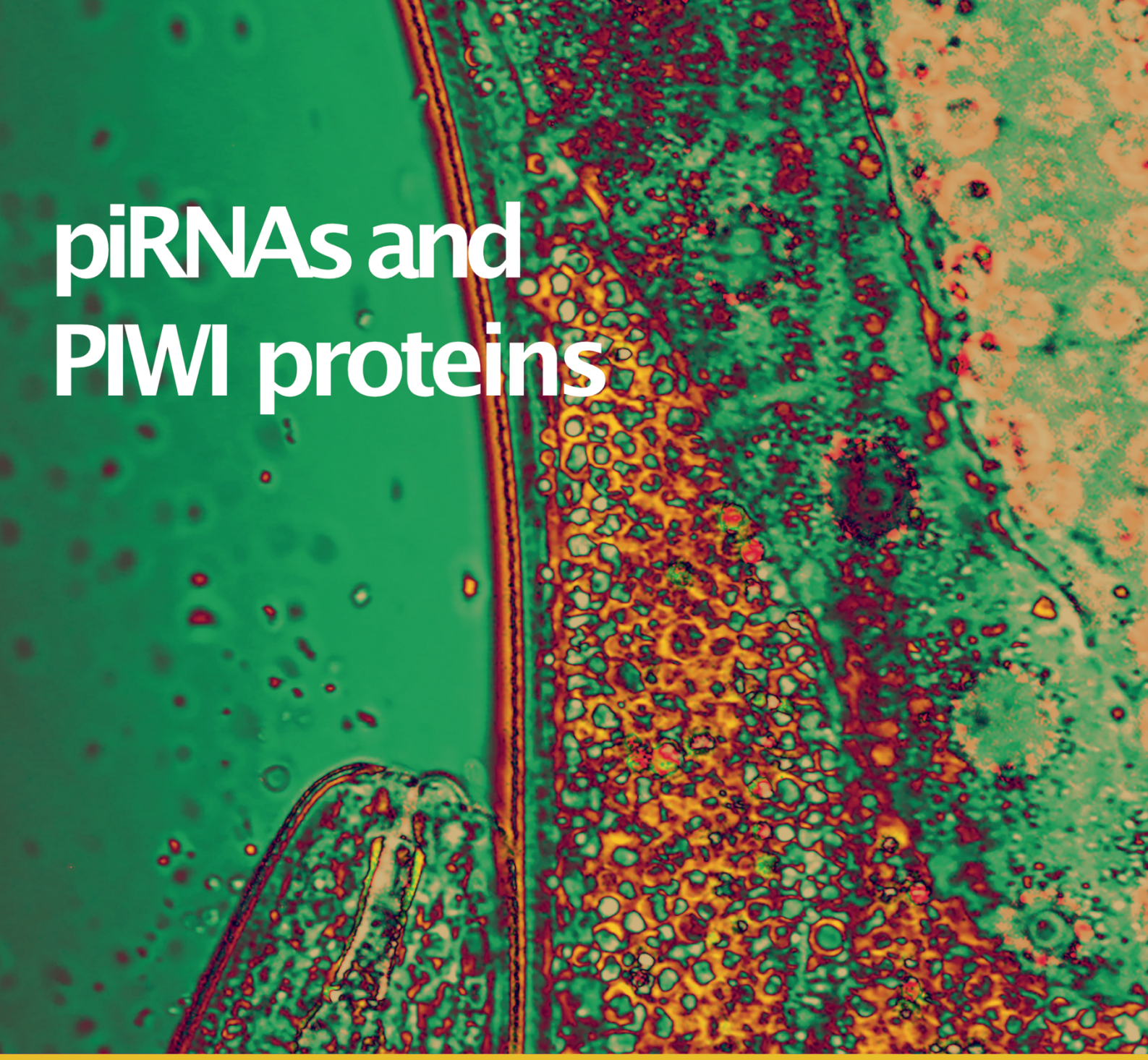

# piRNAs and PIWI proteins

**PROGRAM** 3-5 April 2024 | Montpellier (France)

ORGANIZERS

Severine Chambeyron – Julie M. Claycomb – Ramesh Pillai – Martine Simonelig

https://event.fourwaves.com/piwi2024

| Time | Program category | Speaker | Chairs |
|---|---|---|---|
| WEDNESDAY, APRIL 3 | | | |
| 12:00-14:00 | **Registration** | | |
| 14:00-14:15 | **Opening remarks** | Séverine Chambeyron, Julie Claycomb, Martine Simonelig, Ramesh Pillai | |
| 14:15-18 :30 | **Session 1** | **piRNAs in stem cells and development** | Martine Simonelig & Haifan Lin |
| 14:15-14:40 | **Talk** | Mo-Fang Liu | |
| 14:40-15:05 | **Talk** | Haifan Lin | |
| 15:05-15:30 | **Talk** | Josien Van Wolfswinkel | |
| 15:30-15:45 | **Short talk** | Malte Grewoldt | |
| 15:45-16:00 | **Short talk** | Azad Alizada | |
| 16:00-16:25 | **Coffee Break** | Networking | |
| 16:25-16:50 | **Talk** | Katalin Fejes Toth | |
| 16:50-17:05 | **Short talk** | Ian MacRae | |
| 17:05-17:20 | **Short talk** | Nicole Raad | |
| 17:20-17:35 | **Short talk** | Ansgar Zoch | |
| 17:35-18:00 | **Talk** | Mandy Jeske | |
| 18:00-18:25 | **Talk** | Heng-Chi Lee | |
| 18:30-22:00 | **Welcome reception & Dinner** | Brasserie du Corum* | |
| THURSDAY, APRIL 4 | | | |
| **8:45-10:45** | **Session 2** | **Biogenesis of piRNAs** | Clément Carré |
| 8:45-9:10 | **Talk** | Mikiko Siomi | |
| 9:10-9:35 | **Talk** | René Ketting | |
| 9:35-10:00 | **Talk** | Yukihide Tomari | |
| 10:00-10:15 | **Short talk** | Dominik Handler | |
| 10:15-10:30 | **Short talk** | Yuica Koga | |
| 10:30-10:45 | **Short talk** | Petr Svoboda | |
| 10:45-11:15 | **Coffee Break** | Networking | |
| **11:15-13 :00** | **Session 3** | **piRNAs in genome defense I** | Miao Tian |
| 11:15-11:40 | **Talk** | Severine Chambeyron | |
| 11:40-12:05 | **Talk** | William Theurkauf | |
| 12:05-12:30 | **Talk** | Emilie Brasset | |
| 12:30-12:45 | **Short talk** | Laure Teysset | |
| 12:45-13:00 | **Short talk** | Abdou Akkouche | |
| 13:00-14:00 | **Lunch** | Genopolys Hall | |
| 14:00-15:15 | **Poster session I** | Rooms Rotonde and Espace | |
| **15:15-18:10** | **Session 4** | **piRNAs in genome defense II** | Susanne Bornelöv & Jeremy Luban |
| 15:15-15:40 | **Talk** | Astrid Haase | |
| 15:40-16:05 | **Talk** | Yang Yu | |
| 16:05-16:30 | **Talk** | Nelson Lau | |
| 16:30-16:45 | **Short talk** | Marie Fablet | |
| 16:45-17:10 | **Coffee Break** | Networking | |
| 17:10-17:35 | **Talk** | Julius Brennecke | |
| 17:35-18:00 | **Talk** | Kensaku Murano | |
| 18:00-18:15 | **Short talk** | Anahi Molla-Herman | |
| 18:15-18:40 | **Talk** | Alexei Aravin | |
| 19:30-22:00 | **Dinner** | Brasserie du Corum* | |
| FRIDAY, APRIL 5 | | | |
| **9:00-10:20** | **Session 5** | **piRNA functions beyond the germline and in diseases** | Rajani Gudipatti |
| 9:00-9:25 | **Talk** | Claus Kuhn | |
| 9:25-9:50 | **Talk** | Ronald Van Rij | |
| 9:50-10:05 | **Short talk** | Michael Mitchell | |
| 10:05-10:20 | **Short talk** | Sebastian Riedelbauch | |
| 10:20-10:45 | **Coffee Break** | Networking | |
| **10:45-12:15** | **Session 6** | **RNA granules in piRNA biology** | Eric Cornes |
| 10:45-11:10 | **Talk** | Martine Simonelig | |
| 11:10-11:25 | **Short talk** | Chen Chen | |

| | | | |
|---|---|---|---|
| 11:25-11:50 | **Talk** | Julie Claycomb | |
| 11:50-12:15 | **Talk** | Carolyn Phillips | |
| 12:15-14:15 | **Lunch** | Restaurant: Table de la Lyre** | |
| 14:15-15:15 | **Poster session II** | Rooms Rotonde and Espace | |
| **15:15-16:45** | **Session 7** | **piRNAs in genome rearrangements** | Maria Rosa Garcia Silva |
| 15:15-15:40 | **Talk** | Laura Landweber | |
| 15:40-16:05 | **Talk** | Sandra Duharcourt | |
| 16:05-16:30 | **Talk** | Kazufumi Mochizuki | |
| 16:30-16:45 | **Short talk** | Margarita Angelova | |
| 16:45-17:15 | **Coffee Break** | Networking | |
| **17:15-18:35** | **Session 8** | **piRNAs in gene/transposon regulation** | Ding Degiang |
| 17:15-17:40 | **Talk** | Pei-Hsuan Wu | |
| 17:40-17:55 | **Short talk** | Eric Lai | |
| 17:55-18:10 | **Short talk** | Zuzana Loubalova | |
| 18:10-18:35 | **Talk** | Phillip Zamore | |
| | **Concluding remarks** | Séverine Chambeyron, Julie Claycomb, Martine Simonelig, Ramesh Pillai | |

SFG and RNA SOCIETY awards :
Prizes by SFG and RNA society will be awarded for the best short talks and posters.

*Brasserie du Corum : Esplanade Charles de Gaulle 🚊TRAM 1 direction « Odysseum », Stop at « Corum » station
** Restaurant Table de la Lyre : 48 rue Georges Denizot 🚶🚶

## Invited speakers

Mo-fang Liu (CN)

**HSP90α-mediated TDRD9 stabilization is essential for male fertility.**

TDRD9, as a functional partner of MIWI2 protein for piRNA-mediated retrotransposon silencing, is abundantly present in spermatogonia and spermatocytes but sharply reduced in abundance in haploid spermatids, showing a highly spatiotemporal regulation during spermatogenesis in mice; however, its metabolism has remained largely unclear. Here, we show that TDRD9 is degraded through the HERC2-mediated ubiquitin-proteasome pathway in haploid spermatids, while the spermatocyte-highly expressing chaperone protein HSP90α protects TDRD9 from HERC2 ubiquitination in spermatocytes. Interestingly, we identify a homozygous mutation (c.1037C>T, p.P346L) of TDRD9 gene in infertile men, and further discover in Tdrd9 knock-in mice that this genetic mutation impairs TDRD9 binding to HSP90α and in turn results in its advanced ubiquitination and degradation in spermatocytes, leading to male infertility in mice. These findings reveal a critical role of HSP90α in controlling the stability of TDRD9 protein during spermatogenesis, which we show is essential for male germ cell development and male fertility.

Haifan Lin (US)

**Piwi regulates the usage of alternative transcription start sites in the Drosophila ovary.**

Alternative transcription initiation, which refers to the transcription of a gene from different transcription start sites (TSSs), is prevalent in mammalian systems and has important biological functions. Although transcriptional regulation has been extensively studied, the mechanism that selects one TSS over others in a gene is still poorly understood. Using the cap-analysis gene expression sequencing (CAGE-seq) method, we identified 87 genes with altered TSS usage in the Drosophila ovary upon loss of Piwi and termed these genes as Altered TSS Usage (ATU) genes. Bioinformatic analysis revealed no differential targeting of Piwi-interacting RNAs (piRNAs) on ATU genes versus non-ATU genes, indicating this Piwi-dependent TSS usage regulation is not guided by piRNAs. RNA Polymerase II (Pol II) ChIP-seq data of germline-specific piwi-knockdown ovaries revealed that Piwi affects Pol II density and binding profile at TSSs of ATU genes. Mass spectrometry of Piwi-immunoprecipitated interactors in the nuclear fraction of fly ovaries revealed several epigenetic factors. These results indicate that Piwi might interact with epigenetic regulators to regulate TSS usage in Drosophila ovaries by affecting Pol II occupancy.

Josien Van Wolfswinkel (US)

**PIWI proteins and the eternal life of planarian stem cells.**

Katalin Fejes Toth (US)

Heng-Chi Lee (US)

**Nucleoporin complex shape perinuclear germ granule architecture and coordinate piRNA silencing.**

Mikiko Siomi (JP)

**The piRNA pathway and transposons in cultured fly ovarian somatic cells.**

In our laboratory, we are using cultured fly ovarian somatic cells (OSCs) to elucidate the molecular mechanisms of the somatic piRNA pathway. Recently, we found that a LTR-type TE *springer* escapes piRNA-mediated repression in OSCs and is inserted to host genes such as L(3)mbt, affecting the transcriptome via host-TE hybrid splicing. In the case of L(3)mbt, host-TE hybrid splicing produces a novel isoform L(3)mbt-S that lacks the N-terminus of authentic L(3)mbt, L(3)mbt-L, but retains its original function; L(3)mbt-S shows high similarity to the higher eukaryotic L(3)mbt orthologs. Thus, hybrid splicing can be viewed as a novel mode of TE-host co-option and an evolutionary driver.

René Ketting (DE)

**Functional characterisation of Alg13 in piRNA pathway in Danio rerio.**

Transposons are mobile DNA sequences that pose a particular threat to germ cells, as a germline mutation is heritable and may be detrimental to the offspring. To ensure the genome integrity for future generations, most organisms employ germline-specific small RNA-based immune machinery. It employs a subfamily of Argonaute nucleases, termed PIWI proteins that are bound by small RNAs (piRNAs). While targeting transposons that are

present in the genome, the piRNA system also needs to be able to initiate silencing of novel sequences, as transposons are known to spread also horizontally (i.e. between species). How such de novo piRNA-mediated silencing is established is largely unknown. To address this question, we established an in vivo piRNA/target reporter system in the zebrafish, whereby an eGFP tol2 insertion serves as a template that leads to the production of de novo GFP-targeting piRNAs. We track the inheritance of silencing over generations by examining the readout of a germline-specific eGFP reporter.
All piRNA pathway components, as well as germ-cell specific transcripts and factors, localise to an embryonic structure known as the germ plasm. This structure is inherited over generations from the mother to the embryo and gives rise to the germline. We have identified new factors that are inherited through the germ plasm and are necessary for a functional piRNA pathway. One of these factors is a Tudor domain-containing deubiquitinase termed Alg13. Using CRISPR-Cas9 mutagenesis, we have generated alg13 mutants in zebrafish. To date, we have found that heterozygous alg13 fish have a substantial loss of GFP-targeting piRNAs. We aim to use the alg13 mutants to further investigate the effects on the piRNA pathway.

---

Yukihide Tomari (JP)

**Autonomous Shaping of the piRNA Sequence Repertoire by Competition between Adjacent Ping-Pong Sites.**

PIWI-interacting RNAs (piRNAs) are crucial for silencing transposable elements (TEs). In many species, piRNAs are generated via a complex process known as the ping-pong pathway, which couples TE cleavage with new piRNA amplification. However, the biological significance of this complexity and its impact on the piRNA sequence repertoire remain unclear. Here, we systematically compared piRNA production patterns in two closely related silkworm cell lines and found significant changes in their piRNA sequence repertoire. Importantly, the changeability of this repertoire showed a strong negative correlation with the efficiency of piRNA biogenesis. This can be explained by competition between adjacent ping-pong sites, as supported by our mathematical modeling. Moreover, this competition can rationalize how piRNAs autonomously avoid deleterious mismatches to target TEs. These findings not only unveil the intrinsic plasticity and adaptability of the piRNA system to combat diverse TE sequences but also highlight the universal power of competition and self-amplification to drive autonomous optimization.

---

Séverine Chambeyron (FR)

**Temporal and spatial partitioning of retrotransposon niches in *Drosophila melanogaster*.**

Transposable elements (TE) abundance within the genome suggests that they have certainly adapt their modes of expression/integration upon genome colonization, to insure their proper maintenance and propagation. To explore further these mechanisms, we constructed a Drosophila melanogaster line in which the mobility of different TE families can be induced due to the ovarian somatic relief of the piRNA pathway (Barckmann et al., 2018). Thanks to long-read DNA sequencing and a bio-informatic pipeline established in the laboratory (TrEMOLO, https://github.com/DrosophilaGenomeEvolution/TrEMOLO) (Mohamed et al., 2023), we precisely determined novel integration sites for four TE families belonging to the endogenous retrovirus group. Interestingly, two of them (ZAM and gtwin) exhibit a dynamic choice of their landing sites. Indeed, we showed that these TEs, to limit competition, have specific expression patterns and integration sites within the host genome. Additionally, we established that their timing of integration during embryogenesis varies.

---

William Theurkauf (US)

---

Emilie Brasset (FR)

**Comprehensive investigation of the somatic piRNA pathway in the control of TEs during the development.**

Metazoan genomes are largely composed of repeated sequences, including transposable elements (TEs). To limit the activity of transposable elements, selective pressure has led to the evolution of numerous epigenetic mechanisms to repress their expression. In the animal germline, expression of TEs is restricted by the piRNA pathway. piRNAs target TEs by sequence complementarity, preventing their expression and mobilization. While piRNAs are involved in germline cells, their expression and function in somatic follicle cells highlight the intricate interactions between germline and soma. We have recently shown that a threat to the germline emanates from transposable elements (TEs) that have acquired the ability to be expressed not in the germline but in somatic follicle cells. These TEs have an infective capacity that enables them to reach the germline. To counteract this somatic activation of TEs, a simplified piRNA pathway is active in these somatic cells of adult gonads. The

formation of the gonads starts at the embryonic stage. We show that at this stage, certain TEs are expressed both at the transcript and protein levels in the somatic gonadal cells surrounding the germ cells. Indeed, we report that the somatic piRNA pathway is progressively established during embryogenesis and becomes fully functional at the larval stage, then ensuring long-term repression of TEs. These results show that, despite the selective pressure that lead to the evolution of various mechanisms dedicated to the silencing of TEs, developmental windows exist where TEs are expressed. The potential functions (if any) of these transcripts and proteins detected during this spatio-temporal window have not been investigated. Are they functional or by-products? Further research is under investigation to elucidate this question.

---

Astrid Haase (US)

**The piRNA Cluster Builder (piCB) maps piRNA generating regions and reveals a model for the origin of pre-pachytene piRNA precursors in mammals.**

From flies to mammals, millions of piRNAs are processed from thousands of long piRNA-precursors with no sequence conservation. What determines piRNA generating genomic regions (piRNA clusters) and marks their transcripts for processing into piRNAs remains largely unknown. Here, we developed a computational tool to identify and characterize piRNA clusters. Our systematic comparison of piRNA clusters in different organisms revealed similarities and puzzling differences, and suggests a possible mechanism for the birth of piRNA clusters in response to novel genomic invaders.

---

Yang Yu (CN)

---

Nelson Lau (US)

---

Julius Brennecke (AT)

---

Kensaku Murano (JP)

**Sov represses retrotransposons in a heterochromatin-independent manner.**

In the fly ovary, the functional complex of Piwi-piRNAs recognizes the nascent transcripts of retrotransposons and forms aggregated heterochromatin, thereby inhibiting their transcription. The functional analyses of the Panx-Nxf2 complex, identified as a factor associated with the Piwi-piRNA pathway, have revealed the mechanism for repressing retrotransposons that is independent of heterochromatin formation. However, the detailed mechanisms of this retrotransposon suppression by the Panx-Nxf2 complex remain elusive.
To dissect the silencing mechanism by the Panx-Nxf2 complex, we established a reporter system that combines a doxycycline-induced expression system with a lambdaN-boxB tethering system. The luciferase activity is suppressed when lambdaN-Nxf2 or lambdaN-Panx is tethered to the nascent transcripts carrying the boxB sequence in linker histone H1 and HP1a independently. Using this reporter system and mass spectrometry, we found that the Panx-Nxf2 complex on the nascent transcripts undergoes SUMO (Small Ubiquitin-like Modifier) modification. Knockdown of SUMO and the SUMO E3 ligase Su(var)2-10 dampened the silencing activity of the Panx-Nxf2 complex. In addition, the enforced tethering of Sov (Small ovary), which has been reported to interact with SUMOylated Panx, repressed the reporter gene transcription in a heterochromatin-independent manner. In this report, we discuss the role of Sov in the region-specific transcriptional repression mechanism before heterochromatin formation on retrotransposons.

---

Alexei A. Aravin (US)

**Evolutionary innovations in the piRNA pathway.**

The piRNA pathway performs a conserved function in the suppression of selfish genetic elements in different organisms, however, it is also characterized by fast evolution. New piRNAs are frequently evolved to regulate novel targets that differ among closely related species. First, I will discuss the origin of the eukaryotic piRNA pathway from diverse Argonaute proteins that serve in genome defense in prokaryotes. Next, I will talk about the mechanisms and consequences of rapid piRNA pathway evolution in animals.

---

Claus Kuhn (DE)

**The role of piRNAs in planarian stem cells.**

PIWI proteins utilize small RNAs called piRNAs to silence transposable elements,thereby protecting germline integrity. In planarian flatworms, PIWIproteins are essential for the animals' fantastic regenerative abilities, whichdepend on their abundant stem cell population, termed neoblasts. We previouslycharacterized planarian piRNAs and examined their role in conjunctionwith their PIWI binding parterns in neoblast biology. We found the planarianPIWI proteins SMEDWI-2 and SMEDWI-3 to cooperate in degrading activetransposons via the ping-pong cycle. Moreover, we unexpectedly discoveredan additional role for SMEDWI-3 in planarian mRNA surveillance. Here, Iwill report on our latest findings on the role of planarian piRNAs in mRNA surveillance and genome defense.

---

Ronald Van Rij (NL)

**Transposon control and beyond: piRNA biology in mosquitoes.**

---

Martine Simonelig (FR)

**Functions of germ granules in the Drosophila embryo.**

In Drosophila, the PIWI protein Aubergine (Aub) has a key function in the localization of maternal mRNAs at the posterior pole of the oocyte and early embryo. Aub binds maternal mRNAs through piRNA targeting, leading to their anchoring and stabilization at the posterior pole. Aub is a core component of germ granules that compose the cytoplasm at the posterior pole. Germ granules are specific RNP granules involved in the storage and regulation of mRNAs required for germ cell specification and development. Here, using super-resolution microscopy and single-molecule imaging approaches, we show that germ granules have a biphasic organization. Aub and other protein core components are enriched in their outer phase. In the absence of Aub, mRNAs are not recruited to germ granules and germ granules lose their biphasic organization. Using the Suntag approach to visualize translation, we find that translation takes place in the outer phase. The localization, directionality and compaction of mRNAs within germ granules depend on their translation status. Translated mRNAs are enriched in the outer phase with their 5' end oriented towards the surface, whereas repressed mRNAs accumulate in the inner phase. In addition, altering germ granule biphasic organization using a tudor point mutant represses translation. These findings demonstrate the role of Aub and RNAs in the architecture of germ granules and reveal the importance of their biphasic organization in coordinating their different functions.

---

Julie M. Claycomb (CA)

**Proximity Labeling Identifies New Germ Granule and Germline Small RNA Factors in *C. elegans***

Phase separated germ granules are found across animal germlines and are crucial for ensuring fertility, and passing on epigenetic information and germline determinants to progeny. These non-membrane bound organelles are enriched in RNA and RNA binding proteins, particularly those involved in small RNA mediated gene regulation. *C. elegans* possesses at least four germ granule sub-domains: P granules, Z granules, Mutator Foci, and SIMR Foci. Each of these sub-domains has been associated with different sets of small RNA factors, implying that different small RNA pathway activities *may* occur in different regions of germ granules. While some granule sub-domains, like P granules have been long studied and are comparatively better characterized, the full complement of germ granule associated proteins and RNAs has yet to be identified. In doing so, we may better be able to pinpoint specific molecular activities to specific germ granule sub-domains going forward, and we may uncover new proteins that contribute to fertility and small RNA pathway function. To tackle the characterization of germ granule proteins, we have taken an *in vivo* proximity labeling approach using TurboID. With this method, we have characterized the proteomes of the germ granule Argonautes CSR-1 and WAGO-4, along with the Z granule-associated helicase, ZNFX-1. Overlapping our data with TurboID data from the lab of Wen Tang on the P granule-associated proteins, DEPS-1 and GLH-1 revealed a high confidence set of "core granule" factors, including a number of previously uncharacterized proteins. We are in the process of characterizing these putative germ granule proteins, and have found roles for them in fertility and RNAi inheritance, thus indicating the utility of *in vivo* proximity labeling as an approach for comprehensively mapping the germ granule proteome.

---

Carolyn Phillips (US)

**NRDE-3 switches small RNA targets during embryogenesis coincident with the formation of somatic SIMR-1 granules.**

In C. elegans, there are 19 functional Argonaute proteins, many of which bind distinct classes of small RNAs and modulate gene expression through distinct mechanisms. Correct small RNA binding is essential for accurate gene regulation. However, it is unknown how the small RNA binding specificity is achieved for different Argonaute

proteins. Many components of the RNAi pathway reside in specialized compartments within the cell, where each step, from small RNA production to RNA surveillance, is spatially partitioned. The protein SIMR-1 is found in one of these compartments, though its precise function and significance are not understood. Interestingly, our data demonstrates that the nuclear Argonaute protein NRDE-3 is found in the SIMR-1 compartment only when not bound to a small RNA. Curiously, this compartment is visible only during the middle stages of embryonic development. By sequencing small RNAs associated with NRDE-3, we found that NRDE-3 binds two distinct classes of small RNAs, CSR-class 22G-RNAs in early embryonic development and WAGO-class 22G-RNAs in late embryonic development. We currently hypothesize that NRDE-3 first binds CSR-class 22G-RNAs inherited from maternal oocytes to transcriptionally shut down germline-expressed genes in the somatic cells of early embryos; NRDE-3 then gets loaded with WAGO-class 22G-RNAs during mid embryogenesis at the SIMR-1 compartment which promotes specificity or efficiency of small RNA loading; and in late embryos NRDE-3, binds WAGO-class 22G-RNAs to turn off other types of detrimental genes, like pseudogenes and retrotransposons.

---

Laura Landweber (US)

**RNA-mediated genome reorganization in the ciliate Oxytricha.**

The ciliate Oxytricha has two nuclear genomes, and massive DNA rearrangements construct a highly fragmented but functional somatic genome from a complex germline genome. This process eliminates nearly all noncoding DNA and rearranges over 225,000 short DNA segments to produce over 18,000 somatic chromosomes. Small and long noncoding RNAs regulate the entire process of genome rearrangement. 27nt piRNAs provide the critical information to mark and protect the retained DNA segments in the genome. Maternally-inherited, long, non-coding RNAs provide templates for genome remodeling. In this talk I will discuss our current understanding of this process.

---

Sandra Duharcourt (FR)

**small-RNA-guided genome elimination in the ciliate Paramecium.**

---

Kazufumi Mochizuki (FR)

**Ema2-mediated SUMOylation Facilitates Long Non-Coding RNA Transcription for Epigenetic Regulation of Programmed DNA Elimination in *Tetrahymena***

Piwi-associated ~29-nt small RNAs, called scnRNAs, epigenetically regulate the removal of approximately 12,000 DNA segments during the programmed DNA elimination (PDE) process in *Tetrahymena*. Target-directed small RNA degradation, mediated by the interaction between scnRNAs and nascent long non-coding RNA (lncRNA) transcripts from the somatic genome, plays a central role in this epigenetic regulation. In this study, we demonstrate the essential role of SUMO E3 ligase Ema2 in facilitating the accumulation of lncRNAs derived from the somatic genome, thereby enabling TDSD and completion of DNA elimination, crucial for the production of viable sexual progeny. Our findings reveal that Ema2 interacts with the SUMO E2 conjugating enzyme Ubc9 and enhances SUMOylation of the transcription regulator Spt6. We further show that Ema2 promotes the association of Spt6 and RNA polymerase II with chromatin. These results suggest that Ema2-directed SUMOylation actively promotes lncRNA transcription, which is a prerequisite for communication between the genome and small RNAs to regulate PDE.

---

Pei-Hsuan Wu (CH)

**Beyond spermatogenesis: germline small RNAs in sperm.**

PIWI-interacting RNAs (piRNAs) are the most abundant and diverse mammalian germline small RNAs. Pachytene piRNAs are the primary class of piRNAs in adult mouse testis, accounting for more than 90% of all small RNAs at the peak of expression in meiotic spermatocytes. It is well established that pachytene piRNAs play an indispensable role in male mouse fertility and spermatogenesis, the latter transforming a germ stem cell into a spermatozoon of drastically different morphology and equipping the cell with fertilizing potential. In recent years, genetic elimination of subsets of mouse pachytene piRNAs produced by defined loci on different chromosomes by CRISPR/Cas revealed a requirement for piRNAs in the male gamete after completion of spermatogenesis. piRNAs produced from a locus on Chromosome 6 (pi6) are crucial for sperm capacitation, a post-testicular sperm maturation step required for fertilizing the oocyte in vivo. By degrading transcripts necessary for sperm capacitation, pi6 piRNAs regulate a biological step previously thought to be beyond piRNA function. Furthermore, although the mouse soma does not produce or require piRNAs, embryos derived from sperm without pi6 piRNAs displayed a partially penetrant developmental arrest. It has been reported that sperm carry diverse small RNAs

that are further reshaped during post-testicular sperm maturation. However, how the maturation process influences sperm piRNAs and the biological significance of this influence is not fully understood. We will discuss our ongoing investigation of sperm piRNAs and PIWI proteins that might underlie the effect of paternal pachytene piRNAs on embryogenesis.

---

Phillip Zamore (US)

**Your Pachytene piRNAs Probably Don't Love You.**

Pachytene piRNAs, which comprise >80% of small RNAs in the adult mouse testis, have been proposed to bind and regulate target RNAs like miRNAs, cleave targets like siRNAs, or lack biological function altogether. Our genetic, molecular, and biochemical evidence support the view that pachytene piRNAs bind their target RNAs through extensive complementarity, rarely if ever act like miRNAs, and do not detectably alter the density of ribosomes transiting mRNAs. Instead, pachytene piRNAs generally repress their targets by siRNA-like endonucleolytic cleavage. Despite thousands of pachytene piRNA-directed mRNA cleavage events in vivo, the overwhelming majority of pachytene piRNAs appear to have no biological function. Our data are consistent with pachytene piRNA-producing loci representing a novel class of selfish genetic elements. We suggest that in response to the proliferation of pachytene piRNAs, the mouse genome has (1) undergone purifying selection that has eliminated deleterious target cleavage events; (2) tolerated neutral target sites or piRNAs that have no measurable effect on steady-state mRNA abundance; and (3) retained a miniscule number of piRNA:target site pairs that improve sperm fitness. Despite comprising a few hundred small RNAs, this final set of piRNAs and target sites may explain why pachytene piRNAs persist in mammalian genomes.

---

## Short Talks

Malte Grewoldt (DK)

**Exploring piRNA Evolution and Functional Innovation in Bradysia coprophila.**

In recent years, research in piRNA biology has made significant findings, revealing more and more insights in this crucial pathway and its role in transposon control, germ cell development and fertility. However, these results are mainly limited to examples in well-established models. In this study, we aim to complement the existing knowledge of piRNA biology with a novel emerging research organism: the black-winged fungus gnat, Bradysia coprophila , a so far cytological model for chromosome transmission patterns that is now being advanced using modern methods.
Our approach involves two main aspects: firstly, we explore the evolutionary dynamics of piRNA biology in this species, and secondly, we investigate the innovation of piRNA-related genes beyond their canonical functions. Studies in Dipteran species have shown that duplicated genes within the highly adaptive gene family of Argonautes have undergone neofunctionalization and evolved functions in for instance maternal mRNA degradation, spermatogenesis or antiviral defense.
We hypothesize that such neofunctionalization has also occurred in fungus gnats, where we have identified a notable expansion of Argonaute genes. Analyses of expression patterns and evolutionary rates support diverse functional models for the different Argonaute genes, thereby distinguishing conserved canonical function, robustness through increased gene copy number, and gain of new functions. Notably, we find that the expanded Argonaute family genes are expressed mainly in the germline. Based on this, we will investigate putative novel roles of these genes in Bradysia chromosome biology, with a specific focus on their potential involvement in programmed DNA elimination, chromosome imprinting, and their connection to maternal sex determination. By integrating insights from evolutionary history, phylogenomic analyses and molecular methods, our objective is to expand our knowledge of piRNA-mediated genome regulation across animals.

Azad Alizada (UK)

**The Ovo transcription factor family controls the germline piRNA pathway in animal ovaries.**

The gene-regulatory mechanisms behind the germline PIWI-interacting RNA (piRNA) pathway in the gonads of metazoan species remain largely unexplored. In contrast to the male germline piRNA pathway, which in mice is known to be driven by the testis-specific transcription factor A-MYB, the identity of the ovary-specific gene-regulatory network controlling the female germline piRNA pathway remains a mystery. Here, using Drosophila as a model in combination with multiple genomics approaches, we reveal the transcription factor Ovo as the master regulator of the germline piRNA pathway in ovaries. The ectopic expression of Ovo in somatic cells leads to activation of the germline piRNA pathway components including the ping-pong factors Aub and Ago3. Ovo ChIP-seq and motifs analysis show binding to genomic CCGTTA motifs at the regulatory elements of the germline piRNA pathway, suggesting a feed-forward regulation by Ovo in ovaries analogous to that of A-MYB in testes. Our results show consistent engagement of Ovo TF family at ovarian piRNA clusters across metazoan species, reflecting a deep evolutionary conservation of this regulatory mechanism from flies to humans.

Ian MacRae

Nicole Raad (CH)

**PIWI slicer replacements in mice as a strategy to uncover their physiological functions.**

Germline-specific PIWI proteins associate with small RNAs called PIWI-interacting RNAs (piRNAs) to silence transposable elements in the animal gonads. In the adult male germline, within meiotic and post-meiotic germ cells, another unique set of piRNAs called the pachytene piRNAs become abundant, however their targets are unknown. Two of the mouse PIWI proteins, MILI and MIWI, harbour RNA-guided endonuclease slicer activity that cleaves target RNAs. They are essential for male mouse fertility as mutant germ cells arrest at distinct early meiotic and post-meiotic stages. We previously showed that pachytene piRNA-guided slicer endonuclease activity of mouse MIWI is essential for male fertility and its loss phenocopies the complete knockout. Here we investigated whether these functions of MIWI can be rescued by any PIWI slicer. We generated knock-in mice where the MIWI coding sequence is replaced with that encoding for MILI or the oocyte-specific human PIWIL3 slicer. The results our studies into the rescue of spermatogenesis, piRNA biogenesis and regulation of MIWI targets, will be discussed during my presentation.

Ansgar Zoch (UK)

**C19ORF84 connects piRNA and DNA methylation machineries to defend the mammalian germline.**

In the male mouse germline, PIWI-interacting RNAs (piRNAs), bound by the PIWI protein MIWI2 (PIWIL4), guide DNA methylation of young active transposons through SPOCD1. However, the underlying mechanisms of SPOCD1-mediated piRNA-directed transposon methylation and whether this pathway functions to protect the human germline remains unknown. We identified loss-of-function variants in human SPOCD1 that cause defective transposon silencing and male infertility. Through the analysis of these pathogenic alleles, we discovered that the uncharacterised protein C19ORF84 interacts with SPOCD1. DNMT3C, the DNA methyltransferase responsible for transposon methylation, associates with SPOCD1 and C19ORF84 in foetal gonocytes. Furthermore, C19ORF84 is essential for piRNA-directed DNA methylation and male mouse fertility. Finally, C19ORF84 mediates the in vivo association of SPOCD1 with the de novo methylation machinery. In summary, we have discovered a conserved role for the human piRNA pathway in transposon silencing and C19ORF84, an uncharacterised protein essential for orchestrating piRNA-directed DNA methylation.

---

Dominik Handler (AT)

**Molecular determinants governing primary piRNA biogenesis in Drosophila.**

The piRNA pathway, an essential genome surveillance mechanism in animal gonads, plays a pivotal role in silencing transposable elements (TEs). Central to the efficacy of this pathway is the precise determination of the piRNA repertoire loaded onto PIWI-clade Argonaute proteins, ensuring robust TE silencing while minimizing off-target effects.
In germline cells, this process involves the intrinsic action of PIWI proteins, where piRNA-guided target RNA slicing induces the formation of ping-pong responder plus 3' trailing piRNAs from the target transcript. However, in several cellular or developmental contexts, piRNA biogenesis occurs in the absence of pre-existing piRNAs. A prominent example is the Drosophila somatic follicular epithelium. Here, efficient piRNA biogenesis processes piRNA cluster transcripts and mRNAs into mature piRNAs that are loaded into the nuclear, slicer-incompetent Piwi protein.
I will present our efforts toward understanding the molecular determinants that underlie piRNA precursor selection in cultured ovarian somatic cells. Through comprehensive analyses of piRNAs populations in both wild-type and piRNA biogenesis-deficient cells, we established a robust correlation between the local U content of an RNA, piRNA biogenesis efficiency, and the dependency on the piRNA biogenesis factor Fs(1)Yb. We validated these findings with an extensive set of sensor constructs.
Notably, retrotransposons display a pronounced A bias on their genomic sense strand. This observation implies that the piRNA pathway has evolved to exploit a vulnerability in retrotransposons, specifically their abnormally high A content, by generating piRNAs preferentially from transposon antisense transcripts, which are U-rich. While Fs(1)Yb is not conserved outside of Drosophilids, the A bias of retrotransposons extends across a wide range of organisms. This suggests the possibility of alternative mechanisms in other species, capitalizing on the intrinsic Achilles heel of retrotransposons.

---

Yuica Koga (JP)

**Mitochondrial protein Daed optimize Zuc endonucleolysis in piRISC maturation.**

In Drosophila ovarian somatic cells (OSCs), Piwi and PIWI-interacting RNAs (piRNAs) assemble piRNA-induced silencing complex (piRISC) to silence transposons to maintain germline genome integrity. The current model of piRISC production is as follows: Piwi first binds to 5’ end of piRNA precursor (pre-piRNA) to assemble piRISC precursor (pre-piRISC) in Yb bodies. RNA helicase Armi binds to pre-piRISC and translocates to mitochondria. Finally, mitochondrial endonuclease Zuc cleaves the pre-piRNA to produce active piRISC (piRISC maturation). Daed, a mitochondrial protein which act as the scaffold for piRISC maturation with another mitochondrial protein Gasz, features in this maturation phase, but the details remain unclear.
In this study, we found that Daed stabilizes its partner Gasz through protein-protein interaction, and tethers Armi to mitochondria for piRISC maturation. Daed interacts with two regions of Armi: N-terminal 34 amino acids and Asp1015-Arg1026-Arg1037 (D-R-R) in the helicase domain of Armi. When the former interaction was inhibited, Armi does not localize on the mitochondria and piRISC is little produced. We then perturbated latter interaction by producing an Armi mutant in which the D-R-R was altered to K-E-E. This mutant still has ability to localize on the mitochondria and functions in the production of piRISCs, but piRNAs became slightly longer. Structural predictions predicted that Armi D-R-R would bind SAM domain of Daed. We found that overexpression of the SAM domain-deletion mutant of Daed resulted in the production of similarly long piRNAs. Consequently, it

reduced Piwi-piRISC's level and caused transposon derepression. Daed is suggested to disambigulate Zuc cleavage in orchestrating with Gasz and Armi to optimize Piwi-piRISC production and its function.

Petr Svoboda (CZ)

**Initial analysis of the piRNA pathway in terrestrial slugs.**

Molluscs have the piRNA pathway present not only in the germline but also in somatic cells of their bodies. We have been developing terrestrial slugs into a handy laboratory model system and we have analyzed small RNAs of slugs Arion vulgaris, Deroceras laeve and Deroceras invadens. Deroceras slugs have ~ 1Gbp genomes where the most abundant mobile elements are LINEs (~17%), while LINEs in Arion vulgaris occupy approximately twice as much of the genome. Preliminary analysis of the pathway indicates a simple organization with two PIWI proteins and piRNAs of a uniform size. At the same time time, there are several interesting aspects of somatic and germline piRNAs, such as existence of specific amplifying long non-coding RNAs, which appear to give rise to secondary piRNAs in a cell type-specific manner. Another interesting feature is a possible cross-talk between piRNA biogenesis and Dicer-dependent small RNAs derived from small hairpins. As the analysis is ongoing, the latest data will be presented at the meeting.

Laure Teysset (FR)

**Study of piRNA cluster adaptation.**

A fraction of all genomes is composed of transposable elements (TEs) whose mobility needs to be carefully controlled. In gonads, TE activity is repressed by PIWI-interacting RNAs (piRNAs), a class of small RNAs synthesized by heterochromatic loci enriched in TE fragments, called piRNA clusters. Maintenance of active piRNA clusters across generations is secured by maternal piRNA inheritance providing the memory for TE repression. On rare occasions, genomes encounter horizontal transfer (HT) of new TEs with no piRNA targeting them, threatening the host genome integrity. Naïve genomes can eventually start to produce new piRNAs against these genomic invaders, but the timing of their emergence remains elusive.
Using TE-derived transgenes inserted in germline piRNA clusters, functional assays and playing with maternal and paternal inheritance of TE-derived transgenes, we have modeled a TE HT occurring in Drosophila melanogaster. We have found that the complete co-option of these transgenes by germline piRNA clusters can occur within a limited number of generations with a clear heterogeneity in piRNA profiles, never identify. These underlies the high potential of adaptation of piRNA cluster which is fundamental for the maintenance of genome integrity.

Abdou Akkouche (FR)

**A dual histone code specifies the binding of heterochromatin protein Rhino to a subset of piRNA source loci.**

Animal germ cells deploy a specialized small RNA-based silencing system, called the PIWI-interacting RNA (piRNA) pathway, to prevent aberrant expression of transposable elements and maintain genome integrity. In Drosophila germ cells, the majority of piRNA populations originate from dual-strand piRNA clusters, genomic regions highly enriched in transposon fragments, via an elaborate protein machinery centred on the heterochromatin protein 1 homolog, Rhino. Although Rhino binds to peptides carrying trimethylated H3K9 in vitro, it is not fully understood why it only occupies a fraction of H3K9me3-decorated heterochromatin in vivo. Recent work uncovered that Rhino is recruited to subsets of piRNA source loci by the zinc finger protein Kipferl. Here we identify a Kipferl-independent mode of Rhino targeting that is dependent on the histone H3 lysine 27 methyltransferase Enhancer of Zeste and the presence of H3K9me3 and H3K27me3 marks. Using a Kipferl-independent system, we find that Rhino, through a chromodomain dimer, specifically binds to loci marked by both H3K9me3 and H3K27me3. These results expand our understanding of the characteristic binding profile of the heterochromatin protein Rhino and reveal a role for dual histone modifications in defining the specificity of a chromatin binding protein.

Marie Fablet (FR)

**Transposable elements are pivotal in the arms race between virus and host.**

Transposable elements (TEs) are parasite DNA sequences that are controlled by RNA interference pathways in many organisms. In insects, antiviral immunity also is achieved by the action of small RNAs. In the present study, we analyzed the impacts of an infection with Drosophila C Virus (DCV), which has a long coevolutionary history with its Drosophila host. We found that TEs are involved in a dual response: on the one hand TE control is released upon DCV infection, and on the other hand TE transcripts help the host fight back against the viral suppressor of

RNA interference. This discovery highlights a pivotal role for TEs in the long-term arms race between a virus and its host.

---

Anahi Molla-Herman (FR)

**H2Av controls genome integrity in the female germline.**

Transposable Elements (TEs) are mobile genetic elements representing 15% of D. melanogaster genome and present in almost all genomes. TEs can create mutations upon insertion, which is especially deleterious in the germline since mutations are transmitted to the next generation. Fortunately, the genome has its own immune system based on small non-coding RNAs which target TEs by sequence complementarity: piRNAs and siRNAs. They are associated with Piwi family proteins to regulate TEs expression.
Epigenetic marks and chromatin structure are important for TEs silencing at two levels: piRNAs expression and TEs silencing. For example, H3K9me3 is crucial for heterochromatinization, which is important for piRNAs clusters expression and also TEs repression. However, the role of histone proteins and histone variants in the piRNA pathway is not well understood.
Histone variants play several important functions in eukaryotic genome regulation: genome organization, gene expression control, cell division, DNA repair... Interestingly, while many organisms have several H2A variants to play different functions, D. melanogaster has a single H2A variant called His2Av, involved in heterochromatin assembly, DNA damage response and transcription regulation. A potential role in drosophila oogenesis and the piRNA pathway remains to be uncovered.
We have found that His2Av plays a role in drosophila oogenesis, since its depletion leads to a developmental delay, oogenesis arrest and sterility. Interestingly, we observe that several TEs are highly expressed (such as I-ele, Max and gypsy12) and that the “nuage” is strongly altered. Moreover, by using different genetic tools and GFP biosensors, we show that p53 and mnk checkpoint proteins are activated in His2Av mutant ovaries, together with signs of replication stress. Altogether, we show that genome integrity is altered in His2Av mutant ovaries, causing the oogenesis arrest, highlighting a new role of His2Av in genome integrity defense or maintenance.

---

Michael Mitchell (FR)

**EXD1 inactivation causes severe oligozoospermia and male infertility in human and mouse.**

Oligozoospermia, low sperm count, is a common cause of human male infertility. Although the underlying reasons for oligozoospermia remain unknown, in most cases, assisted reproductive technology (ART) is frequently used to achieve conception. Nevertheless an oligozoospermia associated with genome instability could carry an increased risk of transmitting de novo secondary mutations to the conceptus.
In human, we have identified a homozygous nonsense mutation in the exonuclease 3′-5′ domain-containing 1 gene, EXD1, in five brothers with severe oligozoospermia. C57BL/6 males lacking EXD1 show weakened LINE-1 repression in spermatocytes, but have normal spermatogenesis and fertility (Yang et al. 2016 Mol. Cell).
To investigate whether loss of EXD1 function might be the cause of oligozoospermia in the human family, we developed an Exd1-KO mouse model carrying the Eucomm Exd1$^{Tm1d}$ allele on an outbred genetic background. Most Exd1$^{Tm1d}$ homozygous males had normal fertility and testis size, but 25% were infertile with small testis. Infertile Exd1-KO males show severe oligozoospermia, or azoospermia, with partial meiotic arrest at zygonema. Compared to fertile homozygotes, all infertile Exd1$^{Tm1d}$ homozygous males exhibit strong de-repression of LINE-1 retransposons in spermatocytes. Unexpectedly, in the prospermatogonia of a proportion of newborn Exd1-KO mice, we show that MIWI2 is exclusively cytoplasmic, implying that a lack of MIWI2-mediated DNA methylation at LINE-1 promoters may be the cause of infertility in our Exd1-KO mice.
We are using our model to identify the genetic factor that sensitizes to the loss of EXD1 function through whole genome sequencing and transcription profiling. Our mouse model provides a means of studying how LINE-1 activity in the germ line affects genome and epigenome quality in the male gamete.

---

Sebastian Riedelbauch (DK)

**Recurrent molecular adaptation revealed by systematic cross-species protein interaction analyses of the Drosophila piRNA pathway.**

The piRNA pathway's core function in suppressing transposable elements in cells of the germ line is conserved among animals. However, despite their essential function for fertility, piRNA pathway genes of animals with diverse active transposon families such as fruit flies and teleost fish are known to be rapidly evolving. The functional impact of such rapid evolution of essential genome defense genes remains enigmatic. To address this question, we performed a yeast-two-hybrid screen to systematically test protein-protein interactions of eleven

orthologous genes involved in piRNA precursor expression from five Drosophila species. Our data identify several conserved protein-protein interactions not impacted by rapid sequence evolution, but also reveal two types of molecular innovation within the Drosophila piRNA pathway: (1) co-evolution of PPIs as shown by species incompatibilities in protein-protein interactions that are otherwise conserved between orthologs from the same species, and (2) protein interaction rewiring exemplified by the species-specific recruiters of CtBP, a co-factor required to suppress canonical transcription of transposons at Rhino-occupied loci. Combined with evolutionary analyses and complementary protein-protein interactions assays our data uncover how an arms race, such as the one between transposons and the piRNA pathway in Drosophila, can lead to recurrent innovation of conserved protein interaction networks while preserving the pathway's core function.

---

Chen Chen (US)

**MIWI N-terminal RG motif promotes efficient pachytene piRNA production and spermatogenesis independent of LINE1 transposon silencing.**

PIWI proteins and their associated piRNAs act to silence transposons and promote gametogenesis. Murine PIWI proteins MIWI, MILI, and MIWI2 have multiple arginine and glycine (RG)-rich motifs at their N-terminal domains. Despite being known as docking sites for the TDRD family proteins, the in vivo regulatory roles for these RG motifs in directing PIWI in piRNA biogenesis and spermatogenesis remain elusive. To investigate the functional significance of RG motifs in mammalian PIWI proteins in vivo, we genetically engineered an arginine to lysine (RK) point mutation of a conserved N-terminal RG motif in MIWI in mice. We show that this tiny MIWI RG motif is indispensable for piRNA biogenesis and male fertility. The RK mutation in the RG motif disrupts MIWI-TDRKH interaction and impairs enrichment of MIWI to the intermitochondrial cement (IMC) for efficient piRNA production. Despite significant overall piRNA level reduction, piRNA trimming and maturation are not affected by the RK mutation. Consequently, Miwi$^{RK}$ mutant mice show chromatoid body malformation, spermatogenic arrest, and male sterility. Surprisingly, LINE1 transposons are effectively silenced in Miwi$^{RK}$ mutant mice, indicating a LINE1-independent cause of germ cell arrest distinctive from Miwi knockout mice. These findings reveal a crucial function of the RG motif in directing PIWI proteins to engage in efficient piRNA production critical for germ cell progression and highlight the functional importance of the PIWI N-terminal motifs in regulating male fertility.

---

Margarita Angelova (US)

**DNA N6-methyladenine (6mA) involvement in programmed genome rearrangement in the ciliate Oxytricha trifallax.**

Programmed genome rearrangements are important for adaptive immunity, and aberrant rearrangements are a hallmark of cancer. The ciliate Oxytricha trifallax provides a compelling model system to study genome rearrangement, as it undergoes extensive RNA-guided DNA deletion and chromosome reorganization, during development of a somatic macronucleus (MAC) from a copy of a germline micronucleus (MIC). This process involves DNA fragmentation, extensive elimination of the germline genome, and DNA rearrangement of the remaining DNA sequences[1-3]. piRNAs, together with long non-coding RNAs, mediate transgenerational transfer of the information necessary to decipher the germline genome during nuclear development[4-6].
Recently our lab discovered MTA1, a methyltransferase that catalyzes DNA N6-adenine methylation (6mA). MTA1 is involved in both nucleosome positioning and post-zygotic development in Oxytricha[7]. MTA1 expression peaks during the sexual cycle and mutants fail to complete development. Immunofluorescence analysis of 6mA subcellular localization shows specific enrichment in the developing MAC, suggesting its possible involvement in programmed genome rearrangements. To understand the mechanisms underlying the impaired development of MTA1 mutants, we analyzed the genome-wide 6mA distribution during conjugation and detect enrichment on retained sequences, suggesting a novel protective role for this epigenetic mark in Oxytricha. The distribution of 6mA on retained sequences resembles the distribution of piRNAs that are detected prior to 6mA accumulation. Moreover, our preliminary data suggest a possible association between the PIWI protein Otiwi1 and the methyltransferase machinery. We hypothesize that 6mA is a novel piRNA-guided mark for DNA retention that facilitates programmed genome rearrangements of Oxytricha.

---

Eric Lai (US)

**Silencing endogenous selfish genes.**

Selfish meiotic drive systems (SMDs) can distort progeny sex-ratio (SR) and/or induce sterility. Although widespread in nature, the molecular mechanisms of SMDs, and how they are silenced to restore Mendelian segregation, remain largely mysterious. Importantly, their rapid evolution means that classic model organisms

may not be suited to reveal their fundamental features, breadth and impact. Instead, we used non-model Drosophila species to uncover critical roles for hpRNA class endo-siRNAs in suppressing incipient sex chromosome conflicts. Genetic studies reveal that these involve de novo meiotic drive loci encoded by the X chromosome. Furthermore, long read genomic data from wild fly lines reveals extraordinary polymorphism and copy number variation in these genes across individuals. We interpret this to reflect an ongoing arms race between SMD distortion and suppression by small RNAs.
These findings reveal parallels between usages of RNAi and piRNAs to suppress rapidly evolving selfish genes. Both regulatory strategies have critical requirements for normal fertility and species maintenance. However, the job of RNAi is more difficult, since its targets lack molecular features that the piRNA system exploits to distinguish transposons from self genes. In addition, we note that while deletion of many deeply-conserved miRNAs that target hundreds of genes often has only subtle consequences, loss of individual newly-evolved hpRNAs evokes profound defects in reproductive biology, due to derepression of specific de novo targets. This highlights the genetic importance of RNA silencing during recent or active speciation.

---

Zuzana Loubalova (US)

**Signatures of cooption in mammalian pachytene piRNAs.**

The piRNA pathway is best known for its essential function in silencing transposable elements (TEs). However, the minimal presence of TE-derived sequences in mammalian pachytene piRNAs suggests a function other than TE control for this class of piRNAs. PIWIL1 initiates its expression during the pachytene stage of meiosis in male germ cells and is essential for fertility in mouse. Its associated ‘pachytene piRNAs’ mostly originate from unique genomic sequences with little targeting potential in trans, and their functional targets remain unknown. Based on our recent result that identified abundance as key for piRNA function, we re-investigated potential targets of pachytene piRNAs. We identified a few protein coding genes as top targets by abundance of complementary piRNAs. Interestingly, each candidate was targeted by thousands of piRNAs from a single cluster that originated from an antisense insertion of the respective gene’s own cDNA. Our observation suggested that individual clusters might have been coopted for gene regulation by the insertion of pseudogenes in antisense orientation. To directly test this hypothesis, we generated knock-out alleles of three pseudogene-containing piRNA clusters. After the required backcrosses, we will assess male fertility and germ cell morphology. Results from our study will reveal the phenotypes of three pachytene piRNA clusters containing pseudogene insertion, directly probe their targets through rescue experiments, and potentially solve the longstanding mystery of mammalian pachytene piRNAs.

## Posters

### Poster #1

Ansgar Zoch (UK)

**C19ORF84 connects piRNA and DNA methylation machineries to defend the mammalian germline.**

In the male mouse germline, PIWI-interacting RNAs (piRNAs), bound by the PIWI protein MIWI2 (PIWIL4), guide DNA methylation of young active transposons through SPOCD1. However, the underlying mechanisms of SPOCD1-mediated piRNA-directed transposon methylation and whether this pathway functions to protect the human germline remains unknown. We identified loss-of-function variants in human SPOCD1 that cause defective transposon silencing and male infertility. Through the analysis of these pathogenic alleles, we discovered that the uncharacterised protein C19ORF84 interacts with SPOCD1. DNMT3C, the DNA methyltransferase responsible for transposon methylation, associates with SPOCD1 and C19ORF84 in foetal gonocytes. Furthermore, C19ORF84 is essential for piRNA-directed DNA methylation and male mouse fertility. Finally, C19ORF84 mediates the in vivo association of SPOCD1 with the de novo methylation machinery. In summary, we have discovered a conserved role for the human piRNA pathway in transposon silencing and C19ORF84, an uncharacterised protein essential for orchestrating piRNA-directed DNA methylation.

### Poster #2

Camille Enjolras (FR)

**piRNAs are regulators of metabolic reprogramming in stem cells.**

Stem cells preferentially use glycolysis instead of oxidative phosphorylation and this metabolic rewiring plays an instructive role in their fate; however, the underlying molecular mechanisms remain largely unexplored. PIWI-interacting RNAs (piRNAs) and PIWI proteins have essential functions in a range of adult stem cells across species. Here, we show that piRNAs and the PIWI protein Aubergine (Aub) are instrumental in activating glycolysis in Drosophila germline stem cells (GSCs). High glycolysis is required for GSC self-renewal and aub loss-of-function induces a metabolic switch in GSCs leading to their differentiation. Aub directly binds glycolytic mRNAs. We investigate the regulation of Enolase, one of the glycolytic mRNA, by Aub and find that it depends on its 5'UTR. Moreover, deletion of a piRNA target site in Enolase 5'UTR leads to GSC loss. These data reveal a new mode of regulation of metabolic reprogramming in stem cells based on activation of gycolytic mRNAs by Aub and piRNAs.

### Poster #3

Shau-Ping Lin (TW)

**Male germ cell developmental stage-dependent expression of DNA methyltransferase 3-like (DNMT3L) isoforms and their relationship with piRNA modulation.**

The nucleus-localized canonical DNMT3L is a well-known cofactor, facilitating de novo DNA methylation, which is necessary for efficient establishment of epigenetic landscape in prospermatogonia. This activity is also implicated in accurate composition of postnatal PIWI-interacting piRNAs, successful meiosis and survival of spermatocytes. The piRNA species that are deregulated in Dnmt3l KO spermatogonial progenitor cells are prevalently mapped to meiosis-specific enhancers, implying their function in modulating meiosis progression. In this presentation, we would emphasize on a novel DNMT3L protein isoform, translated from the 3 Dnmt3l-at (abbreviated from Dnmt3l-adult testes) alternatively spliced transcripts. The DNMT3L_AT immunoreactivity can start to be observed from 2-week-old postnatal mouse testes. DNMT3L_AT is cytoplasmically localized, especially concentrating outside of the nuclear envelop in the mitochondrion-enriched germ granules, which indicates that it is involved in other functions beyond epigenetic mark establishment directly. DNMT3L_AT is heavily co-localized with piRNA processing related germ granule components, MILI and VASA, in the intermitochondria cement of spermatocytes. However, in round spermatid stage, DNMT3L do not concentrate into the chromatoid body where VASA and MILI are enriched. Proximal proteomic profiling followed by gene ontology enrichment analysis revealed that DNMT3L_AT's neighboring proteins are most highlighted in piRNA biogenesis, RNA stability and translational control. We have also observed strong mitochondria association of DNMT3L_AT during spermiogenesis until spermatozoa wherein mitochondrial sheathes at the midpiece are condensing, consistent with the discovery that various proximal proteins of DNMT3L_AT are also highlighted in mitochondrial transport along the intraflagellar microtubules. DNMT3L_AT would eventually be discarded with the residue body at the end of spermiogenesis. Based on the structure prediction, DNMT3L_AT may form dimer or tetramer and is a promising RNA binding

protein. The direct and indirect involvement of the canonical DNMT3L and DNMT3L_AT in modulating piRNA composition and function worth further exploration.

**Poster #4**

Shuo Shi (CN)

**Key Roles of Piwil3 in Rabbit Oogenesis and Early Embryogenesis.**

The Piwi-piRNA pathway is essential for maintaining genome integrity and regulating cell fate in animal gonads. In mammals, the Piwi-piRNA pathway has been widely studied in mice, which possess only three Piwi genes—Piwil1(Miwi), Piwil2(Mili), and Piwil4(Miwi2)—without encoding Piwil3, a gene present in most mammals, including humans. Disruption of any Piwi gene in male mice leads to spermatogenesis arrest and sterility, while in female mice retains fertility. This limits our understanding of the function of Piwi in mammalian female gonads. Recent studies in golden hamsters demonstrated that Piwil3-deficient females have no observable ovarian abnormality but display reduced fertility because their oocytes are defective in supporting zygotic development[1,2,3,4]. To explore potential unknown functions in higher mammals, we generated Piwil3 knock-out (KO) rabbits. Our preliminary analyses indicate that the homozygous PIWIL3-deficient female rabbits display complete sterility and the number of growing ovarian follicles significantly diminishes in Piwil3-KO female rabbits. This suggests an earlier and more important function of Piwil3 in rabbit oogenesis than in golden hamster oogenesis. Furthermore, the development of maternal Piwil3-deficient embryos was arrested at the eight-cell stage in vitro, suggesting the critical role of Piwil3 in early embryogenesis. In rabbit MII oocytes, we identified the predominant small RNA species as the 18nt piRNA, resembling the profile observed in human MII oocytes. Depletion of PIWIL3 in rabbit results in a substantial deficiency of 18nt piRNAs in MII oocytes, accompanied by an overall reduction in the proportion of piRNAs, indicating impaired piRNA biogenesis. This study underscores the crucial role of Piwil3 in the reproductive success of female mammals, shedding light on its unique significance in the intricate processes of oogenesis and early embryogenesis.

**Poster #5**

Olivia Charmant (FR)

**The nuclear PIWI-interacting protein Gtsf1 controls the selective degradation of small RNAs in Paramecium.**

Ciliates undergo developmentally programmed genome elimination, in which small RNAs direct the removal of transposable elements during the development of the somatic nucleus. 25-nt scnRNAs are produced from the entire germline genome and transported to the maternal somatic nucleus, where selection of scnRNAs corresponding to germline-specific sequences is thought to take place. Selected scnRNAs then guide the elimination of transposable elements in the developing somatic nucleus. How germline-specific scnRNAs are selected remains to be determined. Here, we provide important mechanistic insights into the scnRNA selection pathway by identifying a Paramecium homolog of Gtsf1 as essential for the selective degradation of scnRNAs corresponding to retained somatic sequences. Consistently, we also show that Gtsf1 is localized in the maternal somatic nucleus where it associates with the scnRNA-binding protein Ptiwi09. Furthermore, we demonstrate that the scnRNA selection process is critical for genome elimination. We propose that Gtsf1 is required for the coordinated degradation of Ptiwi09-scnRNA complexes that pair with target RNA via the ubiquitin pathway, similarly to the mechanism suggested for microRNA target-directed degradation in metazoans.

**Poster #6**

Xin Wang (CN)

**piRNA loading triggers MIWI translocation from the intermitochondrial cement to chromatoid body during mouse spermatogenesis.**

The intermitochondrial cement (IMC) and chromatoid body (CB) are posited as central sites for piRNA activity in mice, with MIWI initially assembling in the IMC for piRNA processing before transitioning to the CB for functional deployment. The regulatory mechanism underpinning MIWI translocation, however, has remained elusive. We unveil that piRNA loading is the trigger for MIWI migration from the IMC to CB. Mechanistically, piRNA loading facilitates MIWI release from the IMC by weakening its ties with the mitochondria-anchored TDRKH. This, in turn, enables arginine methylation of MIWI, augmenting its binding affinity for TDRD6 and ensuring its integration within the CB. Notably, loss of piRNA-loading ability causes MIWI entrapment in the IMC and its destabilization in male germ cells, leading to defective spermatogenesis and male infertility in mice. Collectively, our findings

establish the critical role of piRNA loading in MIWI translocation during spermatogenesis, offering new insights into piRNA biology in mammals.

**Poster #7**

Deqiang Ding (CN)

**TDRD1 phase separation drives intermitochondrial cement assembly to promote piRNA biogenesis and fertility.**

The intermitochondrial cement (IMC) is a prominent germ granule that locates among clustered mitochondria in mammalian germ cells. Serving as a key platform for piRNA biogenesis, however, how the IMC assembles among mitochondria remains elusive. Here, we reveal that TDRD1 triggers IMC assembly via liquid-liquid phase separation (LLPS). TDRD1 LLPS is driven by the cooperation of its tetramerized coiled-coil domain and dimethylarginine-binding Tudor domains but is independent of its intrinsically disordered region. TDRD1 is recruited to mitochondria by MILI and sequentially enhances mitochondrial clustering and triggers IMC assembly via LLPS to promote piRNA processing. TDRD1 LLPS-deficient mutations in mice disrupts IMC assembly and piRNA biogenesis, leading to transposon de-repression and spermatogenic arrest. Moreover, TDRD1 LLPS is conserved in vertebrates, but not in invertebrates. Collectively, our findings uncover a critical role of LLPS in germ granule formation and establish a new link between membrane-bound organelles and membrane-less organelles.

**Poster #8**

Duo Pan (CN)

**HSP90α-mediated TDRD9 stabilization is essential for male fertility.**

TDRD9, as a functional partner of MIWI2 protein for piRNA-mediated retrotransposon silencing, is abundantly present in spermatogonia and spermatocytes but sharply reduced in abundance in haploid spermatids, showing a highly spatiotemporal regulation during spermatogenesis in mice; however, its metabolism has remained largely unclear. Here, we show that TDRD9 is degraded through the HERC2-mediated ubiquitin-proteasome pathway in haploid spermatids, while the spermatocyte-highly expressing chaperone protein HSP90α protects TDRD9 from HERC2 ubiquitination in spermatocytes. Interestingly, we identify a homozygous mutation (c.1037C>T, p.P346L) of TDRD9 gene in infertile men, and further discover in Tdrd9 knock-in mice that this genetic mutation impairs TDRD9 binding to HSP90α and in turn results in its advanced ubiquitination and degradation in spermatocytes, leading to male infertility in mice. These findings reveal a critical role of HSP90α in controlling the stability of TDRD9 protein during spermatogenesis, which we show is essential for male germ cell development and male fertility.

**Poster #9**

Aissatu BALDE CAMARA (FR)

**Unraveling Genetic Determinants of Azoospermia: A Comprehensive Study on PIWIL1 Function in Male Infertility.**

Infertility, recognized by the World Health Organization as a global health concern, affects millions worldwide. With male infertility accounting for 50% of cases, azoospermia, the absence of spermatozoa in the ejaculate, emerges as a severe manifestation. This study delves into the genetic underpinnings of azoospermia, focusing on non-obstructive azoospermia (NOA), a condition affecting 1% of men, often with unexplained origins.
Presented is the case of a 29-year-old male seeking assistance at Pontchaillou Hospital, Rennes, France, for azoospermia. Hormonal assessments revealed normal levels, while spermogram results confirmed azoospermia, leading to genetic scrutiny. Rigorous filtering identified a rare homozygous variant on the PIWIL1 gene.
PIWIL1, conserved in vertebrates, especially mice, plays a vital role in spermatogenesis. Located on chromosome 12, it regulates reproductive biology, particularly in male germ cell development. With a distinctive PIWI domain, it governs gene expression, suppresses transposable elements, and participates in piRNA formation.
Immunostaining of testicular tissues using an automated system and a specific antibody unveiled the absence of PIWIL1 expression in the patient's samples, confirming the lack of spermatocytes and spermatids. This comprehensive approach sheds light on the intricate genetic landscape of azoospermia, particularly the pivotal role of PIWIL1, offering novel perspectives for understanding male infertility.

**Poster #10**

Joanna Michowicz (DE)

**Functional characterisation of Alg13 in piRNA pathway in Danio rerio.**

Transposons are mobile DNA sequences that pose a particular threat to germ cells, as a germline mutation is heritable and may be detrimental to the offspring. To ensure the genome integrity for future generations, most organisms employ germline-specific small RNA-based immune machinery. It employs a subfamily of Argonaute nucleases, termed PIWI proteins that are bound by small RNAs (piRNAs). While targeting transposons that are present in the genome, the piRNA system also needs to be able to initiate silencing of novel sequences, as transposons are known to spread also horizontally (i.e. between species). How such de novo piRNA-mediated silencing is established is largely unknown. To address this question, we established an in vivo piRNA/target reporter system in the zebrafish, whereby an eGFP tol2 insertion serves as a template that leads to the production of de novo GFP-targeting piRNAs. We track the inheritance of silencing over generations by examining the readout of a germline-specific eGFP reporter.
All piRNA pathway components, as well as germ-cell specific transcripts and factors, localise to an embryonic structure known as the germ plasm. This structure is inherited over generations from the mother to the embryo and gives rise to the germline. We have identified new factors that are inherited through the germ plasm and are necessary for a functional piRNA pathway. One of these factors is a Tudor domain-containing deubiquitinase termed Alg13. Using CRISPR-Cas9 mutagenesis, we have generated alg13 mutants in zebrafish. To date, we have found that heterozygous alg13 fish have a substantial loss of GFP-targeting piRNAs. We aim to use the alg13 mutants to further investigate the effects on the piRNA pathway.

**Poster #11**

Shamitha Govind (DE)

**From PIWI to RdRP: Comparative Analysis of GTSF-1 in Nematodes.**

GTSF-1 is a PIWI-associated protein crucial for piRNA-directed silencing in mice and insects. It plays a pivotal role by enhancing the endoribonuclease activity of catalytic PIWI (Arif et al., 2022). GTSF-1 is deeply conserved in animals, including the nematode C. elegans. C. elegans have piRNAs associated with PIWI protein PRG-1, targeting transposable elements and regulating fertility. However, C. elegans GTSF-1 is shown to associate with an RNA-Dependent RNA Polymerase (RdRP) named RRF-3, deviating from the conventional PIWI association (Almeida et al., 2018). The observation gains significance as several nematode lineages independently lost the entire piRNA pathway, yet retained RRF-3-like RdRPs (Sarkies et al., 2015).
This study seeks to understand the factors conferring specificity of C.elegans GTSF-1 to RRF-3 and investigate the conservation of this association across nematodes. Using immunoprecipitation-mass spectrometry (IP-MS), we describe an interaction between GTSF-1 and RRF-3 in C. briggsae and P. pacificus. In both species, deletion of gtsf-1 resulted in sterility at elevated temperatures and a specific loss of 26G-RNAs (endo-siRNAs), while 21U-RNAs (piRNAs) remained unaffected.
We describe sequence and structure differences in nematode GTSF-1 homologs that may explain loss of PIWI binding. The central region lacks aromatic residues, crucial for PIWI binding in mice and insects. Additionally, C. elegans GTSF-1 does not bind RNA in-vitro (Jonathan Ipsaro, CSHL). This may be attributed to the missing positively charged region around the N-terminus Zn-fingers, known to facilitate in-vitro RNA binding in mice GTSF-1 (Ipsaro et al., 2021).
Using Alpha-Fold2 (AF2), we pinpoint the binding of C. elegans GTSF-1 to RRF-3 through its N-terminal Zn finger region. Furthermore, RRF-3 interacts with GTSF-1 through a unique N-terminal region, positioned away from the RdRP active site. We find comparable interactions in the AF2 multimer models of C. briggsae and P. pacificus. We are validating these interactions through Yeast-two-Hybrid assays, and we also actively creating nematode strains with point mutations in gtsf-1 and rrf-3 to assess binding in-vivo.

**Poster #12**

Lizaveta Pshanichnaya (DE)

**A role for the intrinsically disordered N-terminal region of WAGO-3 in small RNA selection.**

Caenorhabditis elegans has a distinct class of Argonaute proteins known as Worm-specific Argonaute proteins (WAGOs). Operating as secondary Argonautes, WAGOs acquire their small RNA through intricate processes involving 'primary' Argonaute proteins and small RNA amplification[1]. As other Argonaute proteins, WAGOs consist of four major structural domains: PAZ, MID, PIWI, N-terminal domain[2]. In addition, WAGOs have an N-terminal intrinsically disordered region (IDR). Previous crystallography studies have highlighted the significance of the PAZ and MID domains in RNA binding, while the PIWI domain is responsible for RNA cleavage. However, the role of N-terminal IDR remains unclear. Our study is focused on understanding the function of N-terminal IDR in WAGOs, using WAGO-3 as a model protein.

WAGO-3 is known to bind WAGO class 22G small RNA and be important for paternal inheritance[3]. While wago-3 mutants do display defects, the absence of WAGO-3 does not induce a dramatic or acute fertility phenotype. Interestingly, our studies showed that WAGO-3 lacking the N-terminal IDR induces a strong, dominant and acute effect on germline formation, resulting in full sterility. Controlling WAGO-3 expression level using the auxin-inducible degradation system we were able to conduct RNA immunoprecipitation of WAGO-3 lacking its N-terminal IDR region. Results showed that IDR deletion led to the binding of small RNAs beyond the usual WAGO-interacting 22G RNAs, suggesting that the IDR controls WAGO-3 loading. Ongoing investigations support the idea that these non-specific 22G RNAs cause the acute phenotype. We currently aim to better understand the details of how the N-terminal IDR may control WAGO small RNA binding.

**Poster #13**

Ida Isolehto (DE)

**N-terminal processing of WAGO-3 affects paternal RNAi inheritance.**

C. elegans employs various small RNA-mediated pathways to initiate and maintain proper gene regulation throughout development and across generations. These pathways rely on Argonautes (AGOs) for target silencing. Despite being well-studied pathways, little is known about the regulation of AGOs themselves. In germ cells, small RNAs and associated AGOs are particularly crucial for ensuring genome stability and maintaining an immortal germline. Notably, silencing responses can become independent of the triggering small RNAs and persist across generations through epigenetic inheritance.
The Worm-specific Argonaute WAGO-3 has a Proline-rich N-terminal intrinsically disordered region (IDR). It has been demonstrated that the N-terminus of WAGO-3 is being processed by the dipeptidyl aminopeptidase DPF-3 in vivo[1]. Processing by DPF-3 is essential for transposon silencing, fertility, and correct small RNA loading. This highlights the important role of N-terminal processing in WAGO function. However, considering the DPF-3 cleavage pattern and amino acid specificity, DPF-3 alone cannot be solely responsible for the fully processed WAGO-3 N-terminus. We identified another peptidase, the X-prolyl aminopeptidase APP-1[2], that also has potential to cleave the WAGO-3 N-terminus.
Interestingly, we found that both APP-1 and DPF-3 localizes to PEI-granules in spermatids, which we identified as germ granules crucial for paternal RNAi inheritance via WAGO-3[3]. We developed a GFP RNAi-based assay specifically testing paternal contributions to RNAi inheritance. Using this assay, we determined that N-terminal processing is essential for WAGO-3 function in paternal RNAi inheritance. Currently, we are further investigating the role of N-terminal processing in WAGO-3 function and paternal RNAi inheritance.

**Poster #14**

Xin Hu (CH)

**Tracking epigenetic silencing mediated by germline piRNAs.**

PIWI-interacting RNAs (piRNAs) are a class of animal gonad-specific small RNAs, that associate with the PIWI-clade Argonaute proteins to target the transposon transcripts or mRNA targets to control mobile genetic elements or regulate germline gene expression(1-3). Previous research in the field has uncovered how nuclear mouse PIWI protein MIWI2 and associated factors mediate epigenetic silencing by promoting DNA methylation and histone modifications(4-6). It is expected that this silencing that is initiated in the embryonic germline remains stable long after expression of MIWI2 is extinguished and transmitted to the next generation, surviving epigenetic reprogramming events.
Our project aims at tracking piRNA-guided epigenetic silencing in mice. Given that transposons are highly repetitive, we designed unique reporters that are silenced by MIWI2 piRNAs, either artificially or endogenously. This report allows uncoupling of the initiation phase from the maintenance of silencing in the next generation. Fluorescent signal can be followed to verify the silencing. We intend to track DNA methylation and histone modifications on the reporter. To identify maintenance factors in the next generation, we will conduct CRISPR screen with mouse embryonic stem cells (mESCs) prepared from embryos whose father experienced epigenetic silencing of the reporter. This project will define how epigenetic silencing initiated by piRNAs can be maintained for transmission to the next generation.

**Poster #15**

Júlia Portell i de Montserrat (AT)

**Towards a mechanistic understanding of Piwi-RISC.**

PIWI-clade Argonaute proteins, in complex with their bound piRNAs, silence transposons and other selfish elements in animal gonads. Although the piRNA pathway has been extensively characterised at the genetic level, the silencing process itself is poorly understood, particularly at the mechanistic level. I will present my efforts to reconstitute the Drosophila nuclear Piwi protein bound to piRNAs and complementary targets in vitro. As Piwi itself is not an active slicer, I investigate the cytoplasmic Aubergine protein in parallel, using an in vitro cleavage assay as a benchmark. Using in vitro reconstitution experiments, AlphaFold and cellular rescue assays, I aim to understand how Piwi target engagement allows the assembly of an active Piwi silencing complex and which co-factors are required for this. My ultimate goal is to provide structural insight into the active Piwi silencing complex.

**Poster #16**

Adrià Mitjavila-Ventura (ES)

**Transposons and the evolution of piRNA cluster expression in mice.**

Piwi-interacting RNAs (piRNAs) are small non-coding RNAs expressed in the animal germline. They are produced from long single-stranded transcripts that derive from discrete genomic loci called piRNA clusters. piRNAs and piRNA clusters are highly diverged between species showing almost no evidence of selection constraint. Considering their fast turnover, we wondered how the expression of piRNA clusters evolves in short evolutionary time scales. To address this, we focused on differences in postnatal piRNA expression in different inbred strains of mice and closely related murine species. We found significant differences in piRNA clusters within and across species. Comparing the expression of the piRNA clusters across mouse species, we found that piRNA expression level correlated with conservation of the piRNA clusters, while species-specific clusters showed fewer and more variable piRNA production. We found that clusters with polymorphic endogenous retroviruses were overrepresented among those with highly variable piRNA cluster expression, likely contributing to transcriptional activation and post-transcriptional processing of novel piRNA clusters. Taken together our results suggest young endogenous retroviruses as potent drivers of piRNA cluster gains and that piRNA abundance constrains piRNA evolution.

**Poster #17**

Ferenc Jankovics (HU)

**Identification of structural elements of Small ovary (Sov) involved in co-transcriptional transposon silencing in Drosophila.**

Co-transcriptional silencing of transposon (TE) expression is regulated by heterochromatin mediated repression of TE transcription. In Drosophila, heterochromatinization of the TE loci is initiated by Piwi/piRNA mediated recognition of the nascent TE transcripts and subsequent recruitment of piRNA pathway specific recognition complex, composed of Panx, LC8 and Nxf2. Then, the recognition complex tethers heterochromatinisation factors to the transposon loci. The concerted action of the recognition complex and the heterochromatin effector proteins generates a compact heterochromatin structure which efficiently prevents further TE transcription. The initial steps of the co-transcriptional TE silencing are well described, however, the molecular interactions underlying heterochromatin formation are less well understood.
It is known that Panx directly binds to Small ovary (Sov), a recently identified general heterochromatin regulator protein. We aimed to reveal how the recruitment of Sov to the TE loci leads to facilitated heterochromatin formation and efficient TE silencing. To uncover mechanisms, by which Sov fulfils specific tasks associated with TE silencing, we identified specific regions of the protein required for distinct biological activities, such as nuclear localisation, association with subnuclear compartments, protein-protein interactions, phase separation capacity and transcriptional suppression activity. We demonstrated the biological relevance of these functions by phenotypic analysis of the deletion mutants lacking the identified structural elements. We propose a model in which Sov contributes to the regulation of chromatin organisation as a multifunctional molecular hub for processes that influence different transcriptional events.

**Poster #18**

Ralf Jansen (AT)

**Mechanistic insight into how the piRNA pathway hijacks TFIIA for transcriptional control in heterochromatin.**

Initiation of transcription by RNA polymerase II depends on the assembly of the preinitiation complex at promoters. A key player in this process is the TFIIA heterodimer (comprising TFIIA-L and TFIIA-S), which stabilises the TATA box binding protein (TBP) or related factors such as Trf2 on the promoter DNA. The resulting complex

enables the subsequent recruitment of TFIIB and RNA polymerase II. In the context of the Drosophila piRNA pathway, transcription initiation at heterochromatic piRNA clusters requires the germline-specific TFIIA-L paralog Moonshiner. Moonshiner is recruited to chromatin together with TFIIA-S by the piRNA cluster-defining Rhino-Deadlock-Cutoff complex. Through co-immunopurification experiments in cultured germline stem cells coupled with quantitative mass spectrometry, we unexpectedly discovered that Moonshiner forms a trimeric complex with TFIIA-S and a C-terminal fragment of TFIIA-L. Our data indicate that proteolytic cleavage of TFIIA-L by the endopeptidase Tasp1 is a prerequisite for the formation of the non-canonical Moon-TFIIA complex. Flies harboring a non-cleavable TFIIA-L allele are viable but sterile due to the specific loss of piRNAs from Moon-dependent piRNA clusters and concomitant transposon derepression. This research sheds critical light on the biological function of the highly conserved Taspase cleavage site in TFIIA-L, underscoring its importance as a prerequisite for the formation of cell type-specific alternative TFIIA complexes that facilitate non-canonical transcription initiation.

**Poster #19**

Jiaying Chen (US)

**Piwi regulates the usage of alternative transcription start sites in the Drosophila ovary.**

Alternative transcription initiation, which refers to the transcription of a gene from different transcription start sites (TSSs), is prevalent in mammalian systems and has important biological functions. Although transcriptional regulation has been extensively studied, the mechanism that selects one TSS over others in a gene is still poorly understood. Using the cap-analysis gene expression sequencing (CAGE-seq) method, we identified 87 genes with altered TSS usage in the Drosophila ovary upon loss of Piwi and termed these genes as Altered TSS Usage (ATU) genes. Bioinformatic analysis revealed no differential targeting of Piwi-interacting RNAs (piRNAs) on ATU genes versus non-ATU genes, indicating this Piwi-dependent TSS usage regulation is not guided by piRNAs. RNA Polymerase II (Pol II) ChIP-seq data of germline-specific piwi-knockdown ovaries revealed that Piwi affects Pol II density and binding profile at TSSs of ATU genes. Mass spectrometry of Piwi-immunoprecipitated interactors in the nuclear fraction of fly ovaries revealed several epigenetic factors. These results indicate that Piwi might interact with epigenetic regulators to regulate TSS usage in Drosophila ovaries by affecting Pol II occupancy.

**Poster #20**

Melinda Bence (HU)

**Direct interaction of Su(var)2-10 by SIM-binding site of PIWI protein is required for transposon silencing in Drosophila melanogaster.**

Nuclear PIWI/piRNA complexes mediate co-transcriptional silencing of transposable elements by inducing local heterochromatin formation. In Drosophila, sumoylation plays an essential role in assembly of the silencing complex, however the molecular mechanism by which the sumoylation machinery is recruited to the transposon loci is poorly understood. Here, we show that the Drosophila E3 SUMO-ligase Su(var)2-10 directly binds to the PIWI protein. This interaction is mediated by the SUMO Interacting Motif-like (SIM-like) structure in the CTD domain of Su(var)2-10. We demonstrated that the SIM-like structure binds to a special region found in the MID domain of PIWI protein, whose structure is highly similar to the SIM-binding pocket of SUMO proteins. Abrogation of the Su(var)2-10 binding surface of the PIWI protein resulted in transposon derepression in the ovary of adult flies. Based on our results, we propose a model in which the PIWI protein initiates local sumoylation in the silencing complex by recruiting Su(var)2-10 to the transposon loci.

**Poster #21**

Maria Rosa Garcia Silva (UY)

**PIWI Proteins: Guardians of the Cell Cycle in Colorectal Cancer Models.**

The subfamily of Argonaute proteins called PIWI (P-element inducing Wimpy Testes) is found predominantly in germ line and play a fundamental role in the maintenance and renewal of stem cells. Its canonical function, in this context, is the stability and defense of the genome against transposable elements by binding to small RNAs called piRNAs.
Although the piRNA machinery is mainly expressed in germline cells, there is extensive recent evidence that the expression of PIWIs proteins, not necessarily bound to piRNAs, is aberrantly induced in various types of cancer. Therefore, they are considered as Cancer Testis-derived Antigens (CTAs)

However, the piRNA-independent molecular mechanism by which PIWI proteins contribute to cancer initiation and progression remains unclear. Therefore, in the present work, we intend to highlight the relevance of a non-canonical role of PIWI proteins as new regulators of the cell cycle in somatic cells and their impact on malignant transformation.
We have shown that PIWIL1 is over-expressed in colorectal cancer (CRC) patients. Surprisingly, PIWIL1 in CRC cell lines exhibited a dynamic subcellular localization similar to cell cycle-dependent proteins. Furthermore, PIWIL1 downregulation triggers chromosomal disorders and aneuploidy in this cellular context (Garcia-Silva el at in preparation).
It should be remembered that the epigenetic dysregulation that characterizes tumor cells is analogous in certain aspects to that which occurs in germ cells or in poorly differentiated cells, thus opening up the possibility of re-expression of part of this pathway. Hence, we firmly believe that overexpression of PIWI proteins is relevant for tumor maintenance and progression in somatic cellular contexts as colon crypts.

**Poster #22**

Rajani Gudipatti (PL)

**Picd-1 is a novel factor required for small RNA mediated transposon silencing.**

Genome integrity is constantly threatened by internal and external corrosive agents including transposable elements (TEs) and repetitive sequences. Hence, it is vital to understand the mechanism(s) of TE transposition and its regulation given the prevalence of TEs and their negative effects. An unbiased forward genetic screen led to the identification of a novel gene, f56e10.1 (recently named as picd-1) as an essential factor required for TE silencing. Loss of picd-1 function caused dysregulation of several short regulatory noncoding RNAs, de-silencing of transposons, increased DNA damage, and germ cell death. The mutant animals are fully penetrant sterile when maintained at 26.5℃, and their brood size is significantly less than that of wild type (wt) control animals raised at 25℃. Total RNA sequencing of the RNA extracted from synchronized wt and picd-1 mutant animals of the early L4 developmental stage identified the differentially expressed genes. We have seen widespread dysregulation of several classes of repeat sequences and TEs. Small RNA sequencing from these mutant animals revealed that a subset of piRNAs and 22G RNAs are depleted. Interestingly, we have also found out that the steady state levels of specific Argonaute proteins, that bind various small RNAs, are decreased in picd-1 mutant animals. Additionally, the mutant animals revealed significant DNA damage as revealed by anti-RAD-51 antibody staining. Our results indicate that the loss of picd-1 causes dysregulation of small RNAs and specific Argonaute proteins, de-silencing of TEs and repeat elements, and increased DNA damage, all of which contribute to serious reproductive abnormalities. Our findings establish picd-1 as a novel gene playing an important role in small RNA mediated repression of TEs.