RIPM2025

Symposium on 'Repeat-Induced Point mutation'
1st edition

June 30th – July 1st at the Institute of Biology, University of Neuchâtel

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Mechanisms of recombination-independent homology recognition and heterochromatin-driven RIP in Neurospora

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Our genetic studies of RIP in *Neurospora crassa* uncovered a fundamentally distinct mechanism by which DNA sequence homology can be recognized. This mechanism does not require RecA-like recombinases and appears to rapidly match double-stranded DNA segments directly. The same mechanism may also promote recombination-independent pairing of homologous chromosomes in meiosis. We further found that RIP mutation can be mediated by a conserved pathway that assembles heterochromatin on repetitive DNA in eukaryotes. In *N. crassa*, this pathway includes a SUV39 methyltransferase (DIM-5) and a cytosine methyltransferase (DIM-2). Remarkably, this heterochromatin-related pathway preferentially mutates sequences between closely-positioned repeats and appears to be exquisitely sensitive to their relative orientation: direct repeats promote much stronger DIM-5/DIM-2-dependent RIP compared to inverted repeats. These and other results suggested a model in which DNA supercoiling (that cannot be converted into a plectoneme in the case of direct repeats) provides a physical signal for the DIM-5/DIM-2-dependent RIP. Our current work identified additional factors required for this pathway and suggested that RIP may indeed share its basis with repeat-induced gene silencing (RIGS) observed in somatic/vegetative nuclei.

Exploring the Impact of DNA Methylation on three-dimensional genome architecture in a major wheat pathogen

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Genome structure and maintenance are key determinants of the evolvability of organisms. In eukaryotes, including fungi, genomes are organized within three-dimensional (3D) nuclear space, where chromosomes occupy distinct regions. The genome of the wheat pathogen *Zymoseptoria tritici* is partitioned into gene-rich, transposon-poor core compartments and accessory compartments enriched in species-specific genes and transposable elements (TEs). We recently uncovered the 3D conformation of the *Z. tritici* reference genome, revealing topologically associating domain (TAD)-like structures associated with distinct epigenetic and transcriptomic landscapes. Notably, TAD-like boundary regions were enriched in specific histone modifications, depleted in cytosine DNA methylation, and showed low TE content and RIP signatures. Cytosine DNA methylation plays a critical role in silencing TEs and maintaining genome integrity. In natural populations of *Z. tritici*, the loss of the DNA methyltransferase DIM2 led to a nearly complete loss of cytosine DNA methylation, resulting in TE reactivation [1]. However, the influence of DNA methylation variation on 3D genome conformation remains poorly understood. In this study, we combined Hi-C, genome, transcriptome, and methylome sequencing of two pairs of *Z. tritici* strains with active and inactive DIM2 to investigate how variation in DNA methylation affects genome architecture and 3D organization.

1. Möller, M. *et al*. Recent loss of the Dim2 DNA methyltransferase decreases mutation rate in repeats and changes evolutionary trajectory in a fungal pathogen. PLoS Genet 17, e1009448 (2021).

Exploring the relationships between RIP and sexual development in filamentous ascomycetes

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Since its first description, RIP has been experimentally observed in few fungi but traces of RIP are detected in most of Dikarya genomes making RIP a conserved genome defense mechanism. However, the RIP mechanisms leading to DNA repeat recognition and C to T mutagenesis are still mostly unknown. In *N. crassa*, the RID gene (RIP Deficient), which encodes a DNA methyltransferase-like protein, was shown to be essential to introduce RIP mutations and methylate cytosines in repeats. In the Sordariomycetes *Podospora anserina*, traces of RIP mutations are present in the genome and RIP has been experimentally observed. Deletion of the P. anserina's homologue of RID (PaRid) results in female sterility as observed for *Ascobolus immersus*, *Aspergillus nidulans* and *Trichoderma reesei* but not for *N. crassa*. We showed that in the PaRid mutants, several genes are deregulated, many of them being also under the control of the mating-type transcription factor Fpr1. In, addition, to assay RIP, we developed a read-out based on a spore color-regulating gene using a duplication of a gene involved in melanin biosynthesis, which inactivation causes loss of pigmentation. Thanks to this tool, we established that PaRid is required for RIP in P. anserina. From our results, three hypotheses can be proposed to explain the link between RIP and sexual development: i) RIP is an obligate genome integrity checkpoint which controls karyogamy, ii) PaRid regulates gene expression during this developmental process, eventually via an imprinting system and iii) PaRid has been independently recruited in two unrelated processes. We aim at exploring these hypotheses. To identify new RIP actors, we use our readout tool to assay RIP efficiency in various mutant backgrounds. We also explore we will explore the possibility that introducing point mutations in the PaRid allele may affect either female fertility or the RIP, but not both.

Insights into genomic features and variability of *Cenococcum geophilum*, a highly divergent and widespread ectomycorrhizal morphospecies with a yet unknown life cycle

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Cenococcum geophilum is one of the most common ectomycorrhizal (ECM) fungi in temperate and boreal forests. Yet, the molecular mechanisms underlying its ability to interact with diverse host tree species and adapt to a broad range of habitats remain poorly understood. An improved telomere-to-telomere reference genome assembly reveals that its 178Mbp consists of seven chromosomes, all uniformly covered over 70% by transposable elements (TEs). Almost 90% of these TEs are located in regions affected by RIP, whereas only 25% of genes are found in such regions, excluding the heterothallic mating-type locus. De-novo assemblies of 25 additional strains, representing divergent lineages, indicate high genomic variability, with genome sizes ranging between 127–252Mbp and TE content between 72–84%. Population genomic studies in Switzerland indicate a balanced distribution of the two mating types and the coexistence of highly divergent lineages within populations, although sexual structures have never been observed. Ongoing landscape genomic studies of *C. geophilum* and its associated hosts aim to identify genes and environmental factors involved in local adaptation, particularly those related to drought. Understanding the mechanisms by which this ECM species adapts and the genomic features underpinning this capacity, may inform nature-based solutions to mitigate detrimental effects of climate change.

A reference metagenome sequence of the lichen *Cladonia rangiformis* reveals strong RIP signatures and genome compartimentalization

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Lichens are an ancient symbiosis comprising the thalli of lichen-forming fungi, their photoautotrophic partners and their microbiome. So far they were poorly studied at the genome sequence level. Here, we present a reference metagenome for the holobiont of *Cladonia rangiformis*. Using long read sequences from an entire symbiotic complex, plus short read libraries from 28 additional diverse European lichen samples, we were able to separate genome sequences of 20 individual species. We constructed chromosome-scale assemblies of the *C. rangiformis* fungus and its trebouxioid green algal photobiont *Asterochloris mediterranea*. Furthermore, we isolated 18 near-complete bacterial genomes, of which 13 are enriched in the lichen compared to surrounding soil. The genome of the fungus comprises ~40% transposable elements and is highly compartmentalized into genic regions and large TE-derived segments which show extensive signatures of repeat-induced point mutations (RIP). One of the main questions we wanted to address is how TEs manage to “survive” and proliferate in an organism with such high RIP activity. Although most TEs seem to have undergone multiple rounds of RIP, we identified a few potentially intact copies. However, the intact copies represent families with very low copy numbers, while high-copy families contained no more intact elements. One possible explanation is that new TE families get introduced through horizontal transfer from other Cladonia lichens. This still would leave the question unanswered how and how of often such transfers occur.

Advancing rust genomics with telomere-to-telomere haplotype resolution assemblies for two poplar rust fungi

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Rust fungi are remarkable for their heteroecious life cycle, which includes an asexual stage where dikaryotic spores proliferate clonally. During this phase, two genetically distinct haploid nuclei coexist within the same cell without immediate recombination. This nuclear organization, coupled with a highly repetitive genome, has long posed significant challenges for assembling fully phased, telomere-to-telomere (T2T) rust genomes. However, recent advances in long-read sequencing and chromatin conformation capture (Hi-C) now allow for high-accuracy genome reconstruction, providing unprecedented insights into the genomic architecture of rust fungi. We will present our strategy and the first results to reach high-quality, haplotype-resolved genome assemblies of *Melampsora larici-populina* and *Melampsora allii-populina*, two major poplar rust species. PacBio HiFi long reads and Hi-C scaffolding were used to reconstruct complete haplotypes, in order to provide new insights into chromosomal organization, transposable element dynamics, and genome structure The first fully phased genomes of two *M. larici-populina* and *M. allii-populina* fungi will serve as valuable resources for studying genome organization, structural variation, and haplotype evolution in the fungal order Pucciniales. These data will support comparative genomics and enhance our understanding of rust fungal genome architecture.

Impact of RID and Dim2 on Repeat-Induced Point Mutations in *Zymoseptoria tritici*

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Repeat-induced point mutation (RIP) is a genome defense mechanism in filamentous fungi that introduces C:G to T:A transitions in repetitive DNA, typically during the sexual cycle. The DNA methyltransferases RID and Dim2 are essential for RIP in *Neurospora crassa*, but their roles in other fungi remain unclear. In natural populations of the wheat pathogen *Zymoseptoria tritici*, Dim2 is polymorphic: strains with an active copy show elevated RIP-like mutation signatures, both in nature and after clonal propagation, compared to strains with an inactive allele. This suggests that RIP-like mutagenesis may also occur during mitotic growth. We aim to test for mitotic RIP-like activity in *Z. tritici* and to dissect the contributions of RID and Dim2 to both mitotic and meiotic RIP. To enable phenotypic detection of RIP-like mutations, we introduced a RIP-susceptible construct at a melanization gene in strains with single and double knockouts of *rid* and *dim2*. These strains will be clonally propagated for 25 generations, followed by phenotyping and sequencing to assess mutation rates. In parallel, sexual crosses with compatible strains will evaluate the roles of RID and Dim2 in meiotic RIP. This work will provide the first experimental test of RIP in *Z. tritici* and its underlying mechanisms.

An efficient tool to understand RIP characteristics

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Despite being a crucial mechanism, necessary for sexual reproduction in some species, the RIP mechanism remains unknown. To better understand this mechanism, our team has developed a tool to test the RIP efficiency. It is based on a duplication of the *pks1* gene, which product is involved in melanin synthesis. The duplication is recognized by RIP, resulting in the inactivation of both copies of *pks1*, and therefore, a lack in melanin in spores, appearing white, instead of black. The tool has been improved by introducing the GFP gene driven by a strong and constitutive promoter linked with the *pks1* duplication. RIPed spores appears now green where black ones do not show fluorescence. This makes them easier to detect. Pictures of green and black spores are taken with a fluorescence binocular and are analysed with a CellProfiler script. This method allows the counting of a large amount of spores in a short time and the automation of the counting. Thanks to this tool, I can now (i) describe the RIP characteristics in our model fungus *Podospora anserina* (efficiency, timeframe) and (ii) thanks to a large collection of mutant strains available in the lab, I can test the RIP efficiency in different mutant backgrounds to identify new actors of RIP. Here, I show that (i) RIP efficiency increases with time during spores' projection, and (ii) the implication or not of different potential actors, identified in previous studies in other species.

Transposons impact eukaryotic genome size and evolution

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Horizontal transfer of transposons (HTT) is important for their long-term persistence, but has only been systematically studied in animals, and thus the abundance, impact, and factors that shape HTTs in lineages outside animals is unknown. Fungi are at least as ancient and diverse as animals and are characterized by extensive genome size variation caused by transposons. Here, we screened 1,348 genomes across fungal biodiversity, genome sizes, and lifestyles to detect extensive HTTs, that generated on average 7% but up to 70% of the transposon content in some taxa. We in total identified at least 5,518 independent HTTs, mostly involving Tc1/Mariner DNA transposons. While the majority of HTTs occur between closely related taxa, irrespective of their lifestyles, HTTs were particularly common in Mucoromycotina, Sordariomycetes, Dothideomycetes, and Leotiomycetes. Importantly, species lacking fungal-specific defense mechanisms against transposons and those with gene-sparse and repeat-rich genomic compartments are involved in significantly higher number of HTTs, unveiling ecological and genomic factors shaping HTTs. Our findings thus illuminate the dynamic landscape of HTTs in fungi, providing the framework to further study the impact of HTTs on genome evolution and the processes that mediate transposon transfers within and between eukaryotic lineages.

Loss of RIP and divergence of centromeric architecture in the *Podospora anserina* species complex

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Centromeric DNA is highly variable and rapidly evolving among eukaryotes. In fungi, centromeres range from 125 bp point centromeres to regional centromeres reaching hundreds of thousands of base pairs and filled with transposable elements. In addition, fungi have evolved several specialized defense mechanisms against transposable elements making these regional centromeres intriguing sites for investigating genomic conflict. In this study, we investigated the evolution of the regional centromeres of the *Podospora anserina* species complex, which is made up of seven closely related filamentous ascomycetes. P. anserina has been studied for over a century in a number of genetic and molecular biology topics, one of which is the specialized genome defense mechanism called Repeat Induced Point mutations (RIP). RIP is thought to be ancestral to the ascomycetes and induces C-to-T mutations in repetitive regions thereby disrupting transposable elements. In this study, we discovered that the genome of one species in the complex, *P. pseudocomata*, lacks the signature pattern of RIP. Prompted by this finding we characterized the centromeres of *P. pseudocomata*, using chromatin immunoprecipitation targeting the centromere-specific histone variant cenH3. The recent divergence of the seven members of the species complex then allowed us to bioinformatically compare the centromeric regions among them. We found that the centromeric regions of *P. pseudocomata* are smaller than those of the other species and have different transposable element content. The recent divergence of the species complex and the recent loss of RIP in one of the species makes the P. anserina species complex into a model for studying the impact of genome defense for genome organization and centromere evolution in filamentous fungi.

Widespread and recurrent loss of Repeat-Induced Point mutation in Sordariales fungi

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One of the major contributors to new mutations in eukaryotic genomes are transposable elements (TEs) that are capable of duplicating themselves and inserting at new locations within the genome through a variety of mechanisms. TEs are ancient in eukaryotes and, as a response to their proliferation in genomes, various defense mechanisms, both common and highly specialized types, have evolved. In this study we investigated a fungal-specific defense mechanism known as repeat-induced point mutation (RIP), which induces a C→T mutation in any duplicated sequence in the genome. RIP is thought to be ancestral to the phylum Ascomycota, and it is expected to come with the downside of also targeting duplicated genes, which are an important substrate for adaptation and evolution. Here we investigated genomic RIP-signatures in the order Sordariales, in which RIP was first described, and its relation to TE evolution and gene duplications. We found that out of 81 investigated species, at least 17 lack RIP signature in their genomes, and hence, our data suggest that RIP is less ubiquitous than previously thought. We speculate that losing RIP may not be a long-term viable strategy as we only found it in taxa scattered throughout the phylogeny. By comparing pairs of sister-species differing in RIP signature, we found that absence of RIP is not associated with larger TE abundances, suggesting that there is no short-term effect of losing genome defense for maintaining integrity. Furthermore, a higher number of high-similarity paralogous genes was found in the genomes without RIP-signature, suggesting a benefit of allowing gene duplications.