14th International Conference

October 27-29, 2021
in Strasbourg

Levures, modèles et outils

Abstract book

Invited speakers

Valérie Borde, Institut Curie
Anne Carvunis, University of Pittsburgh
Claudio de Virgilio, Université de Fribourg
Maitreya Dunham, University of Washington
Alain Jacquier, Institut Pasteur
Michael Knop, University of Heidelberg
Benoît Palancade, Institut Jacques Monod
Lars Steinmetz, EMBL Heidelberg

Organizers

Joseph Schacherer
Dom Helmlinger

Wednesday, October 27th

13:30 Opening of the meeting

13:45-15:00 **Population, functional and evolutionary genomics (part I)**
Chair: Gilles Fischer (Sorbonne Université, Paris)

Anne-Ruxandra Carvunis - 30'
"Molecular mechanisms of evolutionary innovation"

Anne Lopes - 15'
"Intergenic ORFs as elementary structural modules of de novo gene birth and protein evolution"

Nikos Vakirlis - 15'
"Evolutionary origins of the *S. cerevisiae* pan-transcriptome"

Philippe Marullo - 15'
"Flor yeasts rewire the central carbon metabolism during wine alcoholic fermentation: consequences on the acidification properties of *S. cerevisiae* strains"

15:00-15:30 **Flash talks — session 1**
Chair: Teresa Texeira (LBMCE, Paris)

Oana Ilioaia
"Extra-telomeric functions of the CST complex"

Isabelle Georis
"Differential requirement for SAGA complex subunits to carry out NCR-sensitive gene expression"

George Kapetanakis
"Overlapping roles of yeast transporters Aqr1, Qdr2, and Qdr3 in amino acid excretion and cross-feeding of lactic acid bacteria"

Sandrine Pinheiro
"Deciphering the regulatory mechanisms responsible for the dynamics of gene expression"

Ambre Noly
"Isolation and characterization of wild fission yeast species"

Anna Marzelliusardottir
"The condensation pathway liberates chromosomes from a retention machinery to allow their proper segregation"

Bechara Zeinoun
"Hog1 and the homeostasis of telomeres during replicative senescence in *Saccharomyces cerevisiae*"

Paul Le Montagner
"Intraspecific variability of surface physico-chemical and bioadhesion properties of *Brettanomyces bruxellensis*"

Nuria Bosch-Guiteras
"Mapping an environmental suppression network of essential genes in yeast"

15:30-16:00 Coffee break

16:00-17:30 **Replication, repair and recombination**
Chair: Bertrand Llorente (CRCM, Marseille)

Valérie Borde - 30'
"Functional links between chromosome structure and recombination during meiosis in *S. cerevisiae*"

Hélène Bordelet - 15'
"Cohesin regulates homology search during recombinational DNA repair"

Christophe De La Roche Saint-André - 15'
"Deciphering how H3K4 methylation influences DNA replication fork progression"

Abhishek Dutta - 15'
"Meiotic recombination from the perspective of the non model *Lachancea waltii* yeast"

Jeanne Le Peillet - 15'
"The Polo kinase Cdc5(Plk1) is regulated at multiple levels in the adaptation response to DNA damage"

17:30-18:00 **Flash talks — session 2**
Chair: Dom Helmlinger (CNRS, Montpellier)

Catherine Tricot
"Identifying transporters involved in tryptophan excretion"

Fabien Duveau
"Mutational sources of regulatory variation in *Saccharomyces cerevisiae*"

Alberto Ballin
"Molecular basis of resistance to the metabolic inhibitor 2-Deoxyglucose in *S. cerevisiae*: identification of mutations affecting AMPK signaling"

Luis Sousa
"Amino acid excretion by yeast cells is promoted at the diauxic shift"

Julia Maria Coronas-Serna
"In search for the role of Cdc42 effectors in ensuring the irreversibility of cell-cell fusion"

Lorenzo Tattini
"Pan-genome references: improving yeast genomics with graph-based tools"

Ludovic Monnin
"Primary metabolites yields variations in wine fermentation among strains of *Saccharomyces cerevisiae* species from different origins"

Karine Casier
"Understanding genomic instability induced by telomere shortering"

18:00-18:45 **"André Goffeau" career award (SFG)**
Alain Jacquier
"RNA degradation and quality control in yeast, mechanisms and consequences"

19:00 Cocktail

Thursday, October 28th

09:00 - 10:30 **Tools, resources and databases**
Chair: Romain Koszul (Institut Pasteur, Paris)

Lars Steinmetz - 30'
"Genomes under construction"

Benjamin Dubreuil - 15'
"Principles of proteins and proteome evolution in the yeast phylum"
Andreas Tsouris - 15'
"Species-wide expressivity survey across a *Saccharomyces cerevisiae* population"

Matthias Le Bec - 15'
"Spatio-temporal control of cooperation in yeast communities"

Basile Jacquel - 15'
"Monitoring single-cell dynamics of entry into quiescence during an unperturbed life cycle"

10:30 - 11:00 **Flash talks — session 3**
Chair: Matthias Peter (ETH, Zurich)

Houssam El Barbry
"Experimental exploration of the role of the conserved N-end signature of mitochondrial precursors"

Hassa Mustapha
"Yeast genetic immunity"

Eléonore Pourcelot
"Development of a model consortium of wine yeasts to explore the impact of diversity on wine fermentation"

Shashank Pandey
"Single-cell analysis of the switch from vegetative to filamentous growth in *S. cerevisiae*"

Sylvain Pouzet
"Optogenetic control of beta-carotene production"

Isabelle Georis
"Relief of NCR in a gdh1 mutant due to altered transporter function?"

Raffaele Nicastro
"Indole-3-acetic acid is a physiological inhibitor of TORC1 in budding yeast"

Prisca Berardi
"The shortest telomere in the cell signals senescence through a probabilistic rather than deterministic mechanism"

Zane Ozolina
"Mutation location in the eukaryotic purine synthesis pathway determines response to nitrogen or purine starvation"

11:00-11:30 Coffee break

11:30-13:00 **Pathogenic yeast and filamentous fungi**
Chair: Sadri Znaidi (Institut Pasteur, Tunis)

Cécile Fairhead - 30'
"Evolution of mating and mating-type switching in the *Nakaseomyces* yeasts"

Adnane Sellam - 15'
"Metabolic reprogramming by chromatin remodelers modulates fungal fitness under hypoxia"

Bernard Turcotte - 15'
"Mitochondrial defects result in decreased susceptibility to echinocandins via the transcriptional regulator Pdr1 in *Candida glabrata*"

Ana Sofia Brito - 15'
"Yeast filamentation signaling is connected to a specific substrate translocation mechanism of the Mep2 transceptor"

Hervé Begue - 15'
"Proteomic analysis of *Candida albicans* to identify new virulence factors"

13:00-14:30 Lunch

14:30-16:00 **Transport, sensing and signaling**
Chair: Sébastien Léon (Institut Monod, Paris)

Claudio de Virgilio - 30'
"Metabolic control of TORC1"

Françoise Roelants - 15'
"Phosphorylation of mRNA-binding proteins Puf1 and Puf2 by TORC2-activated protein kinase Ypk1 alleviates their repressive effects"

Ludovic Enkler - 15'
"The small GTPase Arf1 regulates ATP synthesis and mitochondria homeostasis by modulating fatty acid metabolism"

Anna Babour - 15'
"Nuclear regulation of the UPR"

Gwenael Rabut - 15'
"Quantitative and real-time analysis of protein-protein interactions in yeast: from interactomics to drug design"

16:00-16:30 Coffee break

16:30-18:30 **Gene expression regulation**
Chair: Mathieu Rederstorff (Université de Lorraine, Nancy)

Benoit Palancade - 30'
"Multiple translational controls in the biogenesis of nuclear pore complexes"

Nathalie Bastié - 15'
"Smc3 acetylation, Pds5 and Scc2 control the translocase activity that establishes cohesin dependent chromatin loops"

Jérémy Scutenaire - 15'
"Pho92, an m6A-binding protein facilitating recombination and meiotic progression in the yeast *Saccharomyces cerevisiae*"

Pascal Carme - 15'
"New insights into the function of the Spt3 subunit of the SAGA co-activator from studies in fission yeast"

Serge Pelet - 15'
"Correlation of MAPKa activity and gene expression in single cells"

Sara Andjus - 15'
"Translation in the metabolism of antisense long non-coding RNAs"

Laura Natalia Balarezo Cisneros - 15'
"Functional and transcriptional profiling of non-coding RNAs in yeast reveal context-dependent phenotypes and in trans effects on the protein regulatory network"

Friday, October 29th

09:00-10:30 **Population, functional and evolutionary genomics (part II)**
Chair: Gilles Fischer (Sorbonne Université, Paris)

Michael Knop - 30'
"The unusual life of a yeast: sex and evolution in the absence of recombination"

Matteo de Chiara - 15'
"The budding yeast domestication syndrome"

Francisco Cubillos - 15'
"Phylogenomics analyses of divergent *Saccharomyces* lineages from Patagonia"

Salomé Nashed - 15'
"N-end rule in an evolutionary perspective: identification of a conserved N-end signature essential for the destiny of mitochondrial precursors"

Jolanda Van Leeuwen - 15'
"Systematic mapping of natural variants driving genetic context-dependent gene essentiality"

10:30-11:00 Coffee break

11:00-12:15 **New technologies, yeast and industry**
Chair: Warren Albertin (ISVV, Bordeaux)

Pablo Villarreal - 15'
"Bioprospecting in central Patagonia reveals novel *Lachancea cidri* strains for efficient mead production"

Charlotte Vion - 15'
"NMR analysis of *Saccharomyces cerevisiae* endo-metabolome: comparison of yeast strains of different origins during the alcoholic fermentation of a grape must"

Fanny Bordet - 15'
"*Saccharomyces cerevisiae* discrimination by metabolomics"

Marine Hemmerle - 15'
"Compartment-specific split-fluorescent proteins in baker's yeast"

Jules Harrouard - 15'
"Signatures of phenotypic adaptation: the case of *Brettanomyces bruxellensis*, a yeast associated to anthropized environments"

12:15 - 12:45 **Special iGenolevures talk**
Chair: Cécile Fairhead (Université Paris-Saclay)

Maitreya Dunham - 30'
"Following yeast genome evolution in real time in breweries"

12:45 - 13:00 Singer dissection and flash talk prizes

Closing remarks

13:00 Lunch

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Population, functional and evolutionary genomics (part I)

Intergenic ORFs as elementary structural modules of *de novo* gene birth and protein evolution

Chris Papadopoulos[1], Isabelle Callebaut[2], Jean-Christophe Gelly[3], Isabelle Hatin[4], Olivier Namy[5], Maxime Renard, Olivier Lespinet[6], and ***Anne Lopes***[7]

[1] Institut de Biologie Intégrative de la Cellule – Commissariat à l'énergie atomique et aux énergies alternatives : DRF/I2BC, Université Paris-Saclay, Centre National de la Recherche Scientifique : UMR9198 – France
[2] Institut de Minéralogie et de Physique des Milieux Condensés – CNRS : UMR7590 – Paris, France
[3] Biologie Intégrée du Globule Rouge - Equipe Dynamique des Systèmes et des Interactions des Macormolécules Biologiques (DSIMB) – Université Paris VII - Paris Diderot, Institut National de la Transfusion Sanguine (INTS), Inserm, Laboratoire d'excellence GrEx – 6 rue Alexandre Cabanel 75739 Paris cedex 15, France
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[6] Institut de Biologie Intégrative de la Cellule – Université Paris-Sud - Paris 11, Commissariat à l'énergie atomique et aux énergies alternatives : DSV/I2BC, Université Paris-Saclay, Centre National de la Recherche Scientifique : UMR9198 – Bâtiment 21, 1 avenue de la Terrasse, 91198 Gif/Yvette cedex, France
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The noncoding genome plays an important role in *de novo* gene birth and in the emergence of genetic novelty. Nevertheless, how noncoding sequences' properties could promote the birth of novel genes and shape the evolution and the structural diversity of proteins remains unclear. Therefore, by combining different bioinformatic approaches, we characterized the fold potential diversity of the amino acid sequences encoded by all intergenic ORFs (IGORFs) of *S. cerevisiae* with the aim of (i) exploring whether the structural states' diversity of proteomes is already present in noncoding sequences, thereby investigating the relationship, if any, between the fold potential of the amino acid sequences encoded by IGORFs and the structural diversity of proteins, and (ii) estimating the potential of the noncoding genome to produce novel protein bricks, that can either give birth to novel genes or be integrated into pre-existing proteins, thus participating in protein structure diversity and evolution. We showed that amino acid sequences encoded by most yeast IGORFs contain the elementary building blocks of protein structures. Moreover, they encompass the large structural state diversity of canonical proteins with strikingly the majority predicted as foldable. Then, we investigated the sequence and structural factors determining *de novo* gene emergence with two complementary approaches: (i) we characterized the early stages of *de novo* gene birth by reconstructing the ancestral sequences of 70 yeast *de novo* genes in order to identify the sequence and structural features of IGORFs that indeed gave birth in the past to *de novo* genes, and (ii) we characterized the sequence and structural properties of IGORFs with a strong translation signal through ribosome profiling experiments, in order to identify the properties of candidate IGORFS that could give birth to future novel genes. We showed that ancestral and translated IGORFs display intermediate properties between IGORFs and protein-coding genes and highlighted several sequence and structural features associated with *de novo* gene emergence. In addition, we showed a strong correlation between the fold potential of *de novo* proteins and the one of their ancestral amino acid sequences, reflecting the relationship between the noncoding genome and the protein structure universe. Finally, we showed that most *de novo* genes result from the combination of multiple IGORFs through STOP codon mutations and insertion/deletion events. Altogether, these results enable us to propose a model which gives a central role to IGORFs in the evolution of proteomes where IGORFs can be seen as starting points for *de novo* gene emergence, or protein bricks participating in protein structure evolution and diversity.

Evolutionary origins of the *S. cerevisiae* pan-transcriptome

Nikolaos Vakirlis[1] *and Joseph Schacherer*[2]

[1] Christian-Albrechts University of Kiel – Germany
[2] Génétique moléculaire, génomique, microbiologie (GMGM) – CNRS : UMR7156, Université de Strasbourg

Establishing a species' pan-genome is fundamental to understand its biology and its evolutionary history, both recent and ancient. Adding a transcriptional layer to the pan-genome, establishing the pan-transcriptome, takes us significantly further, as it can reveal expression patterns and reveal the functional importance of the accessory genome. A detailed pan-genome of the budding yeast *Saccharomyces cerevisiae* was recently proposed, based on 1,011 isolates from around the globe. Here, we present a high-quality pan-transcriptome of the species, based on RNA-seq data from 969 of these isolates. Our analysis reveals a rich repertoire of accessory transcripts whose size exceeds that suggested by the pan-genome.Their distribution is sparse, with most of them being present in only a small number of isolates. We discovered hundreds of novel Horizontal Gene Transfer and introgression events from an expanded set of donor species, while at the same time confirming the majority of known ones. Through extensive similarity searches, we uncovered a large number of entirely novel transcripts that appear to have originated since the split of *S. cerevisiae* and its sister species, *S. paradoxus*. Many are highly expressed while others show patterns consistent with birth in specific subpopulations. Our findings emphasize the overall complexity and diversity of the budding yeast accessory transcriptome, and pave the road for future work that will characterize its function in depth.

Flor yeasts rewire the central carbon metabolism during wine alcoholic fermentation: consequences on the acidification properties of *S. cerevisiae* strains

*Emilien Peltier[1,2,3], Charlotte Vion[1,4], Anne Friedrich[3], Joseph Schacherer[3,5], and **Philippe Marullo**[4,6]*

[1] BioLaffort – BioLaffort [Floirac] – France
[2] Université de Bordeaux – Institut des Sciences de la Vigne et du Vin (ISVV) – France
[3] Université de Strasbourg – UMR 7156 GMGM – France
[4] Université de Bordeaux – Institut des Sciences de la Vigne et du Vin (ISVV) – France
[5] Institut Universitaire de France – IUF, (IUF) – France
[6] Biolaffort – BioLaffort [Floirac] – France

The identification of natural allelic variations controlling quantitative traits could contribute to decipher metabolic adaptation mechanisms within different populations of the same species. Such variations could result from human-mediated selection pressures and participate to the domestication. In this study, the genetic causes of the phenotypic variability of the central carbon metabolism of *Saccharomyces cerevisiae* were investigated in the context of the enological fermentation. The genetic determinism of this trait was found out by a quantitative trait loci (QTL) mapping approach using the offspring of two strains belonging to the wine genetic group of the species. A total of 14 QTL were identified from which 8 were validated down to the gene level by genetic engineering. The allelic frequencies of the validated genes within 403 enological strains showed that most of the validated QTL had allelic variations involving flor yeast specific alleles. Those alleles were brought in the offspring by one parental strain that contains introgressions from the flor yeast genetic group. The causative genes identified are functionally linked to quantitative proteomic variations that would explain divergent metabolic features of wine and flor yeasts involving the tricarboxylic acid cycle (TCA), the glyoxylate shunt and the homeostasis of proton. These findings led to the identification of genetic factors that are hallmarks of adaptive divergence between flor yeast and wine yeast in the wine biotope. from an applied point of view these results were used for controling the acidification properties of wine staters by using marker assisted selection with numerous QTLs.

Flash talks - session 1

Extra-telomeric functions of the CST complex

Oana Ilioaia[1], *Jeanne Le Peillet*[1], *Deniz Uresin*[1], *and Zhou Xu*[1]

[1] Biologie Computationnelle et Quantitative = Laboratory of Computational and Quantitative Biology – Sorbonne Universite, Centre National de la Recherche Scientifique : UMR7238, Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique : FR3631 – France

DNA damage, and in particular double-strand breaks (DSB), is a major threat to genome integrity, which eukaryotes deal with by triggering a set of processes to repair or adapt to the damage. Telomeres, the extremities of eukaryotic linear chromosomes, resemble one side of a DSB but must not trigger a DNA damage response (DDR) that would lead to unwanted repair and genome instability. Therefore, they are protected by proteins that inhibit the DDR. One particular complex, composed of Cdc13/Stn1/Ten1 (CST) in *Saccharomyces cerevisiae*, is best known for its essential role in protecting the single-stranded overhang, in assisting replication and in recruiting telomerase. Intriguingly, in addition to canonical telomeric functions, the CST complex also localizes at DSBs where it promotes de novo telomere addition. Moreover, the human CST complex is involved in DSB processing. In this work we aim to investigate the extra-telomeric functions of CST in response to a DSB in yeast, without interference from its telomeric functions. To accomplish this, we take advantage of a strain with a single circular chromosome. We used a yeast strain with all 16 chromosomes fused into one single linear chromosome (Shao et al. 2018), and fused its two remaining extremities together by double CRISPR/Cas9-induced breaks and fusion between the two subtelomeres. Preliminary results show, as expected, that *CDC13*, *STN1* and *TEN1* are no longer essential for a circular genome. Next, we introduced in both the circular and linear chromosome a single inducible DSB at a defined location aiming to investigate the role of CST in DSB metabolism. In addition, a genome-wide screen for essential genes in both strains using SATAY (saturated transposon analysis in yeast; Michel et al. 2017) has been performed on the linear chromosome strain and the circular one. This approach will enable us to have an overview on the fundamental genetic requirements to maintain a linear versus a circular genome and on the factors with dual functions in DSB processing and telomere maintenance, such as the CST complex.

Differential requirement for SAGA complex subunits to carry out NCR-sensitive gene expression

Isabelle Georis[1], *Aria Ronsmans*[2], *Fabienne Vierendeels*[1], *and Evelyne Dubois*[1]

[1] Labiris – Belgium
[2] ULB – Belgium

Transcription by RNA polymerase II relies on PIC (Polymerase Initiation Complex) recruitment. Chromatin constitutes an obstacle for PIC formation, justifying the requirement for gene-specific transcriptional activators and coactivators, whose functions are diverse (adaptor between activators and the transcriptional machinery, histone modification, chromatin displacement, eviction, restructuration...). With its multiple modules, the SAGA complex carries out a certain number of these functions, and Ada1, a core member of the SAGA complex, has previously been shown to contribute to the derepression of genes regulated by Nitrogen Catabolite Repression (NCR). In this work, we have investigated the respective contributions of the different SAGA subunits for NCR-sensitive gene expression: gene and inducer specificities will be discussed.

Overlapping roles of yeast transporters Aqr1, Qdr2, and Qdr3 in amino acid excretion and cross-feeding of lactic acid bacteria

George C. Kapetanakis[1], *Christos Gournas*[2], *Martine Prévost*[3], *Isabelle Georis*[4,5], *and Bruno André*[4,6]

[1] Molecular Physiology of the Cell, Université Libre de Bruxelles (ULB), Biopark, Gosselies – Belgium
[2] Microbial Molecular Genetics Laboratory, Institute of Biosciences and Applications, National Centre for Scientific Research "Demokritos", Agia Paraskevi – Greece
[3] Structure et Fonction des Membranes Biologiques, Université Libre de Bruxelles (ULB), Brussels – Belgium
[4] These authors equally contributed to this work – Belgium
[5] Transport of amino acids, sensing and signaling in eukaryotes, LABIRIS, Brussels, Belgium – Belgium
[6] Molecular Physiology of the Cell, Université Libre de Bruxelles (ULB), Biopark, Gosselies – Belgium

Microbial species occupying the same ecological niche or codeveloping during a fermentation process can exchange metabolites and mutualistically influence each other's metabolic states. For instance, yeast can excrete amino acids, thereby cross-feeding lactic acid bacteria unable to grow without an external amino acid supply. The yeast membrane transporters involved in amino acid excretion remain poorly known. Using a yeast mutant overproducing and excreting threonine (Thr) and its precursor homoserine (Hom), we show that the Aqr1, Qdr2, and Qdr3 proteins of the Drug H+-Antiporter Family (DHA1) family mediate excretion of both amino acids. We further investigated Aqr1 as a representative of these closely related amino acid exporters. In particular, structural modeling and molecular dock- ing coupled to mutagenesis experiments and excretion assays enabled us to identify residues in the Aqr1 substrate-binding pocket that are crucial for Thr and/or Hom export. We then co-cultivated yeast and *Lactobacillus fermentum* in an amino-acid-free medium and found a yeast mutant lacking three DHA1 family members to display a reduced ability to sustain the growth of this lactic acid bacterium, a phenotype not observed with strains lacking only one of these transporters. This study highlights the importance of DHA1 transporters in amino acid excretion and reveals the role of these proteins in mutualistic interaction with lactic acid bacteria.

Deciphering the regulatory mechanisms responsible for the dynamics of gene expression

Sandrine Pinheiro[1] *and Serge Pelet*[1]

[1] Department of Fundamental Microbiology, University of Lausanne – Switzerland

A key question in developmental biology is how cell fate is determined. To investigate the molecular mechanisms involved in such a complex process, the mating pathway of *Saccharomyces cerevisiae* serves as a simplified model. In budding yeast, a Mitogen-Activated Protein Kinase (MAPK) signal transduction pathway regulates the activation of a complex expression program necessary for mating. Gene expression is regulated by one main transcription factor Ste12 which is capable of site specific binding to the consensus DNA motif 5'-TGAAACA-3', commonly termed PRE. The high diversity of PRE-arrangements present in the promoter sequence of mating genes appears as a strategy by which a single transcription factor can modulate the timing and the expression level of a set of genes. Because the dynamics of gene expression are important for achieving proper cell fate de- termination, it is crucial to understand how the promoter architecture and the underlying molecular mechanisms control gene expression dynamics. To decipher the PRE-combinations decoded by Ste12 that dictate the speed of induction of mating genes, we engineered a synthetic pheromone-responsive promoter. The use of synthetic promoters allows to test several PRE-conformations to elucidate the rules governing Ste12 DNA binding and transcription induction. Our data demonstrate for instance that two consensus PREs spaced by 3nt in a tail-to-tail orientation allow for fast gene induction. Further analyses will be carried out to study the impact of variations for this ideal conformation on the gene expression, such as the distance from the TATA box, the orientation of the PRE or point-mutations that may affect Ste12 binding affinity. Overall, understanding how a promoter sequence encodes the level and timing of gene expression based on TF binding sites organization in yeast may give insights on a regulatory mechanism used by eukaryotic cells to regulate cellular processes such as cell differentiation.

Isolation and characterization of wild fission yeast species

Ambre Noly[1]*, Frédéric Landmann*[1]*, and Dominique Helmlinger*[1]

[1] CRBM – CNRS : UMR5237, Université Montpellier II - Sciences et Techniques du Languedoc – France

Understanding the mechanisms governing gene expression is a fundamental question in biology. Our knowledge comes mostly from studies done in a handful of model organisms, particularly the budding yeast *Saccharomyces cerevisiae* and, to a lesser extent, the fission yeast *Schizosaccharomyces pombe*. Laboratory strains originate from domesticated ancestors and, for practical reasons, have been selected for traits that are not fully representative of their wild relatives and of their lifestyle in natural habitats. This domestication might explain seemingly paradoxical findings. For instance, some factors appear essential to transcription regulation in vitro, yet, the corresponding loss-of-function mutants do not show apparent phenotypes. One possibility is that these strains do not fully represent the diversity of gene regulatory mechanisms, highlighting the importance of characterizing the genetic and phenotypic diversity of wild relatives of laboratory strains. Whereas several studies have tackled this question using Saccharomyces species, much less is known about the ecology and natural history of the phylogenetically distant fission yeasts. In order to describe their habitat and their genetic and phenotypic diversity, we developed a protocol to isolate wild fission yeast species. Specifically, we used the ability of generalist pollinators, such as bees, to collect nectar from flowers and to store it in the form of honey, to isolate yeast from the phyllosphere. This strategy allowed us to identify several fission yeast species, despite their poor abundance in the wild. Furthermore, as a proof of concept, we confirmed that some of these yeast species are present in the crop, which is the specialized organ in which bees store the collected nectar. Finally, we will present a preliminary phenotypic characterisation of the species identified. We will discuss how the characterisation of these yeasts will contribute to better understand the diversity of mechanisms controlling gene expression and chromatin organisation in this clade.

The condensation pathway liberates chromosomes from a retention machinery to allow their proper segregation

Anna Marzelliusardottir[1], *Anne C. Meinema*[1], *and Yves Barral*[1]

[1] ETH Zurich – Switzerland

In addition to 16 chromosomes, the budding yeast *S. cerevisiae* also harbours various other DNA elements, such as extrachromosomal DNA circles (ERCs). While chromosomes are symmetrically segregated between mother and daughter cells in mitosis, ERCs are asymmetrically retained in the mother cell by being tethered to nuclear pore complexes (NPCs). Like ERCs, chromosomes interact extensively with NPCs and other factors at the nuclear envelope (NE). Previous studies have shown that in contrast to ERCs, chromosomes need to detach from the NE prior to mitosis in order to be properly segregated. But how cells distinguish between chromosomes and ERCs in mitosis is not known. Interestingly, while chromosomes condense in mitosis, DNA circles (which are used to model ERCs) do not. Moreover, when DNA circles are forced to condense, they no longer stay attached to the NPCs during mitosis resulting in their random segregation. This indicates that condensation might promote the detachment of DNA from the NE and facilitate its symmetric partitioning. Supporting this hypothesis, we found that mutants which fail to condense their chromosomes also fail to detach their chromosomes from the NE in mitosis and have elevated levels of aneuploidy. Furthermore, we identified the nuclear pore basket component Nup60 as a potential 'DNA detachment factor'. Namely, tethering Nup60 to DNA circles causes them to be released from NPCs and randomly segregated. In addition, when Nup60 is deleted, chromosomes are closer to the periphery and are increasingly mis-segregated towards the mother compartment. Together, our data suggest that the condensation pathway and the nuclear basket liberate chromosomes from mother cell retention and thereby facilitate their symmetric segregation.

Hog1 and the homeostasis of telomeres during replicative senescence in *Saccharomyces cerevisiae*

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Telomeres protect linear chromosomes from fusions and degradation. However, the protective functions of telomeres are put at risk when they shorten as the cell divides without telomerase. When telomeres reach a critically short length, they trigger a DNA damage checkpoint-dependent cell cycle arrest in a process called replicative senescence. Data obtained in different organisms show that mitochondrial defects and oxidative stress accelerate telomere shortening and dysfunction. However, how ROS levels are produced and controlled during senescence is still elusive. In *Saccharomyces cerevisiae*, Hog1, the orthologue of p38 MAPK, is a major factor protecting against osmotic stress and regulating ROS levels. Hog1 activates the transcription of detoxifying genes but also interferes with mitochondrial functions and increases the levels of ROS. Here we show that in telomerase-negative cultures, ROS levels increase before senescence crisis, coinciding with the activation of Hog1. Deletion of Hog1 accelerates senescence demonstrating its contribution to cell viability in the absence of telomerase. Adding antioxidants delays senescence and partially suppresses the acceleration of senescence in hog1cells, suggesting a role of Hog1 in regulating ROS levels during this process. ROS levels also increase during senescence when Mec1, the major DNA damage checkpoint in budding yeast, is mutated. However, Mec1 and Hog1 act on ROS through two independent signaling pathways that cooperate to keep ROS in check during senescence.

Intraspecific variability of surface physico-chemical and bioadhesion properties of *Brettanomyces bruxellensis*

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Brettanomyces bruxellensis is a ubiquitous yeast with different fermentation media such as beer and cider where its presence is beneficial and brings an aromatic typicity. Furthermore, its presence in wines is feared because it is considered as a spoilage yeast producing 4- ethyl phenols responsible for organoleptic deviations causing significant economic losses for the sector. The winery is considered like the first source of contamination (Alguacil et al., 1998; Connel et al., 2002). Indeed, it is possible to find *Brettanomyces* in the air, on walls and floors of the cellar, on small wine material, as well as on vats and barrels. Furthermore, specific strains were isolated recurrently in wines of certain winery (Cibrario et al., 2020). Bioadhesion phenomena and biofilm formation could explain the persistence of this yeast in winery and recurrent wine contaminations. A subset of 50 *Brettanomyces bruxellensis* strains, representative of the species genetic diversity (Avramova et al., 2018; Cibrario et al., 2019) were selected to study the intraspecific variability in physico-chemical surface parameters and bioadhesion properties. The determination of hydrophobic character (MATS) and electronegativity (zeta potential) were carried out upstream of the microscopic observation of bioadhesion on stainless steel coupon by confocal microscopy.First results showed a hydrophilic behavior and a negative surface charge but with variation depending on the strain considered. Regard to bioadhesion, contrasting behaviors were observed according to the genetic groups with variable cell morphologies. In the future, these first results should allow an optimization of cleaning procedures adapted to each bioadhesion behaviour.

Mapping an environmental suppression network of essential genes in yeast

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Genes are typically classified as essential based on particular laboratory control conditions. Nevertheless, the phenotypic effect of the lack of an essential gene can be modified by environmental factors, implying that a proportion of the genes catalogued as essential may be dispensable under certain conditions. To systematically assess the fraction of environment-dependent essential genes, we screened complete deletion alleles of 873 genes (~80% of all essential genes in yeast) under 21 environments targeting a variety of fundamental biological pathways and processes including the maintenance of genome integrity, RNA and protein expression, and osmotic, oxidative and reductive stress response pathways. Our preliminary analyses suggest that 1 to 6% of the essential genes are not required for viability in a specific environment, revealing interesting gene candidates with a strong environment-specific suppression effect. For example, we found that specific glycolytic enzymes were no longer essential in the presence of sorbitol as carbon source, and that several proteins with a role in releasing nuclear export proteins from ribosomal subunits were not required for viability at elevated temperatures. Together with further analyses and validations our aim is to provide a novel network of high-confidence environmental suppression interactions in yeast.

Replication, repair and recombination

Cohesin regulates homology search during recombinational DNA repair

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Homologous recombination repairs DNA double-strand breaks (DSB) using an intact ds-DNA molecule as a template. It entails a homology search step, carried out along a conserved RecA/Rad51-ssDNA filament assembled on each DSB ends. Whether, how, and to what ex- tent a DSB impacts the spatial organization of chromatin, and how this (re)organization in turns influences the homology search process, remain ill-defined. Here we characterize two layers of spatial chromatin reorganization following DSB formation in *S. cerevisiae*. While cohesin folds chromosomes into cohesive arrays of ~20kb-long chromatin loops as cells arrest in G2/M, the DSB-flanking regions interact locally in a resection- and 9-1-1 clamp-dependent manner, independently of cohesin, Mec1ATR, Rad52 and Rad51. This local structure blocks cohesin progression, constraining the DSB region at the base of a loop. Functionally, cohesin promotes DSB-dsDNA interactions and donor identification in cis, while inhibiting them in trans. This study identifies multiple direct and indirect ways by which cohesin regulates homology search during recombinational DNA repair.

Deciphering how H3K4 methylation influences DNA replication fork progression

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DNA replication is a highly regulated process that occurs in the context of chromatin structure and is sensitive to several histone post-translational modifications. In *Saccharomyces cerevisiae*, the histone methylase Set1 is responsible for the transcription-dependent methylation of histone H3 lysine 4 (H3K4me) throughout the genome. In recent years, we have been interested in the role of Set1 during DNA replication. We have shown that Set1, along with other chromatin modifiers and remodelers, promotes chromatin accessibility at stalled replication forks (1). This facilitates DNA processing and cohesion loading that facilitates the resumption of DNA replication. Our latest published work shows that H3K4me limits the occurrence of replication-transcription conflicts when replication kinetics is in- creased in response to a replication initiation defect (2). Preliminary results suggest that spontaneous duplication of a particular chromosome mitigates some of the negative effects associated with loss of Set1 in this mutant context. This may result from overexpression of one or more proteins encoded by the duplicated chromosome, as has been shown in other settings (3). We plan to identify, among candidate genes, those whose expression and/or copy number modification can correct the S phase defect in the same mutant context. In this way, we hope to uncover part of the mechanism underlying the influence of Set1 and H3K4 methylation on fork progression during normal replication or in response to replicative stress.

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Meiotic recombination from the perspective of the non-model *Lachancea waltii* yeast

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Meiotic recombination is a driving force for genome evolution, deeply characterized in a few model species, notably in the budding yeast *Saccharomyces cerevisiae*. Interestingly, Zip2, Zip3, Zip4, Spo16, Msh4, and Msh5, members of the so-called ZMM pathway that implements the interfering meiotic crossover pathway in *S. cerevisiae*, have been lost in *Lachancea* yeast species after the divergence of *Lachancea kluyveri* from the rest of the clade. In this context, after having determined the meiotic recombination landscape of *L. kluyveri*, we determined the meiotic recombination landscape of *Lachancea waltii*. Besides an intermediate recombination rate compared to *S. cerevisiae* and *L. kluyveri*, we found an elevated frequency of zero-crossover bivalents and, surprisingly, a weak crossover interference signal in *L. waltii*. However, this weak crossover interference signal is reminiscent of an interference signal resulting from even DNA double strand break patterning. In addition, we found that meiotic recombination hotspots are not conserved between *L. waltii*, *L. kluyveri,* and *S. cerevisiae*, showing that meiotic recombination hotspots can evolve at a rather limited evolutionary scale within budding yeasts.

The Polo kinase Cdc5(Plk1) is regulated at multiple levels in the adaptation response to DNA damage

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DNA damage is a severe threat to genome integrity. When the DNA of a cell is damaged, the DNA damage checkpoint detects the injury and halts the cell cycle, thus providing time for repair. However, if the damage is not repaired after several hours, yeast cells eventually resume their cell cycle. This process, called adaptation to DNA damage, seems to act as the last opportunity for cell survival, although it promotes mutations and genome instability in the daughter cells. Consistently, we recently discovered that adaptation contributes to the increase of mutation rate in senescence. Evidence also suggests that cancer cells may have enhanced adaptation mechanisms, which may contribute to the accumulation of mutations necessary for tumour progression and resistance to chemotherapy. In budding yeast, the Polo-kinase Cdc5, which is involved in many cell-cycle related processes, is critical for adaptation. However, its specific targets during adaptation are not clearly defined yet, and its exact role in this process remains unclear. To provide insights into these questions, we investigate how telomere dysfunction, which induces a DNA damage response, regulates Cdc5. We find that the amount of Cdc5 decreases over time following telomere dysfunction and this downregulation is a direct consequence of checkpoint activation, through the activity of the effector kinase Rad53 and transcription factor Ndd1. Both Cdc5 and the adaptation- deficient mutant Cdc5-ad are phosphorylated in response to telomere dysfunction in a Mec1- and Tel1-independent manner. Some of these phosphosites modulate adaptation efficiency. In addition, we show that PP2A phosphatases are also involved in adaptation. These results indicate that Cdc5 is regulated at the transcriptional and post-translational level in the adaptation response to telomere dysfunction. Further genetic analysis of adaptation-deficient alleles of CDC5 suggests that Cdc5 acts through multiple pathways. We thus performed a genome-wide screen to uncover potential substrates of Cdc5 involved in adaptation, using a saturated transposon analysis method. In-depth characterization of the candidate genes found in this screen will help to unravel the complex function(s) of Cdc5 in adaptation.

Flash talks - session 2

Identifying transporters involved in tryptophan excretion

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Amino acids are among the most abundant nitrogenous compounds in all cells. Their uptake across the plasma membrane involves transporters that have been extensively investigated in many species, including yeast, for decades. Under particular conditions, *S. cerevisiae* can also excrete amino acids, notably when they are overproduced and when their reuptake is impaired. We have now found that tryptophan (Trp) excretion can be detected in a strain lacking the general amino acid permease (Gap1) and the Ssy1 sensor, responsible for the transcriptional activation of several other amino acid permeases. Excretion of Trp is also visible in a strain expressing a feedback-insensitive mutant form of anthranilate synthase (TRP2FBR), the enzyme catalyzing the initial step of Trp biosynthesis. As anticipated, combining in the same strain the gap1, ssy1 and TRP2FBR mutations further strengthened the Trp excretion phenotype. Furthermore, we have identified several members of the DHA1 (Drug H+ Antiporter 1) family which, when overexpressed in a ssy1 mutant, confer increased Trp excretion, suggesting that these transporters mediate tryptophan excretion. Further results of experiments aimed at identifying Trp excretion proteins will be presented.

Mutational sources of regulatory variation in *Saccharomyces cerevisiae*

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Gene expression variation can play an essential role in phenotypic evolution and diseases, but little is known about the genetic changes that contribute to heritable expression differences among individuals. In particular, new trans-regulatory mutations that alter the expression of a distant gene are difficult to study because they can be potentially located anywhere in the genome and because their impact on expression can be subtle. Here, we used genetic mapping approaches based on bulked segregant analysis and deep sequencing (BSA-Seq) to identify 69 mutations that altered the mean expression level and 4 mutations that altered the expression noise of a YFP reporter gene expressed under control of the yeast TDH3 promoter (pTDH3-YFP). The trans-regulatory effects of individual mutations on pTDH3-YFP expression were confirmed by site-directed mutagenesis. We found that these trans-regulatory mutations were enriched in coding sequences of transcription factors previously predicted to regulate the activity of the TDH3 promoter, but that the vast majority of mutations mapped to other types of genes involved in diverse biological processes such as metabolism, chromatin state and iron homeostasis. This finding suggests that regulatory networks describing the interactions between transcription factors and target genes might only capture a small fraction of the potential sources of expression changes. We also observed an enrichment of trans-regulatory mutations in genomic regions associated with natural variation of TDH3 expression (previously characterized eQTL regions). Therefore, this work helps understand how trans-regulatory mutations that give rise to expression variation segregating in natural populations are structured within the genome and within a regulatory network.

Molecular basis of resistance to the metabolic inhibitor 2-Deoxyglucose in *Saccharomyces cerevisiae*: identification of mutations affecting AMPK signaling

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The yeast *Saccharomyces cerevisiae* and cancer cells share a peculiarity: they preferentially drive a fermentative metabolism. The strong reliance on glucose makes them sensitive to the metabolic inhibitor 2-deoxyglucose (2-DG), which has been proposed as an anticancer drug in combinatorial therapies. 2DG resistance can be acquired in HeLa cells. Yeast also develops resistance to the molecule at a high rate. Most of the mutations causing 2-DG resistance characterized to date involve an increased activity of the yeast orthologue of the AMPK nutrient-signaling kinase, Snf1. This study investigates the molecular basis of resistance to 2-DG with two purposes: identify new mechanisms underlying this phenomenon and gain insights into glucose-signaling pathways. First, an unbiased genetic screen has been carried out and 195 spontaneous resistant mutants have been isolated. 51 mutant alleles have been identified in 9 genes via targeted gene sequencing. Most of these encode for actors of the Snf1-regulated glucose-repression pathway, regulating gene expression according to glucose availability. Among them, we found 17 missense alleles of REG1, encoding a regulatory subunit of the protein phosphatase 1 (PP1) complex which is a negative regulator of Snf1. To carry out their functional characterization, tagged versions of the Reg1 mutant proteins were cloned and expressed in REG1-deleted cells. The activation of Snf1, via evaluation of its phosphorylation and the expression of downstream glucose-repressed genes were tested. Most of the mutant proteins do not lead to aberration in those aspects. This suggests the existence of novel mechanisms of 2-DG resistance, which are worth further investigation.

Amino acid excretion by yeast cells is promoted at the diauxic shift

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Amino acids are the most abundant nitrogen compounds in all living organisms, and yeast transporters catalyzing their uptake across the plasma membrane have been widely studied. However, and surprisingly, yeast is also able to excrete amino acids. In 2004, we have characterized the first yeast amino-acid excretion protein, Aqr1 (1), which belongs to the Drug H+-Antiporter (DHA1) family (2). More recently, we have shown that Qdr2 and Qdr3, two other members of this transporter family, also function as amino-acid export proteins (5). The DHA1 family include 12 members many of which were reported to mediate excretion of xenobiotics. Agp1 is an amino acid permease whose gene is transcriptionally induced in the presence of external amino acids (3). This induction is dependent on Ssy1, a permease-like sensor specialized in detection of external amino acids (4). Aiming to develop an amino acid excretion proxy, we used AGP1 transcription as a readout of external amino acid accumulation. We observed that this proxy responds with a higher sensitivity when used in cells lacking Gap1, the general amino acid permease. We observed that excretion of amino acids is induced at the diauxic shift, time at which cells face an extensive metabolic reprogramming by switching their carbon source utilization from glucose to ethanol. How- ever, when using a strain deleted for all the 12 DHA1 family genes (12dha1 D), we observed a strong reduction of amino acid excretion. Additionally, we also observed that the 12dha1 D strain displays an exponential growth rate similar to the w-t strain, but enters the diauxic shift phase – decreasing its growth rate – at a much lower cell density than the w-t. Our results suggest that amino acid excretion by yeast is a natural phenomenon driven by export proteins of the DHA1 family and playing a crucial role in cells facing glucose exhaustion conditions.

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In search for the role of Cdc42 effectors in ensuring the irreversibility of cell-cell fusion.

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In yeast mating, as in other eukaryotic species, cell-cell fusion is a prerequisite to forming a zygote. This is a one-way process, whose irreversibility is crucial. The Rho-family GTPase Cdc42 and its p21-associated kinase (PAK) effectors act in cell fusion, besides orchestrating cell polarization. We work with fission yeast *Schizosaccharomyces pombe*, as its sexual cycle is a well-established model to understand cell-cell fusion. After pheromone signaling and shmoo formation, the two opposite mating-type cells come into close contact. At this stage, the cell wall has to be locally digested to enable plasma membrane merging. Recent evidence shows that the deletion of the Pak2/Shk2 PAK (a homolog to budding yeast Ste20, Cla4, and Skm1) produces transient fusion events, with plasma membranes eventually re-sealing (Vjestica A, et al. Nature. 2018. PMID: 30089908). This indicates that Pak2 ensures the irreversibility of the process. A hypothesis for this function is that Pak2 coordinates precise cell wall removal at the fusion through control of the cell wall integrity MAPK pathway. To test this idea, we present a plan to explore the functions of Pak2, identify its substrates during cell fusion and characterize the ultrastructure of the resealing membranes. This will be achieved by mixing structure-function studies and synthetic biology, with cutting-edge optogenetic, phospho-proteomic, and correlative light-electron microscopy methods. Cdc42 signaling is conserved from yeast to mammals, and PAKs participate in various models of eukaryotic cell-cell fusion, suggesting that studying it in fission yeast will yield generally relevant information. Overall, this plan is designed to open new avenues to understand how the directionality of cell-cell fusion events is guaranteed, as well as the roles that PAK-family kinases may play in this context.

Pan-genome references: improving yeast genomics with graph-based tools

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Resequencing studies are based on mapping short-reads against a reference genome. Short-reads mapping shows intrinsic limits, e.g. in the context of hybrid genomes or for the study of gene flow processes, such as introgression and horizontal gene transfer (HGT). In fact, only one version of each locus can be represented in a conventional linear reference. Thus, resequencing studies are biased against the observation of genomic variants involving sequences which are not included in the reference genome.
A pan-genome is a general solution that includes shared sequences and as well as rare and private variation, from many different individuals, combining them into a comprehensive framework that can be possibly extended to any taxonomic level. The pan-genome can be modelled as a variation graph and used to improve the functionality traditionally provided by linear reference genomes. Our research is currently focused on graph-based approaches to improve the study of several fields of yeast genomics including phylogenetics, genome evolution, and functional genomics.
While we have recently gathered the most complete eukaryotic pan-genome by high-coverage sequencing of 1011 *S. cerevisiae* isolates, we are currently expanding our dataset using: (1) other species from the Saccharomyces genus, e.g. S. paradoxus, (2) intraspecies and inter- species hybrids evolved under different experimental conditions, (3) introgressed clades, as well as (4) strains bearing HGTs. In this work we show how pan-genome variation graphs can be used to characterise several genomic features, such as sequence divergence, structural variants, introgressions, and HGTs, by means a single and comprehensive data structure.

Primary metabolites yields variations in wine fermentation among strains of *Saccharomyces cerevisiae* species from different origins

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Alcoholic fermentation in wine is mostly carried by yeasts from the *Saccharomyces cerevisiae* species from indigenous origin or added by the winemaker. As the major step of winemaking, it shapes wine properties by its direct and indirect products. In the last 30 years, a high interest has been given to yeast central carbon metabolism to understand and eventually reroute carbon fluxes in the cell with the primary aim to reduce ethanol yield of wine fermentation. This target has been driven by the constant need of winemakers to adapt to the wine market. Conjointly, a great interest has been given to non-GM technics, which have a better acceptance from society and have already showed their efficiency. However, these technics require a natural variation on the chosen phenotype and an evaluation of this variation as a prerequisite. Our study aims to define natural phenotypic variation of primary metabolites yield from a wine-like alcoholic fermentation, with a focus on ethanol, glycerol, succinate, and acetate, on *S. cerevisiae* strains from different origins (from techno- logical environment or natural isolate). Our results point out the existence of a great range of variation for glycerol, succinate and acetate yields among our set of strains. Ethanol, even being the first fermentation product by concentration and yield, shows a very low but statistically significant range of variation. Succinate seems to be the metabolite with the more diversity of production. Correlation between ethanol and glycerol yields, which has been clearly established in precedent works, has been confirmed. Finally, our study shows that extreme values of glycerol and ethanol yields in modified or evolved strains are reachable in wine-like fermentation. Therefore, there is room for improvement of natural oenological strains.

Understanding genomic instability induced by telomere shortering

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Telomeres protect chromosomes from fusion and degradations, but this function is threatened in human somatic cells where telomeres are progressively shortened after successive cell divisions in the absence of telomerase activity. When telomeres are critically short, cells enter replicative senescence, in which the combined actions of the DNA damage signaling pathway, the DNA repair pathway, and mitosis mechanisms, can occasionally cause genomic instability and initiate oncogenesis. A difficulty associated with the study of mechanisms involved in genomic instability is the variability in telomere length, the intercellular variation in replicative senescence and the rarity of pathological events. The objective of this project is to study the initiation, expansion and consequences of genomic instability induced by telomere erosion. To circumvent the heterogeneity of genomic instability caused by telomere erosion, we are using a tool derived from CRISPR-Cas9 technology to generate telomeres of defined length in *Saccharomyces cerevisiae*. This tool will be used to study the global chromatin and epigenetic changes associated with telomere erosion that may contribute to the emergence of genomic instability.

Tools, resources and databases

Principles of proteins and proteome evolution in the yeast phylum

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The differential conservation of amino-acid residues in protein sequences is widely used to identify catalytic residues, binding interfaces, sites of post-translational modification, to predict deleterious effects of genetic variants, or to guide in protein design. As such, it is critical to understand the factors that influence sequence conservation, both within and across proteins, i.e. at the residue and protein levels. To that aim, we carried out a proteome-wide analysis of protein evolution in the fungal lineage, which revealed several evolutionary principles. (i) Disordered regions in proteins evolve 2.3 times faster than domain regions and this ratio appears constant among proteins expressed at widely different levels (Dubreuil and Levy 2021). This is surprising, because disordered and domain residues experience different structural-biophysical constraints thought to vary with expression. (ii) We analyzed the determinants of protein evolutionary rates in the fungal lineage and found there exists a strong protein-specific constraint contributing to sequence conservation that is independent of abundance. To examine the origin of this protein-specific effect, we created the yeastOmics repository, which integrates thousands of genomics, transcriptomics, biophysical, structural, and functional information on *Saccharomyces cerevisiae*'s proteome. We identified 12 categorical and 22 quantitative features that together capture most of this protein-specific effect. The combination of those features explained 66% of the total variance in protein evolutionary rate, of which 27% is not related to protein abundance. Together these results will help decipher the forces shaping protein conservation, which is key both for the fundamental knowledge it represents and to allow for optimal use of evolutionary information in practical applications.

Dubreuil B, Levy ED. 2021. "Abundance Imparts Evolutionary Constraints of Similar Mag- nitude on the Buried, Surface, and Disordered Regions of Proteins." Frontiers in Molecular Biosciences 8 (April): 626729.

Species-wide expressivity survey across a *Saccharomyces cerevisiae* population

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Understanding the genetic basis of traits, the underlying genetic complexity and how it varies depending on the genetic background is of prime interest to gain better insight into the genetic architecture of traits. The dichotomy between monogenic and complex traits is overly simplistic as the genetic complexity of a trait can change depending on the genetic background of the individual. Such variations in complexity can often lead to phenotypic expressivity. Until now, no systematic and species-wide assessment of this phenotypic expressivity has been performed.To assess the prevalence of phenotypic expressivity at a population scale, we constructed a half-diallel panel by pairwise crossing genetically diverse *Saccharomyces cerevisiae* natural isolates producing 190 unique hybrids. A haploid progeny of 160 individuals was produced by each hybrid and its mitotic growth was measured in 50 conditions, considered as 50 traits. As the phenotypic distribution of the progeny of a given cross allows to infer the inheritance patterns of a trait in that genetic background, we evaluated these patterns for 9,450 cross/trait combinations and found that 86% displayed complex inheritance, 4.4% monogenic inheritance and 9.6% oligogenic inheritance. In addition, we identified 26 major effect loci for various traits. A special case of variable complexity revealed the dual effect of the *GAL3* gene on growth in galactose media. Some *GAL3* alleles have a high effect and cause large shifts in the phenotype, whereas other *GAL3* allele act as a modifier gene and influence the phenotype only under certain genetic backgrounds. Altogether, these results lay the foundation for a more comprehensive exploration of the genetic variants causing variable complexity and therefore phenotypic expressivity. The dissection of the genetic basis of the different cases observed gives us a better insight into how phenotypic expressivity can occur in natural populations.

Spatio-temporal control of cooperation in yeast communities

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The division of labor is the separation of a system into different parts specialized in one or multiple tasks. This concept is observed in social economics, in multicellular organism or ecosystems, and especially in microbial communities where the complementarity of different species can result in a more productive and robust consortium. Even among clonal colonies, nutrient uptake, inhibitory chemicals excretion or chemical communication considerably affect the individual microenvironment leading to cell-to-cell phenotypic differentiation. While recent works make effort toward designing rational microbial communities, there is however few established methodologies to control, especially spatially, these microbial associations. Here, we aim at studying the role of spatial patterning of cooperation in a microbial population. We chose to control the degree of cooperation of *Saccharomyces cerevisiae* cells in a monoclonal population. For this we use the invertase enzyme (*SUC2*) as a mean for the optogenetic control of public goods production. This enzyme, embedded in the cell wall, allows yeast to grow on sucrose by catalyzing its hydrolysis into usable glucose and fructose, that can diffuse and be shared between cells. We built an optogenetic strain producing Suc2 enzyme upon blue illumination and optimized its induction range. We developed a method to perform simultaneous light patterning and time-lapse recording of yeast growing on agar-plate. We can thus control quantitatively in space and time the production of the Suc2 enzyme, hence the spatial pattern of cooperation in the microbial community. We assessed the influence of the pattern on the global growth behavior and compared our results with a diffusion-reaction model. Our first results outline the fact that our system acts as a spatial low-pass filter with a typical cooperation distance between illuminated and non-illuminated cells, evidencing the importance of microbial species patterning for ecosystem dynamics. Our project provides new insights in the spatial organization of microbial communities by developing new tools with enhanced control over biological parameters, toward a better understanding of microecology.

Monitoring single-cell dynamics of entry into quiescence during an unperturbed lifecycle

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The life cycle of microorganisms is associated with dynamic metabolic transitions and complex cellular responses. In yeast, how metabolic signals control the progressive choreography of structural reorganizations observed in quiescent cells during a natural life cycle re- mains unclear. We have developed an integrated microfluidic device to address this question, enabling continuous single-cell tracking in a batch culture experiencing unperturbed nutrient exhaustion to unravel the coordination between metabolic and structural transitions within cells. Our technique reveals an abrupt fate divergence in the population, whereby a fraction of cells is unable to transition to respiratory metabolism and undergoes a reversible entry into a quiescence-like state leading to premature cell death. Further observations reveal that non-monotonous internal pH fluctuations in respiration-competent cells orchestrate the successive waves of protein super-assemblies formation that accompany the entry into a bona fide quiescent state. This ultimately leads to an abrupt cytosolic glass transition that occurs stochastically long after proliferation cessation. This new experimental framework provides a unique way to track single-cell fate dynamics over a long timescale in a population of cells that continuously modify their ecological niche.

Flash talks - session 3

Experimental exploration of the role of the conserved N-end signature of mitochondrial precursors

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My PhD project is part of a research program that aims to discover, in the yeast *S. cerevisiae*, the structure and functional impact of networks defined by the residue following the initiator methionine of the diverse nascent polypeptide chains. *In silico* analysis of amino acid utilization biases at position 2 in various protein subsets showed that mitochondrial precursors exhibit a strong and position-specific enrichment for amino acids targeted by NatC, an N-acetyltransferase involved in the N-end rule acetylation pathway that controls protein half-life. This N-end signature, which was found to be independent of the well- known amino acid usage bias observed in the N-terminal mitochondrial targeting sequence, is conserved in the Saccharomycotina yeast lineage, suggesting that it could play a key role in the destiny of the mitochondrial precursors. Selective Translating Ribosome Affinity Purification validated mitochondrial precursors as the main co-translational targets of NatC, providing for the first time an explanation for the previously published growth-defective phenotype in respiratory medium of NatC-deficient strains. Finally, the crucial importance of N-end signature of mitochondrial precursors was revealed by the drastic effects observed in mutagenesis experiments of position 2 of the precursor of the mitochondrial chaperonin Hsp60p.

Yeast Genetic Immunity

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Bacteria are known to target exogenous DNA through the innate immunity of the restriction and modification system, as well as the acquired CRISPR/Cas immunity. Both mechanisms selectively attack the incoming DNA element while keeping the cellular genetic material untargeted. In eukaryotes, DNA sensors such as cGAS have evolved recently that are able to detect DNA in the cytoplasm and trigger an inflammatory response against the infected cell. Nevertheless, such cell non-autonomous sensors are particular to metazoans and thus whether such recognition mechanism exists in all other eukaryotes remains unknown. In this project, we study the fate of exogenous DNA in budding yeast *Saccharomyces cerevisiae* cells. We use the variety of genetic and biochemical tools available in yeast to ask whether an ancestral exogenous DNA recognition mechanism exists in other eukaryotes in which we do not know of any DNA senors such as cGAS. Using fluorescently labelled plasmid trans- formed into yeast cells we found that at least one plasmid was observed in around 50% of transfected yeast cells in a population. Nevertheless, only 0.01-1% of those cells express and stably maintain this plasmid. To understand what could be the possible fate of the plasmid in almost 99.9% of the cases in which it is not maintained in the population, we used live cell imaging to track the plasmid in the population of transfected yeast cells. The results showed that the plasmid focus could take one of three possible fates in the cells: potential degradation in the cytoplasm, potential degradation in the vacuole, or rare persistence in the cell. The first two fates show a possible encapsulation of the plasmid DNA. To study the possible players involved in this targeting of the exogenous DNA, we performed a transposon screen SATAY (Michel et al. 2017). Using this screen, we were able to find genes involved in restricting yeast transformation. Interestingly, those genes expressed peptides involved at different levels in the cell from cell wall maintenance to endocytosis and lipid homeostasis reaching nuclear import and chromatin remodeling. We thus hypothesize that exogenous DNA targeting in yeast cells is a multi-layered barrier mechanism that the DNA has to pass through all for a transfection event to end in a stably maintained transformed DNA element in the cell.

Development of a model consortium of wine yeasts to explore the impact of diversity on wine fermentation

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Currently, there is a growing interest in describing and understanding natural microbial community involved in fermented food and beverages such as wine. Hence a lot of studies on wine investigated the diversity and the dynamic of the different yeasts present during the alcoholic fermentation. Must is generally rich in yeast species of the genera Hanseniaspora, Metschnikowia, Pichia, Torulaspora. There are then outcompeted by *S. cerevisiae* which dominates the active phase of fermentation. It is suggested that several environmental factors, as well as viticultural and winemaking practices can influence the microbial diversity. Due to the yeast diversity and the great variations in environmental conditions, it is often difficult to study the interaction mechanisms driving microbial dynamics during fermentation. The development of a model consortia could help to analyse the microbial community more easily by reducing the number of species and using defined environmental conditions. Our work aims to build a model consortium of yeasts representing the diversity found during winemaking to study the impact of abiotic stress. We have selected 7 species representing the initial diversity of must and chosen according to bibliography their starting proportion. We show the labelling strategy that enables us to follow individually each species during fermentation by using different fluorescent protein detectable with a flow cytometer. This microbiological tool will be a great help to understand and experiment on the impact of diversity on the oenological fermentation.

Single-cell analysis of the switch from vegetative to filamentous growth in *S. cerevisiae*

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Opportunistic pathogenic fungi pose a great threat to human health, particularly to immune compromised individuals. In the pathogenic fungi like *C. albicans*, filamentation is associated with host-cell attachment, tissue invasion and virulence. Thus, there is a need to understand the molecular machinery causing the switch from vegetative to filamentous growth. In the model organism *S. cerevisiae*, exposure to low-nutrient media can trigger evolutionarily conserved signaling pathways that lead to the formation of filament-like structures called pseudohyphae. This growth pattern can be compared to filamentation in other fungi. Most of our knowledge on this process comes from agar invasion assays and colony morphology analysis. These studies allowed to identify the main players that allow this morphological transition. However, we still lack a clear understanding of how the different signaling pathways interact to promote this cell fate transition. Therefore, our objective is to develop a microscopy assay where we can follow the dynamic transition from vegetative to filamentous growth. We have established fluorescent protein reporters to quantify the induction of the *FLO11* gene to monitor the temporal dynamics of filamentation in individual cells. When combined with specific gene deletions, these reporters will help us to understand the contribution of various signalling pathways to cell fate decision.

Spatio-temporal control of cooperation in yeast communities

Matthias Le Bec[1]*, Sylvain Pouzet, Alvaro Banderas, Céline Cordier, Simon Barral, Benoit Sorre, and Pascal Hersen*

[1] Physico-Chimie-Curie – Institut Curie [Paris], Sorbonne Universite, Centre National de la Recherche Scientifique : UMR168 – France

The division of labor is the separation of a system into different parts specialized in one or multiple tasks. This concept is observed in social economics, in multicellular organism or ecosystems, and especially in microbial communities where the complementarity of different species can result in a more productive and robust consortium. Even among clonal colonies, nutrient uptake, inhibitory chemicals excretion or chemical communication considerably affect the individual microenvironment leading to cell-to-cell phenotypic differentiation. While recent works make effort toward designing rational microbial communities, there is however few established methodologies to control, especially spatially, these microbial associations. Here, we aim at studying the role of spatial patterning of cooperation in a microbial population. We chose to control the degree of cooperation of *Saccharomyces cerevisiae* cells in a monoclonal population. For this we use the invertase enzyme (*SUC2*) as a mean for the optogenetic control of public goods production. This enzyme, embedded in the cell wall, allows yeast to grow on sucrose by catalyzing its hydrolysis into usable glucose and fructose, that can diffuse and be shared between cells. We built an optogenetic strain producing Suc2 enzyme upon blue illumination and optimized its induction range. We developed a method to perform simultaneous light patterning and time-lapse recording of yeast growing on agar-plate. We can thus control quantitatively in space and time the production of the Suc2 enzyme, hence the spatial pattern of cooperation in the microbial community. We assessed the influence of the pattern on the global growth behavior and compared our results with a diffusion-reaction model. Our first results outline the fact that our system acts as a spatial low-pass filter with a typical cooperation distance between illuminated and non-illuminated cells, evidencing the importance of microbial species patterning for ecosystem dynamics. Our project provides new insights in the spatial organization of microbial communities by developing new tools with enhanced control over biological parameters, toward a better understanding of microecology.

Relief of NCR in a gdh1 mutant due to altered transporter function ?

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Yeast cells have evolved an exquisite network of regulations aimed at the adjusted utilization of accessible nitrogen sources and metabolic adaptation to nitrogen availability. One of these, consisting in general transcriptional reprogramming by Nitrogen Catabolite Repression (NCR) pathway, is considered as the major controller of nitrogen use and targets several hundreds of genes (mostly transporter and catabolic enzyme genes) in response to the quality of the nitrogen source. Despite its very early initial characterization and the extensiveness of the relevant literature since then, the primary mechanism initiating NCR is still subject to debate and remains largely undiscovered to date. In an attempt to identify novel players of NCR, we determined that the glutamate dehydrogenase activity of Gdh1 was required to downregulate the transcriptional GATA activators (Gln3 and Gat1) independently of the glutamine and glutamate levels [54]. To further understand the causes of GATA factor repression relief in a strain lacking *GDH1*, we carried out a second-site suppressor screen. This screen led to the identification of 3 complementation groups. The mutants respectively carried the following alleles: rsp5C1204T, bul1G1039A/bul2C340T, and doa4G2120A. The three mutants did not impact Gln3 function and specifically targeted Gat1 in ammonium + glutamine-grown cells, and not in ammonium alone, suggesting that glutamine could be the amino acid that is sensed and generates the signal. Detailed analysis of the role played by the three putative glutamine transporters (*GAP1*, *AGP1*, *GNP1*) in this process will be provided.

Indole-3-acetic acid is a physiological inhibitor of TORC1 in budding yeast

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In eukaryotes, the Target of Rapamycin Complex 1 (TORC1) is a conserved kinase complex responsible for the regulation of crucial cell functions, such as growth and metabolism, acting on processes like protein synthesis, ribogenesis and autophagy. The signals impinging on TORC1 vary from basic building blocks like amino acids, which are sufficient to activate TORC1 in organisms like the budding yeast *S. cerevisiae*, to hormones and growth factors, which, combined with amino acids, are primarily important to activate TORC1 in higher eukaryotes. Whether TORC1 can also be negatively regulated by physiologically relevant metabolic inputs is currently not known. In this context, we have discovered that indole- 3-acetic acid (IAA), best known for its role as a hormone that regulates cell division and growth in plants, potently inhibits growth of yeast cells. Furthermore, through a chemical- genetic screening and a SAturated Transposon Analysis in Yeast (SATAY), we pinpointed the TORC1 pathway as a target of IAA in vivo. We thus measured the effects of IAA on TORC1 activity in vivo (by measuring the phosphorylation of its target Sch9) and in vitro (by assaying the kinase activity of highly purified TORC1 complexes). Surprisingly, IAA caused a rapid, dose-dependent inactivation of TORC1 both in vivo and in vitro. In addition, we observed that a yeast strain which bypasses the essential function of TORC1 was highly resistant to IAA, indicating that TORC1 is the major target of IAA in yeast. Notably, we demonstrated that yeast cells appear to synthesize IAA at levels that are relevant for TORC1 inhibition, supporting the intriguing possibility that the physiological levels of IAA may in fact represent an endogenous signal to modulate TORC1 activity in vivo.

The shortest telomere in the cell signals senescence through a probabilistic rather than deterministic mechanism

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Telomeres ensure genome integrity and are maintained by telomerase. During replicative senescence, telomerase is inactivated, telomere sequences progressively shorten, and set the limit for cell proliferation. As telomeres shorten, they are thought to lose their protective caps at a critical short length, activating the DNA damage response and recruiting DNA damage repair activities that would degrade, fuse, or recombine dysfunctional telomeres. However, the structure(s) of short and dysfunctional telomeres, which respectively trigger permanent replicative senescence or potentially promote genome instability, remain unclear. To define the structure of telomeres at the point of dysfunction and the fate of cells carrying them, we developed a system called FinalCut to induce a single telomere of defined length in cells in which we can conditionally inactivate telomerase. This allows structural analysis of this telomere, and combined with the use of our microfluidic system to track consecutive cell cycles from telomerase inactivation to cell death, we can achieve single telomere and single cell resolution. Our results show that the loss of telomere stability is progressive as the telomere shortens, and that telomeres of a given length can be protected or unprotected in separate cells. We conclude that telomere length is not the sole determinant of the switch between functional and dysfunctional telomeres. We therefore propose that the minimum length of telomeres to maintain cell viability follows a probabilistic, rather than deterministic, model.

Mutation location in the eukaryotic purine synthesis pathway determines response to nitrogen or purine starvation

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When starved for ”natural” nutrients, such as nitrogen, budding yeast induce specific stationary phase phenotype and become extremely resilient to environmental stresses. Besides, these cells survive prolonged starvation periods.
Intracellular metabolites are typically synthesised in multi step reactions where loss of any component evokes auxotrophy. For example, mutation in any gene coding protein in a given synthesis pathway would lead to an auxotrophic phenotype and, in this case, growth of organ- ism depends on external supply of auxotrophic nutrients. Examples of typical auxotrophic markers of laboratory strains of budding yeast are uracil, leucine, adenine and others. In contrast to ”natural starvation” lack of auxotrophic nutrient (uracil, leucine) supply usually doesn’t evoke resilient phenotype: cells don’t stop their metabolism, culture loses viability rapidly. We explored effects of purine auxotrophic starvation in several purine synthesis pathway single gene knock-out mutants. WT and knock-outs were cultivated in (1)full SD media, without (2)nitrogen or (3)adenine. Phenotypically, purine starved cells have similar or even higher stress resistance than nitrogen starved and it doesn’t always correlate with cell cycle arrest in G1/G0. There is also a connection between stress resistance and location of muta- tion within purine synthesis pathway. In transcriptomic level, however, downregulated gene groups are similar across starvations and knockouts, while upregulated gene groups differ. We think that purine deprivation evokes physiological effects, which mimic lack of carbon or nitrogen sources and therefore are at least partly signalised through pathways typically used to signal onset of ”natural” starvations. Purine synthesis pathway is highly conserved across eukaryotes; besides, there are ample examples of purine auxotrophic organisms - typically these are intracellular parasites which rely on purine supply from the host cell. Probably, the loss of purine synthesis capacity together with the resilience phenotype when purines are scarce is in fact ”adaptation” which is an old trick of evolution. In this research we have created a solid basis of observations that can help to understand reactions of eukaryotic cells to purine deprivation and reasons why this auxotrophic starvation strongly differs from other auxotrophic starvations.

Pathogenic yeast and filamentous fungi

Metabolic reprogramming by chromatin remodelers modulates fungal fitness under hypoxia

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Inside the human host, the opportunistic yeast *Candida albicans* colonizes predominantly oxygen-poor (hypoxic) niches such as the gastrointestinal and vaginal tracts. So far, the impact of hypoxia in the overall metabolism of this important human pathogenic yeast was not investigated. We have undertaken a time-resolved metabolomics and transcriptomic analyses to uncover the metabolic landscape of fungal cells experiencing hypoxia. Our data showed a dynamic reprogramming of many fundamental metabolic pathways such as the glycolysis, the pentose phosphate pathway and different metabolic routes related to the fungal cell wall biogenesis. The *C. albicans* lipidome was highly affected by hypoxia with increased level of free fatty acids in addition to metabolites and biochemical intermediates of membrane lipids. Depletion of oxygen-dependent lipids such as ergosterol or phosphatidylcholine with longer and polyunsaturated lateral fatty acid chains was observed as well. Most importantly, the hypoxic metabolome reflected different physiological alterations of the cell wall and plasma membrane of *C. albicans* that were confirmed by different approaches. We found that the chromatin remodelling complex SWI/SNF was required for the reprogramming of different metabolic routes to preserve the fitness of *C. albicans* under hypoxia. SWI/SNF was essential for the maintenance of *C. albicans* as a commensal and also for the expression of its virulence. The oxygen-sensitive metabolic pathways identified in this work provide a framework to comprehensively understand the virulence of human fungal pathogens and represent a therapeutic value to fight fungal infections.

Mitochondrial defects result in decreased susceptibility to echinocandins via the transcriptional regulator Pdr1 in *Candida glabrata*

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In *Candida glabrata*, the transcription factor Pdr1 controls the expression of genes encoding drug efflux pumps such as Cdr1. Pdr1 also controls its own expression through an auto-regulatory loop. Decreased susceptibility to azoles is often due to mutations in *PDR1* that render the factor hyperactive. Mitochondrial defects also result in decreased susceptibility to azoles. Resistance to another class of antifungals, the echinocandins (e.g. micafungin), is generally due to mutations in the *FKS1* and *FKS2* genes that encode the catalytic subunit of 1,3-beta-D-glucan synthase, the echinocandin target. We have observed that mitochondrial defects also result in decreased susceptibility to echinocandins and we have shown that this process is mediated by Pdr1. Mitochondrial defects result in increased levels of *PDR1* mRNA. Overexpression of *PDR1* by replacing its native promoter by the strong ADH1 promoter resulted in decreased susceptibility to micafungin. However, mutations in PDR1 that alter resistance to azoles generally have little effect on echinocandin resistance. We randomly mutagenized the *PDR1* gene and screened for mutants with altered resistance to micafungin. Single amino acid changes downstream of the DNA binding domain result in increased resistance to micafungin. These mutants show 10-fold increased levels of *PDR1* mRNA due to higher promoter activity of this gene. In summary, we have identified a novel role for the transcriptional regulator Pdr1.

Yeast filamentation signaling is connected to a specific substrate translocation mechanism of the Mep2 transceptor

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Diploid *Saccharomyces cerevisiae* cells can switch from the yeast to a filamentous form of growth in conditions of nitrogen scarcity. This occurs for instance when a limiting ammonium concentration is provided as sole nitrogen supply (Gimeno et al., 1992). The transmembrane transport of ammonium (hereafter referring to NH4+ + NH3) is mediated by proteins of the conserved Mep-Amt-Rh family including the human Rhesus factors. *S. cerevisiae* possesses three members of this family (Mep1-3) (Marini et al., 1997). In several fungi, Mep2-type proteins are specifically required for filamentation, in contrast to Mep1/3 orthologues, and are proposed to act as ammonium sensors activating the dimorphic switch (Lorenz et al., 1998). However, the precise molecular mechanism of Mep2-mediated signal transduction remains unclear. Here, our data sustain a close link between ammonium transport efficiency of Mep2 and its capacity to allow filamentation (Brito et al., 2020). We show that, while the C-terminal domain of Mep2 contributes to maximal transport and signaling efficiencies, it is not absolutely required for conveying the signal of filamentation. Hence, filamentation signaling appears to occur in the absence of exclusive binding of partners to the Mep2 C-terminus. Our data further support that a conformational change accompanying substrate translocation in the hydrophobic core of Mep2 is required for the signaling property. Functional characterization in *Xenopus oocytes* reveals that substrate translocation via Mep1 and Mep2 operates via distinct mechanisms. Our findings highlight that the Mep2 specific transport mechanism is related to filamentation induction, suggesting a role of pH in signal mediation. Finally, we show that the signaling process is conserved for the Mep2 protein from the human pathogen *Candida albicans*.

Proteomic analysis of *Candida albicans* to identify new virulence factors

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The fungus *Candida albicans* is a commensal member of the human gastrointestinal tract, but also an important cause of infections. When the intestinal microbiota is disturbed or the immune defenses are compromised, the dissemination of *C. albicans* from the intestinal tract to the blood stream leads to systemic candidiasis. The known strategies employed by the fungus to overcome the intestinal barrier include (i) active penetration of fungal hyphae, and (ii) host-driven induced endocytosis of hyphal forms of the fungus, both of them involving the expression of specific proteins at the fungal cell wall or secretion of proteins and peptides. Concerning the active penetration of hyphae, in addition to the expression of several proteins including invasins, adhesins and peptidases, *C. albicans* secretes a small peptide called candidalysin, responsible for pore-induced damage of epithelial cells. In this present study, we identified within the secretome of *C. albicans* a peptide, which had the capacity to increase the permeability of intestinal cells (IEC), correlated to a down-regulation of proteins involved in the macromolecular structure that keep IEC tightly close. Moreover, mass spectrometry (MS) analysis of the supernatant led to the identification of the whole peptides composing it. Gene ontology (GO) analysis revealed that those peptides were mainly from proteins located at the cell wall, membrane and extracellular region of the fungus. Biological process GO terms associated with those peptides were the filamentous growth, pathogenesis and the fungal cell wall organization. Finally, thanks to supernatants prepared from knocked-out mutant yeasts of proteins identified during MS experiments, we were able to better target the peptide responsible for the increase of the paracellular permeability of IEC. The capacity of the peptide to modulate the permeability of IEC was finally confirmed thanks to a synthetic version of it. During the treatment, events occurred one hour after treatment of IEC, but were not associated with membrane damage of Caco-2 cells. Collectively, those data helped us to establish a new strategy for *C. albicans* aimed at crossing the intestinal barrier using the paracellular route.

Transport, sensing and signaling

Phosphorylation of mRNA-binding proteins Puf1 and Puf2 by TORC2-activated protein kinase Ypk1 alleviates their repressive effects

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Members of the Puf family of RNA-binding proteins typically associate via their Pumilio homology domain with specific short motifs in the 3'-UTR of an mRNA and thereby influence the stability, localization and/or eciency of translation of the bound transcript. In our prior unbiased proteome-wide screen for targets of the TORC2-stimulated protein kinase Ypk1, we identified the paralogs Puf1/Jsn1 and Puf2 as high-confidence substrates. Earlier work by others had demonstrated that Puf1 and Puf2 exhibit a marked preference for interaction with mRNAs encoding plasma membrane-associated proteins, consistent with our previous studies documenting that a primary physiological role of TORC2-Ypk1 signal- ing is maintenance of plasma membrane homeostasis. Here, we show, first, that both Puf1 and Puf2 are authentic Ypk1 substrates both in vitro and in vivo. Fluorescently-tagged Puf1 localizes constitutively in cortical puncta closely apposed to the plasma membrane, whereas Puf2 does so in the absence of its Ypk1 phosphorylation, but is dispersed in the cytosol when phosphorylated. We further demonstrate that Ypk1-mediated phosphorylation of Puf1 and Puf2 upregulates production of the protein products of the transcripts to which they bind, with a concomitant increase in the level of the cognate mRNAs. Thus, Ypk1 phosphorylation relieves Puf1- and Puf2-mediated post-transcriptional repression mainly by counteracting their negative effect on transcript stability. Using a heterologous protein-RNA tethering and fluorescent protein reporter assay, the consequence of Ypk1 phosphorylation in vivo was recapitulated for full-length Puf1 and even for N-terminal fragments (residues 1-340 and 143-295) corresponding to the region upstream of its dimerization domain (an RNA-recognition motif fold) encompassing its two Ypk1 phosphorylation sites (both also conserved in Puf2). This latter result suggests that alleviation of Puf1-imposed transcript destabilization does not obligatorily require dissociation of Ypk1-phosphorylated Puf1 from a transcript. Our findings add new insight about how the TORC2-Ypk1 signaling axis regulates the content of plasma membrane-associated proteins to promote maintenance of the integrity of the cell envelope.

The small GTPase Arf1 regulates ATP synthesis and mitochondria homeostasis by modulating fatty acid metabolism

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Lipid mobilization through fatty acids ß-oxidation is a central metabolic process essential for energy production in times of nutrient shortage. In yeast, this catabolic process starts in the peroxisome from which the product of √ü-oxidation, acetyl-CoA, is transferred to mitochondria and enters the TCA cycle to fuel ATP synthesis. Despite the well-characterized metabolic enzyme cascade involved in this process, little is known about the physical and metabolic cooperation between these two organelles and how their interactions impact cell growth. Here, we report that the small GTPase Arf1 regulates fatty acids √ü-oxidation at the peroxisome level. Using a predominantly active form of Arf1, we show that expression of both fatty acid transporters (Pxa1/Pxa2) and of the first and rate-limiting enzyme involved in √ü-oxidation (Pox1) were decreased, leading to an accumulation of fatty acids in lipid droplets in the form of triacylglycerol. As a consequence, mitochondria become fragmented and ATP synthesis is decreased. Genetic and pharmacological depletion of fatty acids phenocopied Arf1 mutant phenotype, confirming the link between Arf1 and fatty acid utilization. Even though √ü-oxidation occurs in mitochondria in mammalian cells, we find that the general role of Arf1 in fatty acid metabolism is conserved. Together, our results indicate that Arf1 integrates cellular metabolism to mitochondrial energy production by regulating fatty acid storage and utilization, and presumably organelles contact-sites.

Nuclear regulation of the UPR

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Cellular stress induced by the abnormal accumulation of unfolded proteins in the endoplasmic reticulum (ER) is recognized as a hallmark of many pathological conditions including cancer, diabetes, obesity and neurodegeneration. Maintenance of ER proteostasis is controlled by a highly conserved signaling network known as the unfolded protein response (UPR). While UPR activation mitigates protein misfolding, continued ER stress induces persistent UPR signaling, leading to cell death. Chronic ER stress thus arises as central to the pathophysiology of a wide range of human diseases. Hence, understanding the molecular mechanisms that control UPR intensity and duration is of utmost importance. We now identified nuclear factors, that regulate UPR termination and duration in a previously un- recognized manner. In yeast, the UPR is set in motion by a three-component system which includes a stress sensor (Ire1), a downstream transcription factor (Hac1/XBP1), and down-stream target genes. Ire1-mediated cytoplasmic splicing of the HAC1 transcript constitutes the key trigger that turns on the UPR. We previously characterized the yeast chromatin remodeler Isw1 as a mRNP nuclear export quality control (QC) factor that retains export- incompetent transcripts in the nucleus. Here, we define a novel mechanism of UPR regulation that involves Isw1. ISW1 inactivation does not influence UPR activation but instead impairs UPR attenuation and cell viability upon ER stress. Mechanistically, we demonstrate by using the CRAC technique (*in vivo* "UV cross-linking and analysis of cDNA") that Isw1 directly binds the HAC1 mRNA and identify Isw1 binding motifs in HAC1. The interaction of Isw1 and HAC1 restricts HAC1 mRNA nuclear export, cytoplasmic splicing and thereby UPR termination. Moreover, we observe that ISW1 itself is induced by ER stress. We, therefore, propose a model in which ER stress-induced Isw1 acts as a previously un- recognized key effector of the negative feedback loop that abates UPR signaling to allow for homeostatic adaptation to ER stress. Importantly, this novel Isw1-mediated pathway of UPR termination is independent of the previously described "Ire1 deactivation" pathway and can compensate for it (Matabishi et al., under review).

Quantitative and real-time analysis of protein-protein interactions in yeast: from interactomics to drug design

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Gene expression regulation

Smc3 acetylation, Pds5 and Scc2 control the translocase activity that establishes cohesin dependent chromatin loops

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Past decade has shown that the cohesin is essential for the regulation of mammalian's genomes 3D organization. This genome organization is important to regulate or influence DNA related processes like DSB repair or gene transcription. Cohesin is a ring-shaped complex that interacts with three related hook-shaped proteins composed of HEAT repeats namely Pds5, Scc3 and Scc2 that regulate its functions. Cohesin shapes mammalian genomes by establishing chromatin loops along chromosomes, most likely through an extrusion mech- anism. Recent single molecules experiments suggest that loop expansion is mediated by cohesins' ATPase activity stimulated by Scc2. We have recently showed that yeast mitotic chromosomes are also organized by cohesin-dependent loops (Dauban et al 2020). Length of these loops is regulated by two pathways: the Wapl-mediated releasing activity and a mechanism dependent of the acetyltransferase Eco1. Our data also demonstrate that in vivo, Scc2 is essential for cohesin translocation pro- cess expanding DNA loop. Moreover, Smc3 acetylation mediated by the acetyltransferase Eco1 counteracts this activity through the stabilization of Pds5, which finely tunes the size and stability of loops in G2.

Pho92, an m6A-binding protein facilitating recombination and meiotic progression in the yeast *Saccharomyces cerevisiae*

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N6-methyladenosines (m6A) are the most abundant internally modified bases of eukaryotic messenger RNAs (mRNAs). This epitranscriptomic mark is co-transcriptionnally in- stalled by a conserved methyltransferase complex. m6A are then recognized by "reader" proteins, sharing a YTH domain, which mostly control mRNA stability but also regulate alternative splicing, nuclear RNA export, and RNA localization. Many studies have shown that in metazoans m6A is critical for development, cell differentiation or gametogenesis but also contributes to multiple pathologies such as cancer development, neurological diseases and diabetes. Yet, molecular details of the mechanisms by which m6A modulates gene ex- pression aren't completely understood. In the budding yeast *Saccharomyces cerevisiae*, m6A is only formed during meiosis that is part of the sporulation program, the equivalent of mammalian gametogenesis. Deletion of the methyltransferase factor impairs meiotic activation showing that m6A is required for the sporulation program. To decipher the mechanisms by which m6A regulates gene expression, we investigated the impact of Pho92 (the only protein carrying a YTH domain in *S. cerevisiae*) on the transcriptome across meiosis and identified its mRNA targets. Absence of Pho92 delays the recombination process starting from double-strand break formation and leads to an extended meiotic prophase I. Our results suggest that the physiological and molecular role of Pho92 is to facilitate the meiotic progression by accelerating the decay of timely-regulated mRNAs during recombination.

New insights into the function of the Spt3 subunit of the SAGA co-activator from studies in fission yeast

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Transcription is a highly regulated process involving many factors, including transcriptional co-activator complexes. Two such complexes, SAGA and TFIID, have both distinct and overlapping roles in TBP loading at promoters, which is an essential step for pre-initiation complex assembly. Genetic, biochemical and structural evidence indicate that, in SAGA, the Spt3 and Spt8 subunits are involved in TBP delivery. Most studies of Spt3 function have been performed in *Saccharomyces cerevisiae*, in which its deletion causes mild growth phenotypes despite affecting RNA polymerase II transcription globally. In contrast, we found that deleting spt3 in the distantly related fission yeast *Schizosaccharomyces pombe* severely affects cell proliferation, suggesting that studying Spt3 in fission yeast will bring new insights into its functional roles. We first hypothesized that Spt3 might have a SAGA- independent function in *S. pombe*, which would be absent or less important in *S. cerevisiae*. However, tandem affinity purifications followed by quantitative mass-spectrometry analyses (TAP-MS) revealed that Spt3 interacts with SAGA only. Next, we tested whether Spt3 is essential for SAGA structural integrity in S. pombe, in contrast to what is observed in *S. cerevisiae*. TAP-MS analysis of SAGA in yeast conditionally depleted for Spt3 showed that SAGA subunit composition is largely unaffected in the absence of Spt3. We then performed a structure-function analysis of *S. pombe* Spt3 and found, unexpectedly, that a mutation detaching Spt3 from SAGA causes milder phenotypes than the spt3 deletion mutants, suggesting that Spt3 can fulfill its crucial role in cell proliferation independently of SAGA. We are currently investigating the genome-wide contribution of Spt3 to TBP loading, PIC assembly, and nascent transcription in *S. pombe*. We will discuss the implication of our work in our understanding of gene regulation by SAGA and TFIID activities.

Correlation of MAPK Activity and Gene Expression In Single Cells

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In higher eukaryotes, Mitogen-Activated Protein Kinases (MAPK) control the induction of complex transcriptional programs. These newly transcribed proteins will in turn play a key role in multiple cellular processes including transient adaptation to stresses and cell-fate decisions. Misregulation of MAPK gene expression has been linked to numerous diseases. It is therefore important to understand how the MAPKs control their downstream transcriptional targets. In order to achieve this, we use the HOG MAPK cascade in budding yeast as a prototypical stress-response pathway. We developed fluorescent biosensors to monitor in single cells the dynamics of signaling activity and the induction of transcription. The activation of multiple stress-response promoters upon mild osmotic shock was compared, allowing to precisely quantify the timing of gene expression. This comparison allowed to observe which parameters are dependent on the MAPK Hog1 and which ones are controlled directly by the promoter identity. In addition, we perturbed the nuclear relocation of the MAPK, in order to observe how the enrichment of the MAPK in the nucleus contributes to the efficient activation of stress responsive genes.

Translation in the metabolism of antisense long non-coding RNAs

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The pervasive transcription of eukaryotic genomes produces plethora of long non-coding (lnc)RNAs, including antisense (as)lncRNAs synthesized from the DNA strand antisense to 'sense' protein-coding genes. Despite their regulatory potential, aslncRNAs have been poorly studied. This is probably due to their low cellular abundance, as they are extensively degraded by the nuclear Exosome and the conserved cytoplasmic Xrn1 5'-3' exoribonuclease. Recent works in budding yeast *Saccharomyces cerevisiae* further revealed that aslncRNAs are mainly targeted to Xrn1 through the Nonsense-Mediated mRNA Decay (NMD), a translation-dependent RNA decay pathway degrading transcripts with premature translation termination stop codon and/or long 3'-UTR. This suggests that aslncRNAs are translated, raising the question of their coding potential. Using *S. cerevisiae* as a model, here we present the results of classical genetic engineering experiments coupled with multiple high-throughput analyses showing that translation is a key determinant of aslncRNAs degradation, which can also lead to the production of cryptic micropeptides. Our work contributes to re-consider the idea that aslncRNAs are devoid of coding potential, addressing how translation of small ORFs determines their degradation, but also can be used to produce micropeptides.

Functional and transcriptional profiling of non-coding RNAs in yeast reveal context-dependent phenotypes and in trans effects on the protein regulatory network

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Non-coding RNAs (ncRNAs) are increasingly being shown to play pivotal roles in the transcriptional and post-transcriptional regulation of genes in eukaryotes. Stable Unannotated Transcripts (SUTs) and Cryptic Unstable Transcripts (CUTs) have been shown to affect nearby genes by physically interfering with their transcription (cis mode of action), or interact with DNA, proteins or other RNAs to regulate the expression of distant genes (trans mode of action). Here, we carried out a large-scale screening on the ncRNA *Saccharomyces cerevisiae* deletion collection and provided evidence for SUT and CUT function. Phenotypic data on 372 ncRNA deletion strains in 23 different growth conditions were collected, identifying ncRNAs responsible for significant fitness variations. Transcriptome profiles were collected for 18 haploid ncRNA deletion mutants and 2 essential heterozygote ncRNAs showing a high correlation between altered phenotypes and global transcriptional changes in an environmental dependent manner. By analysing the expression network, new functional ncRNAs acting in trans by modulating transcription factors were identified. Furthermore, we described the impact of SUTs and CUTs in modulating coding genes in response to different environmental conditions, acting on critical biological processes such as ethanol tolerance, mitochondrial function, and respiration (SUT125, SUT126, SUT035, SUT432), plasma-membrane fluidity and sterol biosynthesis. (CUT494, SUT530, SUT468) or rRNA processing (SUT075 and snR30). Overall, this data captures and integrates the regulatory and phenotypic network of ncRNAs and protein-coding genes, supporting the notion of ncRNAs' involvement in fine-tuning cellular expression via regulation of transcription factors as an advantageous RNA-mediated mechanism that can be fast and cost-effective for the cells

Population, functional and evolutionary genomics (part II)

The budding yeast domestication syndrome

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Domestication of plants and animals is the foundation for feeding the world human population, but can profoundly alter the biology of the domesticated species. Here, we investigated the effect of domestication on one of our prime model organisms, the yeast *Saccharomyces cerevisiae*, at a species-wide level. We tracked the capacity for sexual and asexual reproduction and the chronological lifespan across a global collection of 1011 genome-sequenced yeast isolates and found a remarkable dichotomy between domesticated and wild strains. Domestication had systematically enhanced fermentative and reduced respiratory asexual growth, altered the tolerance to many stresses, and abolished or impaired the sexual life cycle. The chronological lifespan remained largely unaffected by domestication and was instead dictated by clade-specific evolution. We traced the genetic aetiology of the yeast domestication syndrome using genome-wide association analysis and genetic engineering and disclosed causative effects of aneuploidy, gene presence/absence variations, copy number variations, and single nucleotide polymorphisms. Overall, we propose domestication to be the most dramatic event in budding yeast evolution, raising questions on how much domestication has distorted our understanding of the natural biology of this key model species.

Phylogenomics analyses of divergent *Saccharomyces* lineages from Patagonia

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Saccharomyces species are found all across the world, including anthropogenic niches, temperate forests, and extreme environments. Two of the species in the clade, *S. eubayanus* and *S. uvarum*, predominantly inhabit the South Hemisphere, mainly associated with Nothofagus forests in Patagonia and Australasia. Previous studies in *S. eubayanus* suggested a Patagonian origin of the species, supported by the larger number of lineages in Patagonia, nucleotide diversity, and past admixture events. A similar observation was also established for *S. uvarum*. To date, three *S. uvarum* populations have been described: South America, Holarctic, and Australasia. Among these, South America harbors the greatest genetic diversity, suggesting that both species could be native to the Southern Hemisphere. Interestingly, *S. uvarum* presents a highly divergent Australiasian lineage, with a 4% genetic divergence and partial reproductive isolation. However, there is no evidence of shared lineages between Patagonia and Australasia, precluding further conclusions and analyses of the local origin of the species. A recent survey throughout the southern Pacific coast in South America revealed *S. uvarum* isolates belonging to the Australian lineage. Interestingly, these new isolates clustered within a separate branch in the Australasia lineage, which correlates with Gondwana's footprint on *S. uvarum* diversity around the planet. The construction of a de novo assembly using Nanopore technology coupled with illumina sequencing, demon- strated the presence of large translocations in these isolates, which are genetically divergent from those currently described in Patagonia, both from *S. eubayanus* and *S. uvarum*. These isolates are only present in rainforests that surround the coastal zone of southern Chile and not in the Andes, suggesting a specific ecological adaptation. Indeed, this biological niche is characterized by various endemic species whose ancestors are associated with the supercontinent Gondwana. Considering this, we hypothesize that this endemic subpopulation is in the process of speciation and suggests a likely origin of a common ancestor for *S. eubayanus* and *S. uvarum* in southern Patagonia.

N-end rule in an evolutionary perspective: identification of a conserved N-end signature essential for the destiny of mitochondrial precursors

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The residue following the initiator methionine is an important signal for the recruitment, during translation, of selective nascent chain associated factors. In particular, N-terminal modification enzymes were shown to be key players in the early control of proteins stability via the N-end rule pathways. Despite its importance, the N-end rule has never been explored in a global and evolutionary perspective by analyzing biases in the distribution of the 20 amino acids at position 2 in proteomes. To tackle this issue, we developed a R package to detect Gene Ontology terms showing, among their proteins, a significant and specific overrepresentation of a given amino acid at position 2. Applied to the *S. cerevisiae* proteome, this approach revealed, for the first time, cellular functional categories with a particular distribution of N-termini, suggesting a critical role for this signal in controlling the fate of proteins belonging to these functional groups. Notably, our study revealed a dramatic increase in the use at position 2 of mitochondrial precursors of amino acids targeted by the N-acetyltransferase NatC, an enzyme involved in the acetylation N-end rule pathway. This signature is independent of the well-known amino acid usage bias observed in the N- terminal mitochondrial targeting sequence, and its importance has been emphasized by its strong conservation in the Saccharomycotina yeast lineage and the drastic effects observed in mutagenesis experiments in *S. cerevisiae*.

Systematic mapping of natural variants driving genetic context-dependent gene essentiality

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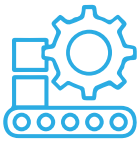
Mutations often show phenotypic differences across genetically distinct individuals. In the most extreme case, a gene can be essential in one genetic background but have no effect on viability in another. Here, we investigated the frequency and underlying causes of differences in gene essentiality by examining 20 genetically diverse yeast strains. First, we generated a collection of 755 haploid query strains in the laboratory background S288c, each deleted for an essential gene, but viable because of the presence of the gene on a plasmid. We crossed this collection to a diverse set of wild yeast strains from various sources and tested for survival of haploid segregant progeny in the absence of the essential genes. We observed viable progeny in at least one cross for 30 essential genes (4%), suggesting that the wild strain contained variants that could bypass the requirement for the essential gene. Although some genes appeared to be essential in S288c only, the vast majority of genes were nonessential in only 1 or 2 genetic backgrounds. We did not observe a correlation between the genetic distance between strains and the number or identity of context-dependent essential genes. Finally, we are identifying and validating causal bypass suppressor variants using bulk segregant analysis and allele replacements. Ultimately, we aim to learn the general mechanisms that drive conditional essentiality and the frequency at which they occur during evolution. Understanding how the genetic background of an individual can affect the phenotype of a mutation of interest will provide insight on how genetic variance accumulates during evolution and affects genetic traits.

New technologies, yeast and industry



Bioprospecting in central Patagonia reveals novel *Lachancea cidri* strains for efficient mead production

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Evaluating the genetic diversity of non-model species is essential for a thorough understanding of biodiversity and the complexity of the genotype-phenotype relationship in nature. In this context, the quest for new wild yeasts has increasingly gained attention because of their potential ability to provide unique attributes to fermented beverages. Patagonia offers a wide diversity of ethanol-tolerant yeasts and stands out as a bioprospecting alternative. Recently, *L. cidri* was recovered from wine fermentations in Europe, Eucalyptus tree sap in Australia, and Patagonia from Nothofagus forests, exhibiting an interesting phenotypic diversity and biotechnological potential for wine and mead fermentations. However, the availability of many individuals is a prerequisite for exploring the potential new technologies and diversity of the species. Here, we determined the phylogeographic history of *L. cidri* together with its phenotypic diversity. We sequenced the whole genome (Illumina reads sequencing) of 55 *L. cidri* strains and a subset of them using Oxford Nanopore technology. We detected many SVs, demonstrating a high level of genomic rearrangements mostly across Patagonian strains. The phylogeny obtained demonstrates that South American (SoAm) strains are genetically separated from European and Australian strains. In parallel, high- throughput micro-cultivation assays demonstrated the extensive phenotypic diversity among Patagonian isolates and the potential of the species to tolerate high ethanol concentrations. Interestingly, we found that *L. cidri* is a novel candidate species to ferment mead, exceeding the fermentation capacity of a commercial strain (*S. cerevisiae* Nottingham). Unlike commercial strains, we found that *L. cidri* does not require nutritional supplements (Fermaid ® K) for efficient mead fermentation. In addition, *L. cidri* produces succinic and acetic acids, providing a distinct profile to the final fermented product. Finally, we analyzed the production of volatile compounds under mead fermentation conditions. We found a wide diversity of compounds in *L. cidri* strains compared to the *S. cerevisiae* commonly used in the industry, giving a novel character to the mead. Altogether, these results show the significant genetic and phenotypic diversity present in Patagonian *L. cidri* and demonstrate the importance of bioprospecting efforts in Patagonia to isolate novel wild yeast strains with extraordinary biotechnological potential for the fermentation industry.

NMR Analysis of *Saccharomyces cerevisiae* Endo-metabolome: Comparison of Yeast Strains of Different Origins during the Alcoholic Fermentation of a Grape Must

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NMR spectrometry is a valuable analytical chemistry tool for achieving metabolomic investigations. Thanks to the simplicity of sample preparation, the variety of chemical families that can be assayed and the reproducibility of the approach, NMR is routinely used for quantifying the endo-metabolism of many cells and tissues. In this study, we developed a (NMR)-based strategy aiming to identify and quantify the mains intracellular metabolites of the yeast in a culture volume of less than 10 ml during wine alcoholic fermentation. After several validations, our method allows the routine and reliable quantification of more than 20 intracellular compounds including the organic and amino acids, alcohols and polyols as well as sugars. As a matter of proof, we investigated at different culture times the phenotypic variability of endo-metabolism of *S. cerevisiae* by comparing 10 wine-related strains. As expected, a large phenotypic variability was observed for fermentation kinetics and metabolites production which is quite usual for this species. In contrast, endo-metabolomic variations are milder and were not particularly correlated to the content of yeast metabolites found in wine. This reliable method will be very useful for investigating the metabolic consequences of environmental changes or genetics modifications in the context of enological fermentation.

Saccharomyces cerevisiae discrimination by metabolomics

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Alcoholic fermentation is known to be a key stage in the winemaking process that directly impacts the composition and quality of the final product: the wine. Microorganisms are the key players, the major ones being *Saccharomyces cerevisiae*. The impact of different strains of *S. cerevisiae* on the composition and diversity of volatile compounds in wine is well described in the literature. However, no approach has been taken to study the impact of non-volatile metabolism of different strains of *S. cerevisiae* on the matrix. Ultra-high-resolution mass spectrometry (uHRMS) and multivariate analysis were used for a non-targeted metabolomics approach for the high precision. uHRMS and multivariate analysis appear as a method of choice in the compositional characterization of utmost complex samples on a molecular level. Results from fermentations conducted by twelve different strains of *S. cerevisiae* on the same must, showed that, despite similar fermentation kinetics, wines can be discriminated with this original approach. Our study revealed hundreds markers specific to each strain of *S. cerevisiae*, and more generally, a chemical composition specific to each wine. Extracted markers were annotated using databases and metabolic pathways and expressed numerous metabolic differences within the same yeast species. This indicates that despite their closeness, the metabolisms of these strains are associated to unique chemical fingerprints.

Compartment-specific Split-fluorescent proteins in Baker's yeast

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Aminoacyl-tRNA synthetases (aaRSs) are ubiquitous and essential enzymes responsible for the formation of aminoacyl-tRNAs used during translation. In addition to their cytosolic localization, eukaryotic cytosolic aaRSs can have various other subcellular localizations, making them multi-localized proteins and these nonconventional localizations are often associated with non-canonical functions. The eukaryotic aaRSs can also assemble into " multisynthetasic" complexes that act as cytosolic reservoirs for relocating aaRSs. The yeast *S. cerevisiae* contains such a multisynthetasic complex, called the AME complex, composed of methionyl- and glutamyl-tRNA synthetases (MRS and ERS) bound to the Arc1 cofactor. Even if this complex has been described as exclusively cytosolic, ERS can relocate in the mitochondria, while MRS can enter the nucleus. These organellar pools of MRS and ERS are referred as organellar echoforms. Moreover, Arc1 was found to interact with different lipid species in vitro, and especially with vacuolar lipids, raising the possibility of an additional vacuolar echoform for this protein as well as for the AME complex. However, the study of organellar echoforms of cytosolic proteins remains challenging because of technical limitations and the impossibility to use conventional GFP fusion for epifluorescence microscopy observations. We therefore engineered two epifluorescence microscopy tools, the BiG Mito-Split-GFP and the Vacuolar-Split-CFP, that respectively enable the identification and visualization of mitochondrial and vacuolar echoforms of multi-localized proteins. In addition to the identification of a new mitochondrial echoform for two aaRSs, we could identify the region of ERS that encompasses the MTS of this protein. We also investigated the aaRSs' vacuolar anchoring and could identify a vacuolar echoform for 18 aaRSs, including MRS, ERS and the leucyl-tRNA synthetase, for which a vacuolar localization related to TORC1 regulation had already been demonstrated.

Signatures of phenotypic adaptation: the case of *Brettanomyces bruxellensis*, a yeast associated to anthropized environments

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The yeast *Brettanomyces bruxellensis* has gained more and more attention in the two last decades for numerous raisons. First, this yeast is deeply associated with anthropized environments. *B. bruxellensis* is a fermentation spoiler in some processes: it is considered as the main wine cellar spoiler and it is problematic in bio-ethanol and industrial beer production as well. Conversely, *B. bruxellensis* is regarded as beneficial in some traditional fermentations (e.g. Kombucha, Kefir...) and for the production of specific beers (spontaneous and in craft beers). Secondly, recent genomic approaches unveiled a complex species, including diploid individuals but also allo- and auto-triploid ones organized in subpopulations associated to specific ecological niches. This unusual population structure mingled with manifold hybridization events drew up *B. bruxellensis* as a valuable yeast model. However, despite its industrial impact, *B. bruxellensis* phenotypic traits are insufficiently assessed in the light of its large genomic diversity. So far, seven genetic groups were identified, associated with wine, beer, bioethanol/tequila, etc. processes. In this study, a large-scale phenotypic analysis was conducted on > 100 strains, in five natural fermentation media (grape must, wine, wort, beer, kombucha wort). The growth and fermentation kinetics as well as the main exometabolites were analysed. Our results highlight variable growth rate and ability in wine particularly, or variable maltose assimilation in wort and beer depending on the different sub-populations. Surprisingly, some extracellular metabolites are also produced or consumed in a differential way in the genetic subpopulations (e.g. malic acid, SO2, acetic acid). The phenotypic variability was fluctuant depending on the subpopulations and on the combination of subpopulations and substrates. These results provide new evidences that *B. bruxellensis*' evolutionary history shows adaptation and domestication to different anthropized process.