genomic variation in eukaryotes. However, the factors shaping rates of HT, as well as the mechanisms and vectors underlying these transfers remain largely unknown. My PhD project aims to explore the extent to which various factors may drive HT, and to assess whether viruses can act as vector of HT. I will focus on HT of transposable elements (TEs), which are more frequent than HT of genes, and I will study insects, in which HT of TEs are rampant. First, I will quantify HT of TEs among 58 insect species that have been collected in 7 localities around the world and I will test whether rates of HT correlate with geographical distance, sympatry, and content in endogenous viruses. Second, I will use several large double-stranded DNA viruses to infect moth and drosophila species and I will monitor the frequency of TE insertion in viral genomes throughout infection cycles. The aim here will be to assess what forces act on the fate of TE insertions in viral genomes, as well as to test whether TEs can jump from one virus to another and from a virus to its host genome. Altogether, my work will shed light on the molecular mechanisms and ecological factors shaping HT of TEs in insects.

4 - Locus Pocus! Uncovering copy-level somatic TE expression through population genomics approaches

Louis Barolle¹, Mickael POIDEVIN², katarzyna Siudeja³

¹PhD student graduate school Paris Saclay University

²CNRS/I2BC

³INSERM/CNRS/I2BC

Transposable elements (TEs) make up a large part of eukaryotic genomes, but only some copies are expressed in somatic tissues. Why these copies are active while many similar ones remain silent is still not well understood. Here, somatic TE expression was studied at copy level in natural *Drosophila melanogaster* genomes. Four fully assembled and TE-annotated genomes were combined with matched RNA-seq and histone ChIP-seq data from adult gut and head. TE expression was quantified at locus level using probabilistic assignment of multi-mapping reads, allowing direct comparison between expressed and non-expressed copies across genomes and tissues. Somatic TE expression is highly heterogeneous, even within the same TE family, and most expressed copies correspond to polymorphic or unique insertions. Internal sequence similarity alone does not explain these differences, since highly similar full-length copies can still show very different expression states. In contrast, expressed loci are more often associated with specific local chromatin environments and upstream transcription factor binding. The strongest signal comes from combinations of local features rather than from any single factor taken alone. Overall, these results support a context-driven model of somatic TE expression, where local genomic environment matters more than TE internal sequence alone. This provides a useful framework to prioritize candidate loci and test their regulation experimentally in vivo.

5 - DNA methylation links lagging strand replication to transposable element control

Manon Coulée¹, Nora Fajri¹, Antoine Pigeon¹, Nataliya Petryk¹

¹Université Paris-Saclay, CNRS, Gustave Roussy, Genome Integrity and Cancers, Villejuif, France

DNA methylation is an essential epigenetic mark that silences transposable elements (TEs) in mammalian genomes. Following DNA replication, methylation patterns must be faithfully restored, yet how the two processes are coordinated remains unclear. Here, using strand-specific, genome-wide analyses in mouse embryonic stem cells, we show that DNA methylation maintenance is coupled to the lagging-strand synthesis in TE-rich regions. Unexpectedly, although DNA methylation maintenance targets the lagging strand, this strand is more vulnerable to TE integration than the leading strand. Notably, retention of full-length LINE-1s on the lagging strand has increased over evolutionary time. Consequently, most TEs, particularly young elements, are oriented head-on relative to replication forks in the mouse genome, creating an unfavorable genomic configuration that is preferentially targeted by DNA methylation maintenance. Mechanistically, DNA methylation maintenance is coordinated with the lagging-strand replication via PCNA-PAF15 interactions, suggesting that interference between these processes may facilitate TE retention. Together, we show a mechanism of TE control during DNA replication with an unexpected evolutionary interplay in which DNA methylation may facilitate, rather than prevent, TE insertion.

6 - Impact of endosymbiosis on transposable element expression and regulation in cereal weevils

Andrew Hsiao^{1,2}, Clément Goubert³, Nicolas Parisot¹, Marie Fablet⁴, Rita Rebollo¹

¹Univ Lyon, INRAE, INSA Lyon, BF2I, UMR 203, 69621 Villeurbanne, France,

²Laboratoire de Biométrie et Biologie Evolutive, UMR 5558, Université Lyon 1, Université Lyon, Villeurbanne, France.,

³R. Ken Coit College of Pharmacy, University of Arizona, Tucson, United States of America,

⁴Laboratoire de Biométrie et Biologie Évolutive, Université Claude Bernard Lyon 1, CNRS, UMR5558, Villeurbanne, France

One of the most destructive cereal pests worldwide, *Sitophilus oryzae* has a remarkably repeat-rich genome, composed of over 72% transposable elements (TEs), many of which remain transcriptionally active. Additionally, *S. oryzae* harbors a recently acquired intracellular endosymbiont, *Sodalis pierantonius*, that is maintained within a tightly regulated developmental association. During the first week of adult life, *S. pierantonius* undergoes rapid proliferation in midgut bacteriocytes, providing amino acids for host cuticle synthesis, before being eliminated - remaining only in the ovaries for vertical transmission. Preliminary transcriptomic data show that bacterial presence is associated with the differential expression of multiple TE families, and that symbiotic and aposymbiotic weevils display distinct small RNA (sRNA) profiles, suggesting that the endosymbiont perturbs host TE regulation. This PhD investigates how endosymbiosis shapes host TE expression and regulation through three complementary work packages. WP1 characterizes differentially expressed TE families between symbiotic and aposymbiotic individuals across developmental stages using RNA-seq datasets (including dual RNA-seq and bacteriocyte microdissection data) to determine which TE families are affected by bacterial presence and how their expression profiles change across development. WP2 investigates the impact of bacterial presence on host TE regulation by examining whether the large proportion of endosymbiont-derived sRNAs in bacteriomes disrupts the host's piRNA and siRNA pathways. Using sRNA-seq datasets, we aim to identify the TE families that are differentially regulated by sRNAs in the presence or absence of the endosymbiont. Finally, WP3 extends these analyses to three related species, *S. zeamais*, *S. granarius*, and the asymbiotic *S. linearis*, spanning a gradient of TE content (54-79%) and varying symbiont loads, to assess how endosymbiosis shapes TE expression across evolutionary contexts. Together, this project will provide new insights into the impact of host-endosymbiont interactions on TE regulation, and how these processes contribute to genome dynamics and host evolution.

7 - Structure-function characterization of Gtwin endogenous retrovirus integration

Mila Villatte^{1,4}, Maelys Lemoine^{2,4}, Yury Zgadzay^{3,4}, Delphine Lapailleur^{1,4}, Bruno Mugat^{2,4}, Paul Lesbats^{1,4}, Charlotte Grimaud^{2,4}, Marc Ruff^{3,4}, Severine Chambeyron^{2,4} and Vincent Parissi^{1,4}.

¹Fundamental Microbiology and Pathogenicity Lab (MFP), Mobility of pathogenic genomes and chromatin dynamics, mobilVIR team, UMR 5234 CNRS-University of Bordeaux, SBM department. Bordeaux, France.

²Institute of Human Genetics, Non-coding RNA, epigenetics and genome stability team, UMR9002 CNRS-University of Montpellier, France.

³IGBMC (Institut de Génétique et de Biologie Moléculaire et Cellulaire), Département de Biologie Structurale intégrative, UDS, U596 INSERM, UMR7104, CNRS, Strasbourg, France,

⁴Dynamic of the interactions between viral and cellular chromatin CNRS GDR 2194 (DYNAVIR).

The human genome is under constant threat from invasion by mobile genetic elements, such as retroviruses and retrotransposons. The majority of eukaryotic genomes contain numerous transposable elements (TEs). If these elements are activated, they can relocate within the host genome or even multiply, significantly increasing the risk of harmful genetic changes. One might expect that natural selection would have eliminated these genomic intruders. Surprisingly, they are prevalent in eukaryotic genomes, suggesting a coexistence between host and parasite that can sometimes resemble a symbiotic relationship. Examining how a host genome responds when these parasites invade, including identifying where they insert themselves and the regions where they become active while maintaining host survival, represents a fundamental question in biology. Indeed, answering this question is vital for comprehending the true impact of TE insertions on the function of the host genome. Our RehosTE project seeks to elucidate the functional relationships between host genomes and endogenous retrovirus (ERV) integration, using *Drosophila* as a model system in collaboration with the Institut de Génétique Humaine (IGH) in Montpellier. Our focus is on the Gtwin ERV integrase (IN), a key viral enzyme responsible for inserting retroviral DNA into the host genome. Through structural and functional characterization, we have uncovered critical insights into Gtwin IN's integration mechanics and chromatin-binding properties. X-ray crystallography revealed that the putative catalytic domain of Gtwin IN adopts a fold characteristic of the DDE retroviral integrase family, confirming its evolutionary and functional kinship with other retroviral INs. In vitro integration assays using reconstituted nucleosomes demonstrated that Gtwin IN efficiently catalyzes the insertion of viral sequences into both naked DNA and nucleosomal templates. This allowed us to define its chromatin structure preferences and map the functional interfaces through which Gtwin IN associates with the nucleosome surface. To further probe its chromatin engagement, we employed a direct Chromosome Binding Assay (diCBA) setup in our team, which confirmed Gtwin IN's intrinsic chromatin-binding function. Preliminary proteomic analyses led us to propose Pontin, a cellular AAA+ ATPase, as a putative chromatin-targeting factor for the Gtwin intasome complex. Its role in Gtwin chromatin targeting and integration is currently under investigation. By comparing this new integration model with other retroviral systems studied in our team, we reinforce the concept that ability of interpreting chromatin cues to optimize integration. Intriguingly, our findings suggest that host cells may co-opt these viral mechanisms to regulate and balance genome invasion, ensuring a controlled integration process. ANR-24-CE12-5869-02 RehosTE "Functional Relationship between the *drosophila* Host genome and ERV integration"

8 - Evolutionary dynamics of transposable elements within the species *Coffea canephora* genome (Robusta coffee plant)

Jasmine Ryan¹, Valérie Poncet¹, Rémi Tournébeize¹, Christine Meynard², Yann Bourgeois¹

¹IRD

²INRAE

Sources of genetic and epigenetic variability, transposable elements (TEs) have played a role in genome evolution since their discovery by Barbara McClintock in 1948. To better understand the differential impact of TE families on their host's fitness, as the effects of demographic, geographical and environmental factors on the intensity of selection pressures, we studied the genome of *Coffea canephora* Pierre ex A. Froehner, a species with high genetic diversity, wide geographical distribution and occupying a wide range of environments. We tested the hypothesis that LTR retrotransposons (TEs LTR-RT) have predominantly weakly deleterious effects, with frequency and abundance negatively correlated with effective population size. This highlighted the effects of geographical barriers, notably the Congo River and the Dahomey Strait, as well as population expansion, but also that the selection acting on TEs varies according to the intrinsic properties of the different TE families.

9 - Tracking somatic endogenous retrovirus activity in vivo.

Darshika SINGH¹, Mickael Poidevin¹, Katarzyna (Kasia) SIUDEJA¹

¹I2BC Paris-Saclay

Retrotransposons are multi-copy DNA sequences that are capable of replicating within the host genome via copy-and-paste mechanisms. In the soma, retrotransposon expression and mobility are increasingly proposed to play important roles in development, adult tissue homeostasis, aging and disease. However, regulation of retrotransposon activity in somatic cells is not well understood. Till now, transcriptomic and DNA-sequencing datasets obtained from tissue extracts from diverse species allowed to reveal varying levels of retrotransposon transcripts and rare events of mobility. Yet, these approaches are costly, do not conserve the intact tissue architecture and, for most, lack single cell or sub-cellular resolution. In our recent work (Rubanova et al., 2025), using sequencing approaches, we have identified a polymorphic donor locus of an endogenous LTR retroviral element (ERV) rover that is transcribed and mobile in the intestinal tissue of *Drosophila melanogaster*. We have demonstrated that the activity of the rover donor copy is influenced by its genomic environment, through tissue-specific transcription factor binding to the sequence upstream of the locus. Here, we present our ongoing efforts towards tracking the transcriptional activity, protein expression and mobilization of the active rover-ERV in vivo. First, we used RNA FISH as well as previously developed antibody against rover Gag protein (gift from K. Senti), to detect rover-derived transcripts and protein products in intact tissues. The results obtained show different rover RNA localization and Gag protein expression in intestinal progenitor vs differentiated cell types. This suggests cell type-specific post-transcriptional regulation of rover-ERV expression in vivo. Next, we constructed a rover mobility reporter, by inserting an intron-GFP cassette, as previously used for mammalian L1 and other *Drosophila* retrotransposons. With this design, a positive fluorescent signal of the reporter is obtained only when it undergoes expression, reverse transcription and integration to a new genomic location. This tool, integrated in the fly genome, has allowed us to track rover mobility at different development stages as well as upon aging with higher resolution than the sequencing approaches and preserving the tissue context, revealing the dynamics of mobilisation events in vivo. Altogether, the presented approaches allow us to zoom into the (sub)cellular distribution of the different retrotransposon-derived products as well as events of somatic mobility, thus revealing different stages of the endogenous retrovirus life-cycle in vivo.

10 - PMD protein-DNA motif pairs: from recruitment to function

Gabin Daijoux¹, Thierry Pélissier¹, Lucas Jarry², Margaux Olivier¹, Marie-Noëlle Pouch-Pélissier¹, Charles Courtois¹, Julie Descombin², Nathalie Picault², Guillaume Moissiard², Olivier Mathieu¹

¹*Institut Génétique Reproduction & Développement,*

²*Laboratoire Génome et Développement des plantes*

The genome is the unique carrier of hereditary information and is shared by all cells of the organism. The identity and functions of these cells are defined by the precise regulation of gene expression. The principal mechanism of transcriptional repression - silencing - involved in this regulation is the Polycomb pathway, which operates through chromatin structural modifications, notably the trimethylation of lysine 27 on histone H3 (H3K27me3). Recruitment of Polycomb complexes is guided by specific DNA motifs known as Polycomb responsive elements (PREs). However, the widespread presence of PRE motifs in active genes suggests the existence of protective mechanisms safeguarding them from Polycomb-mediated silencing. Here, we show that the Plant Mobile Domain C (PMD-C) proteins MAINTENANCE OF MERISTEMS (MAIN), MAIN-LIKE 1 (MAIL1), and MAIL2 act as antisilencing factors that counteract Polycomb repression at actively transcribed genes in *Arabidopsis thaliana*. Loss-of-function mutations in MAIN, MAIL1, or MAIL2 lead to ectopic H3K27me3 deposition and transcriptional repression in a Polycomb-dependent manner. While MAIN and MAIL1 function together, MAIL2 targets a distinct subset of loci through association with specific DNA sequence motifs whose integrity is required to maintain transcriptional activity and prevent H3K27me3 accumulation. Evolutionary analyses further reveal that PMD-C domains and their cognate DNA motifs are enriched within plant DNA transposons. Importantly, in DNA hypomethylated backgrounds, a large reservoir of MAIL2-associated DNA motifs becomes accessible to MAIL2. These newly accessible motifs are predominantly embedded within DNA transposons, suggesting that DNA methylation normally restricts MAIL2 access to transposon-derived sequences and supporting the idea that this antisilencing module may have originated from, or been co-opted by, transposable elements during evolution. Together, our results reveal a novel antisilencing system, potentially derived from transposons, involving PMD-C protein-DNA motif pairs, and extend our understanding of eukaryotic gene-regulatory mechanisms.

11 - Chemical screening to identify inhibitors of human LINE-1 endonuclease activity in cancer and aging

Aurélien Doucet^{1, 2, 3, 4}, Salomé Retailleau^{1, 2, 3, 4}, Benoit Michel^{1, 3, 5}, Alain Burger^{1, 3, 5}, Gael Cristofari^{1, 2, 3, 4}

¹*CNRS*

²*Inserm*

³*Université Côte d'Azur*

⁴*IRCAN*

⁵*ICN*

LINE-1 (L1) retrotransposons are highly active and abundant transposable elements in the human genome. They replicate by target-primed reverse transcription, a process by which the target DNA is cleaved and used as primer to initiate L1 RNA reverse transcription. L1 element mobilization leads to genomic variation both in the germline or in somatic cells, such as in tumors, by insertional mutagenesis. In addition, its endonuclease (EN) and reverse transcriptase (RT) activities, encoded by the element itself and necessary for their replication, can be toxic, especially in primary cells, leading to DNA-damage and cellular senescence. Here, we aim to identify small molecule inhibitors of L1 EN activity. More specifically, we set up a fluorescent assay for EN activity using recombinant L1 EN protein and an optimized version of FRET. We have screened small-compound libraries for EN inhibitors (~10,000 compounds) and are currently validating the most promising hits. On the longer term, obtaining such an inhibitor will allow us to test whether inhibiting the very first step of L1 replication can prevent genomic instability in cancer and through tumor formation and progression. We will also determine if inhibiting L1 endonuclease activity can prevent or delay cellular senescence. In sum, our work will provide unique experimental tools to dissect the mechanisms by which L1 retrotransposons contribute to cancer and aging. Work supported by ANR (ANR-15-IDEX-0001), Cancéropole PACA (Emergence-2021-ENDOL1) and CNRS (AAP AIN INSB and GDR 3546). We thank ChemBioFrance for resources and support.

12 - Retrocopies, selection and functionality in livestock species

Carine GENET¹

¹GENPHYSE

Gene duplication is a fundamental evolutionary mechanism that generates novel genetic material for functional innovation. Among the processes that create gene copies, retrotransposition—wherein mature mRNA is reverse-transcribed and re-integrated into the genome—plays a prominent role. In mammals, the vast majority of retrotransposition events are driven by the enzymatic machinery of LINE-1 (L1) retrotransposons, producing intron-less copies that generally lack the regulatory sequences of their parental genes. Retrocopies derived from protein-coding genes are of particular interest because they can impact phenotype. In domestic livestock species, two retrocopy copy-number variants (retroCNVs) identified in sheep exemplify this effect: Both variants display strong signatures of domestication-related selection and have impact on phenotype: one influences fleece type, while the other affects horn morphology. These loci were initially discovered through classical forward-genetics approaches that connected observable phenotypes to their causal genotypes. Here, we adopt a reverse-genetics strategy. First, we prioritize retroCNVs (obtained from RCPedia database) that overlap the top 1 % of structural variants bearing selection signatures (Li et al., 2023). Next, we investigate the phenotypes associated with these candidates. To achieve this, we are currently genotyping the selected retroCNVs across a broad panel of French sheep populations, characterizing their genomic contexts, and profiling their expression in published RNASeq experiments. In parallel, we evaluate the activity of their putative promoter regions using GFP-based reporter assays, and we explore the evolutionary dynamics of the retroCNVs with ancestral recombination graph (ARG) methods. Collectively, this study aims to clarify how retroCNVs contribute to phenotypic diversification and adaptive evolution in domesticated sheep.

13- Tracking Recent LTR Retrotransposon Insertions in Oil Palm (*Elaeis* sp.) Genomes

Nikolai Paniushev¹, Norbert Billotte¹, David Lopez¹

¹ CIRAD

Oil palm (*Elaeis guineensis* and *Elaeis oleifera*) genomes are heavily populated by transposable elements (TEs), which drive genomic diversity and influence key agronomic traits. Previous studies utilizing older, short-read assemblies concluded that the African oil palm genome is transpositionally silent, characterized entirely by ancient, decaying "fossil" repeats. However, fragmented assemblies notoriously collapse young, highly identical repeat sequences, potentially masking recent transposition bursts. In this study, we leverage publicly available, reference-quality, chromosome-scale assemblies of *Elaeis* genomes, alongside with the closest relatives of genus *Elaeis*: coconut (*Cocos nucifera*), and date palm (*Phoenix dactylifera*), to explicitly search for young, intact LTR retrotransposons.

Using a targeted pipeline focused on full-length LTR detection, we isolated intact retrotransposons to evaluate the nucleotide identity between their 5' and 3' sister long terminal repeats. Because sister LTRs are identical upon insertion and diverge over time, this comparative approach acts as a high-resolution genomic clock to identify recent transposition events across these lineages. By scanning these highly contiguous assemblies, we aim to determine whether recent or active transposition waves persist in modern oil palms compared to related monocots. Ultimately, this work provides a clearer picture of recent repeat dynamics, offering valuable insights into the evolutionary stability of the *Elaeis* genome to support ongoing genomic selection and breeding initiatives.

14 - Cellular subversion of retroviral integrases chromatin binding function for optimal host invasion

Vincent Parissi¹

¹MFP, UMR5234 CNRS University of Bordeaux

Our work aims to understand the molecular and cellular determinants for chromatin invasion by retroelements. This process relies on the integration of the viral genome into host DNA by the intasome complex. Retroviral integration is profoundly influenced by the chromatin environment that determine whether the provirus will undergo efficient expression or remain latent. The selection of insertion sites is a complex, multi-faceted process that involves the successive targeting of the viral capsid and intasome by cellular factors such as CPSF6 and LEDGF/p75 in the case of lentiviruses as HIV-1. Preference for specific chromatin structures ultimately modulates the interaction between the retroviral intasome and nucleosomes, thereby serving as an additional regulatory parameter in site selection. To better define the functional interfaces engaged in chromatin integration, we require structural data regarding the HIV-1 intasome/nucleosome interaction and the nucleosomal strand transfer complex (Nuc-STC). In our previous, and ongoing, ANRS-funded project (INterfaces&StructurIN), we employed a combination of biochemical techniques and structural docking methodologies to elucidate the functional interfaces between the intasome and its nucleosomal substrate across various retroviral models, including HIV-1 and the human foamy prototype virus PFV. Our findings demonstrate how chromatin compaction affects accessibility to these interfaces and provide the first molecular insights into the regulatory role of chromatin structures in integration. Additionally, we reveal that LEDGF/p75 and histone H3K36me3 modifications contribute to the proper structuring of optimal nucleosomal templates, addressing long-standing questions regarding the role of this cellular factor during integration (Lapaillerie et al., NAR 2021, Lapaillerie et al., in revision). Our work also identified local chromatin R-loop structures generated during transcription along with the Aquarius helicase involved in their resolution, as novel determinants influencing the selection of HIV-1 integration sites (Penzo et al., Nature Microbiology 2025). Significantly, we discovered that HIV-1 integrase (IN) can recognize and bind to specific histone modifications, suggesting a novel chromatin reader function within the SH3-folded C-terminal domain (CTD) of IN. Further structure-function analyses indicate that this recognition is modulated by LEDGF/p75 both in vitro and in infected cells. Selection of IN mutations and drugs targeting this chromodomain—capable of inhibiting and redirecting integration—highlights their potential as therapeutic targets and tools for controlled gene transfer (Mauro et al., NAR 2019, Mauro et al., mBio 2023). Comparative studies with other retroelements including yeast retrotransposon and endogenous retroviruses imply that retroviral INs act as multifunctional chromatin binders, scanners, and potential "readers"—playing a critical role in chromatin invasion. Our data also reflect a co-evolutionary strategy for regulating and balancing genome invasion, ensuring a controlled integration process.