



10th Francophone Yeast Meeting 'Levures, Modèles & Outils'

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10th Francophone Yeast Meeting ‘Levures, Modèles & Outils’

The sunny city of Toulouse in the south of France welcomed the 10th Edition of the Francophone Yeast Meeting ‘Levures, Modèles & Outils’ (LMO) at the beginning of April 2012. Since its first editions in the early 90s, this conference has gathered together all francophone researchers who are devoting their passion to the same group of organisms: yeasts.

Between the first and tenth editions of this biannual rendez-vous, yeasters were faced with an extraordinary revolution: *Saccharomyces cerevisiae* was indeed the first eukaryotic genome completely sequenced and released to the community, reviving its enormous potential for understanding life and opening the door to new approaches in biology. This revolution was immediately followed by ambitious programmes for sequencing the genomes of its distant cousins, e.g. the ‘Gen-olevures’ program held by a consortium of seven French laboratories, with the first aim of deciphering the different mechanisms of eukaryotic genome evolution over long periods of time. Taking advantage of the knowledge of these new genomes, researchers can now tackle much more easily relevant physiological studies in very different and distant yeasts, the diversity of which has once again been attractively represented at this 10th meeting. In addition to *S. cerevisiae* and *Saccharomyces pombe* still highly valued by researchers, many oral and written communications attested to the growing interest of our community for these ‘less-conventional’ yeasts. This explains why, in parallel with this genomic revolution, a mutation naturally occurred in the title of this meeting, with the recent emergence of three ‘s’ and subsequent transition from ‘Levure, Modèle & Outil’ to “Levures, Modèles & Outils”.

This latest edition of LMO therefore brought together 250 participants from fields that rarely interact in order to discuss the current status of their research interests. This massive participation certified that our community remains very active, but also that it is particularly fond of this appointment. It offers a panoramic view of the extraordinary potential of yeasts, that continue to be driving forces in many fields of biology, from basic research to biotechnological challenges in coming years. Topics therefore ranged from a convincing demonstration of the central role of the mediator complex in RNA-polymerase II-dependent transcription to the engineering of new yeast strains to meet emerging energy problems that humanity will have to face in the near future. This report highlights some of the latest findings presented at this meeting, where two of the

sessions were held in memory of renowned French colleagues Barbara Winsor and Pierre Thuriaux, who sadly passed away on the eve of retirement.

1. Beverages and energy: from ancestral use of yeasts to cutting edge biotechnological challenges

The opening ‘Physiology, Biotechnology & Bioprocess’ session was of course devoted to *S. cerevisiae*, but for several years now, the singular properties of yeasts such as *Kluveromyces lactis*, *Yarrowia lipolytica*, *Candida albicans* and many other yeast species have been increasingly drawing the attention of researchers. Participants heard about yeasts responses to environmental stresses such as oxidative stress, acetic acid and temperature, exposure to heavy metals, CO₂, nitrogen and lipid limitation and energy starvation. We even heard about viability of yeasts in response to ethanol, the main product of *S. cerevisiae* metabolism! These environmental conditions affect signalling, metabolic regulation, redox homeostasis and even cell viability, with all these effects investigated through classical or more modern global approaches. In addition to fundamental knowledge of life, these studies clearly stand as preliminary essential steps towards biotechnological applications and control of bioprocesses, such as production of molecules of interest in dedicated industrial environments. In this context, many of the works presented in this session, particularly those reported below, were jointly presented with industrial partners and therefore led to restrictions in data communication due to confidential constraints. The two aspects that are highlighted in this meeting report present, on the one hand, the use of yeasts for production of alternative and sustainable fuels, and on the other, yeasts as natural or engineered biocatalysts in the beverage industry.

Among the major industrial uses of yeast is production of ethanol as fuel from sugar cane in Brazil and from corn in the USA. But a second generation of sustainable biofuels produced by recombinant yeast strains is estimated to become the main transportation fuel over the coming decades. Current state-of-the-art progress in that field was nicely reviewed in the introductory conference of the meeting by Jack Pronk (Delft University of Technology, The Netherlands). In the context of sustainability, lignocellulosic biomass held the attention of researchers, since this material can be hydrolysed

in a mixture of both pentose sugars and hexoses. Economically viable production of ethanol by yeast nevertheless requires efficient utilisation of this complex mix of sugars. Although most yeasts can ferment hexoses, this is not the case for sugars like xylose and arabinose, which can reach 35% of the total carbohydrate content. This situation becomes even more complicated when considering the significant amount of inhibitors in these lignocellulosic feedstocks, such as acetic acid that interferes with sugar assimilation. Jack Pronk showed that metabolic engineering made this fermentation possible by using, among others, expression of structural genes for the L-arabinose utilisation pathway of *Lactobacillus plantarum* into *S. cerevisiae*. Significant improvement in fermentation parameters was then achieved through repeated cycles of consecutive batch cultivation in media with different compositions. This evolutionary engineering strategy allowed rapid selection of an evolved strain exhibiting improved performance. It resulted in a 40% reduction in the time required to completely ferment glucose–xylose–arabinose mixtures with high ethanol yield (0.43 g/g of total sugar) (Wisselink et al., 2009). Julie Montheard (LISBP, Toulouse, France) also focused on xylose conversion into bioethanol using the alternative yeast *Candida shehatae*, which is able to naturally ferment xylose. The author successfully set up growth conditions to reach an average titre of 40 g/l of ethanol, with 80 g/l of biomass dry weight without any loss of viability, which represents one of the best performances reported in the bibliography.

Still in the field of biofuels, development of new routes for hydrotreated lipid production is also being promoted. Nicolas Morin (MICALIS, Jouy-en-Josas, France) and Maud Babau (LISBP, Toulouse, France) showed that oleaginous yeasts such as *Y. lipolytica* and *Rhodotorula glutinis* could be used for lipid-based biofuels, including aeronautic applications in which specifications are drastically more restrictive than for car engines. By using a well-controlled fed-batch cultivation process, N. Morin integrated both quantitative physiological parameters and transcriptomic data to decrypt gene networks that control lipid synthesis during the transition from biomass formation to lipid accumulation in *Y. lipolytica*. Transcriptome profiling highlighted an early lipid accumulation phase, characterised by an increase in nitrogen metabolism together with strong repression of protein production and activity, followed by a late lipid accumulation phase characterised by the rerouting of carbon fluxes within cells (Morin et al., 2011). Through microbial engineering approaches, the LISBP team first demonstrated that *R. glutinis* could accumulate lipids up to 0.72 g/g of biomass with a yield of 0.22 g/g glucose, i.e. 95% of the theoretical maximal yield of this carbon source. This remarkable performance relative to lipid accumulation led M. Babau to study the possibility of growing *R. glutinis* using a mixture of both pentose sugars and hexoses. Despite a clear reduction in energetic and carbon yields while increasing the xylose/glucose ratio, they showed that xylose could be assimilated for biomass production, therefore confirming the possible use of this alternative yeast for lipid production from renewable non-food resources.

In addition to biofuels, yeast is of primary importance in oenology. In this particular applied context, alternatives to metabolic engineering approaches have been engaged to improve the fermentation potential of wine yeast strains. Bioprocess optimisation, evolutionary engineering and crossing approaches of parental yeasts that strongly diverge for one or more phenotypic properties (or traits) can be used for such purposes. Philippe Marullo (ISVV/SARCO, Bordeaux, France) presented data relative to the field of yeast breeding. His approach relies on heterosis, which represents the superiority of the hybrid in comparison to the progenitor strains for quantitative polygenic traits. While studying a total of 64 strains, both diploid *S. cerevisiae* and *Saccharomyces uvarum* parental strains and their resulting hybrids, he found many cases of punctual heterosis for either kinetic properties or fermentation products, including aromas. Interestingly, he noted that few interspecific hybrids showed high levels of ethyl esters. Another approach based on quantitative trait loci (QTL) analysis was highlighted by Jessica Noble (Lallemand R&D, Blagnac, France). Using functional complementation analysis, J. Noble identified loci implicated in the reduction of SO₂, H₂S and acetaldehyde, which are undesirable compounds in oenology. She also identified another locus that appeared to be determinant for fermentative performances under nitrogen starvation, which is one of the limiting parameters in wine fermentation. Claire Brice (SPO, Montpellier, France) illustrated in her poster the strategy developed to identify mechanisms underlying the diversity of nitrogen use in an oenological context. She tested three strains of *S. cerevisiae* with high nitrogen requirements and four strains with low nitrogen requirements, in both rich and poor nitrogen media. Transcriptome analysis highlighted that efficient *de novo* protein synthesis is of primary importance for strains with low nitrogen requirements, while it discarded the nitrogen storage and amino acid assimilation-based-hypotheses.

2. Evolution and the genomics revolution

These later presentations naturally led us to the ‘Genomes & Evolution’ session, which was opened by Gianni Liti (IRCAN, Nice, France). G. Liti gave us a clear lecture on population genomics and complex traits in yeast, highlighting the advantages of yeast for linking natural genetic variations in a population to phenotypic variations in a given trait. The resequencing of a large collection of *Saccharomyces* strains isolated worldwide and characterised at the genomic and phenotypic levels both provided data on population structure and proved to be a powerful tool for identifying sequence variants accounting for phenotypic variations. In addition, the development of a new QTL mapping method and its immediate advantages were presented. This strategy, which involves several rounds of crosses, artificial selection and whole genome sequencing, enables measuring genome-wide changes in parental allele frequency, hence yielding a much higher resolution than previous genomic linkage studies (Cubillos et al., 2011; Parts et al., 2011). The genes *IRA1* and *IRA2*, encoding two Ras GTPase-activating proteins (GAP) that act

as negative regulators of the Ras–cAMP signalling pathway, were validated as determinant QTLs for heat sensitivity.

The work reported by G. Liti was a perfect example of how the massive use of new sequencing methods has accelerated the production of genomic data, leading to conceptually new approaches, specifically relevant for genome evolution studies. However, the advent of new generation sequencing has also lowered the quality of the resulting data, especially in terms of assembling and annotation. Hence, general sites such as Genbank and EMBL now demand a set of meta-data, information on sequence quality and minimal annotation for publication of a new genome. In that context, Tiphaine Martin (LaBRI – Génolevures, Talence, France) gave a comprehensive presentation of these recent evolutions in the publication policies of sequencing data on public websites. Two examples of genome sequencing and analysis readily meeting these criteria were presented, both from biotechnologically relevant strains. In the first, Jean-Marc Daran (Delft University of Technology, The Netherlands) exposed the sequencing of the laboratory CEN.PK113-7D strain (Nijkamp et al., 2012). Numerous genomic variations have been identified compared to S288c, including copy number variations and, in particular, 6 duplicated clusters, some of them containing *MAL* genes (involved in maltose metabolism). Remarkably, genes encoding transcription factors and DNA binding proteins are over-represented among sequences presenting single nucleotide variations and insertions/deletions. Moreover, the authors reported variations in the number of tandem repeats in genes like *SNF11*, involved in transcription efficiency. Genomic regions present only in the CEN.PK113-7D strain have also been identified, particularly the one on chromosome I that contains the first two genes of the biotin biogenesis pathway, enabling this strain to grow in the absence of biotin in the medium. Then, Virginie Galeote (SPO, Montpellier, France) exposed new results from sequencing of the commercial wine yeast strain EC1118. The genome of this strain contains 34 additional genes compared to S288c, clustered into three large regions that have been horizontally transferred from distant yeasts and may participate in its technological performances (Novo et al., 2009). Most of these genes are unknown, but some of them, encoding high-affinity fructose and oligopeptide transporters, could be implicated in oenological traits. V. Galeote and collaborators discovered that one of these DNA regions, originating from the wine contaminant *Zygosaccharomyces bailii* and recently transferred, is likely amplified in yeast genomes as an extrachromosomal circular DNA molecule which can integrate the *Saccharomyces cerevisiae* genome at different loci by non-homologous end joining (NHEJ) (Galeote et al., 2011). Indeed, karyotypic analysis revealed different possible locations and sometimes different copies of this region in other wine yeasts. Furthermore, gene organisation of these different fragments is variable and the breakpoint sequences show little or no homology, corresponding to insertions by NHEJ.

In addition to their intrinsic scientific interest for deciphering the genetic basis of physiological traits, these fine analyses of particular genomes allow development of new

global approaches to searching for genes or gene networks of possible technological interest, such as that presented by Christian Brion (SPO, Montpellier, France). By analysing segregants from a S288c \times EC1118 cross, he identified several interesting expression QTLs (eQTLs). One of them, triggered by the *PDR8/HAP1* locus, is particularly relevant for its regulation of the *PDR* detoxification gene family, while another contains the oenological form of *THI3* gene and contributes to increased expression of genes involved in the thiamine biogenesis pathway. Finally, a cluster of abnormal transcription on chromosome 16 revealed a translocated fragment associated with a higher fermentation rate at 70% of the fermentation process. From a similar perspective, but on a very different yeast model, Stéphanie Michely (MICALIS, Jouy-en-Josas, France) used comparative physiology and genome sequencing to investigate the basis of growth on hydrophobic substrates and lipid storage of *Y. lipolytica* and its eight closest species. Six complete genomes have been obtained and, together with physiological analysis, enabled identification of the *POX* family (encoding Acyl-CoA oxidases) as being associated with growth on oleic acid. Loss of *POX4* or duplication of *POX5*, for instance, has been mentioned, and divergence of the *POX4* sequence in one strain is linked to better growth on oleic acid.

3. Traffic and organelles: focus on mitochondrial ATP synthase

In the “traffic and organelles” session, mitochondria have been the object of several oral and poster presentations, mainly dealing with different aspects of mitochondrial physiology and metabolism. In particular, two talks were given on ATP synthase, studied from very different perspectives. Daniel Brèthes (IBGC, Bordeaux, France) showed that the two ATP synthase subunits e and g, non-essential for enzyme activity, are required for both the supramolecular organisation of ATP synthase as well as for mitochondrial morphology and cristae formation. He also showed that those conserved in higher eukaryotes properties have significant consequences well beyond ATP synthase activity. Alexandre Martos (IBGC, Bordeaux, France) showed that evolutionary transfer of genes from the mitochondria to the nuclear genome requires modification of the gene product itself, but also adaptation of other cellular pathways allowing protein transfer to its final mitochondrial compartment. By using the mitochondrial *ATP9* gene of *S. cerevisiae* as a case study, these authors successfully complemented the *atp9* mutant through nuclear expression of the filamentous fungus *Podospira anserina* homologue, likely due to the low hydrophobicity of the first transmembrane segment of the protein. This hypothesis is strengthened by the inability to mature the *S. cerevisiae* ATP9 precursor protein, synthesised in the cytosol, by mitochondrial proteases, contrary to a hybrid ATP9 protein carrying the first transmembrane segment of *P. anserina* ATP9. Besides fundamental knowledge of mechanisms of gene transfer during evolution, their results augur well for future treatment of genetic diseases of mitochondrial origin.

4. Let's travel into the nucleus: from DNA organisation to transcription

Microorganisms are particularly exposed to fluctuating environments, but are able to swiftly adjust their behaviour according to their needs. How cells are able to sense these changes and subsequently activate specific regulatory pathways remains subject to debate. In eukaryotes, genomes are physically separated from other cellular components by the nuclear envelope (NE), enabling establishment of a distinct environment for DNA replication, gene expression and maintenance of genome integrity. Genome-wide, molecular and cellular approaches combined in different mutated backgrounds were used to unravel the complex regulation that underlies DNA-based processes in yeast.

The accurate replication of genomic DNA depends on sequential activation of hundreds of replication origins. During replicative stress induced by genotoxic agents, initiation at late origins is inhibited by the DNA replication checkpoint (DRC) (Tourrière and Pasero, 2007). Whether or not the DRC is also active during a normal S-phase is not known. Philippe Pasero (IGH, Montpellier, France) showed that the DRC delays the activation of late origins under normal growth conditions. He also reported that phosphorylated histone H2A, a mark of DRC activation, accumulates at transcribed regions immediately after passage of replication forks. These data suggest that the DRC detects interferences between replication and transcription in order to regulate S-phase progression. Replication forks are fragile structures that frequently encounter obstacles hindering their progression. How paused forks are maintained is still a matter of debate. Mireille Tittel-Elmer (University of Calgary, Canada) discovered that cohesins, a protein complex important for maintaining sister chromatid cohesion, are localised at sites of replication and spread along DNA as forks move. Moreover, Sec1p enrichment at sites of replication is dependent on the structural features of Rad50p and promotes fork restart. This finding suggests a new role for cohesin in the maintenance of replisome integrity.

Another level of regulation is provided by co-activators of transcription such as the Spt-Ada-Gcn5-acetyltransferase complex (SAGA), which regulates the expression of inducible genes in eukaryotes. Dominique Helmlinger (CRBM, Montpellier, France) found that the SAGA subunit Tra1 is not essential in *S. pombe* as compared to *S. cerevisiae*, enabling a functional study of this protein. He showed that rather than having a global role within SAGA, as other subunits do, Tra1 is involved in recruitment of SAGA at only a subset of SAGA-dependent promoters and in transcriptional regulation of the same subset of genes in response to environmental stresses. Further findings on yeast transcription were presented by Jessie Colin (CGM, Gif-sur-Yvette, France), who showed results of a screen aimed at detecting new elements involved in RNAPII transcription termination. In the framework of this screen, J. Colin identified a new termination mechanism relying on a Myb-related transcription activator, Reb1p. This activator binds to an 8-nt conserved motif on DNA and elicits termination of a new class of non-coding unstable transcripts,

providing a fail-safe termination mechanism for a number of mRNAs *in vivo*.

The NE bridges the cytoplasm to the nucleoplasm through the nuclear pores and integral NE proteins, allowing for regulation of cellular events. Hugo Bretes (IJM, Paris, France) focused on Pml39p, a protein anchored to the nuclear pore complex by the NE myosin-like proteins Mlp1p and Mlp2p. Together with Mlp1p and Ulp1p, Pml39p is required for proper export of messenger ribonucleoparticles (mRNPs) resulting from intron-containing genes, thus preventing inappropriate expression of mRNAs derived from these genes. New roles for the NE in chromosome positioning were also presented by Maya Spichal (Institut Pasteur, France) who showed that the outer NE protein Csm4p might have an effect on telomere localisation and dynamics through its interaction with currently unidentified components of the inner NE. Telomere homeostasis is mainly regulated by the well-known telomerase enzyme. Using high-resolution microscopy, Franck Gallardo (LBME, Toulouse, France) demonstrated that telomerase-recruitment clusters (T-recs) are formed on telomeres only at the end of the S-phase. This technique could be used to screen for telomerase activity in cancerous cells (Gallardo et al., 2011).

5. RNA life, from synthesis to translation

The yeast is an easy genetic tool allowing researchers to delve deeper into the understanding of complex mechanisms, particularly relevant for many aspects of the RNA life cycle that were developed in the 'RNA, Ribosome and Translation' session. DEAD-box RNA helicases are ubiquitous proteins involved in virtually all processes involving RNA. They are RNA-dependent ATPases and ATP-dependent RNA binding proteins, but they display only weak helicase activity when assayed *in vitro*; this makes it difficult to characterise their specificity, regulation or target identification. Meriem Senissar and Agnès Le Saux (IBPC, Paris, France) presented some of the data they recently obtained concerning the essential DEAD-box family member Ded1p. This protein is homologous to the human DDX3 and it is one of the most active helicases *in vitro*. However, it shows no specificity *in vitro*, indicating that *in vivo* its specificity is likely due to cofactors that need to be identified. They showed that Ded1p interacts directly with the translation initiation factors eIF4G and eIF4E, present on the 7-methylguanosine CAP of mRNAs, and with Pab1p, which is a polyA binding protein present at the 3'-end of mRNA. They also established that some of these interactions stimulate the ATPase activity of Ded1p *in vitro*. They showed that Ded1p is associated with mRNPs containing eIF4G, eIF4E and Pab1p, but that it is not associated with polysomes. This demonstrates that Ded1p has an affinity for mRNA. The identification of Ded1p partners and substrates should contribute to characterisation of its function and lead to better understanding of the role of RNA helicases in the cell.

Splicing is an important regulatory step in the mRNA life cycle. It can be affected either in *cis*, by the mRNA splice sites, or in *trans*, by regulatory proteins involved in the

splicing event. The spliceosome is an RNA–protein structure composed of five small nuclear ribonucleoproteins (snRNP) associated with Sm proteins. The biogenesis of snRNPs is a complex process mediated by the methylosome and the survival motor neuron (SMN) complex. In his presentation, Adrien Barbarossa (IGMM, Montpellier, France) described the identification of an *S. pombe* ICln homologue. Mutations into *spICln* lead to growth and splicing defects. He noted that some introns with suboptimal polypyrimidine tracts are inefficiently spliced in the *Dicln* mutant.

Ribosomes are RNA–protein macromolecular complexes that require a plethora of factors for their synthesis. During the last two decades, a large number of genome-wide strategies have been employed to identify *trans*-acting factors involved in ribosome biogenesis. This gave rise to the discovery of not less than 200 factors, including proteins and small nucleolar ribonucleoprotein particles (snoRNPs) implicated at different steps of ribosomal RNA precursor (pre-rRNA) maturation and ribosome assembly. The challenge now is to determine the precise function of these 200 factors. Nearly all of the ribosome biogenesis process was covered during this 10th LMO meeting, including the study of the early nucleolar pre-90S, the pre-60S and the late cytoplasmic pre-40S particles, work mainly done in the ‘Laboratoire de Biologie Moléculaire Eucaryote’ (LBME, Toulouse, France), directed by Michèle Caizergues-Ferrer. The very first steps in ribosome assembly were investigated through characterisation of the Utp23p and Kri1p proteins. Indeed, Coralie Hoareau-Aveilla (LBME) showed that these two proteins interact specifically and in a mutually exclusive manner with the snR30-snoRNP. In addition, she reported evidence suggesting that Utp23p plays a crucial role in early pre-ribosome conformation changes to promote snR30 release from the pre-ribosomal particles (Hoareau-Aveilla et al., 2012). To the already long list of early *trans*-acting factors taking part in the small subunit (SSU) processome, Elodie Choque (LBME) added a new member: the Efg1p protein. This nucleolar and non-essential protein is associated *in vivo* with the 35S, 23 pre-rRNAs and the U3 snoRNA. Furthermore, yeast cells depleted for Efg1p cultured at 37 °C showed defective 18S rRNA production. Indeed, E. Choque showed that Efg1p is required for 35S pre-rRNA early cleavages at sites A0, A1 and A2. These cleavages are known to take place very early during the processing pathway of the 35S pre-rRNA and to be essential for the elimination of the 5' external (5'ETS) and internal (ITS1) transcribed sequences surrounding the 18S rRNA sequence. The talk given by Yan-ling Chen (LBME) discussed early pre-60S ribosome biogenesis. Using immunoprecipitation experiments, Yan-ling showed that the Gno1p protein, previously reported to interact with the helicase Prp43p, is associated with pre-60S ribosomal particles and is required for their production. In addition, using *in vitro* enzymatic tests, she demonstrated that PinX1, the human homologue of Gno1p, stimulates the helicase activity of Prp43p. Thus, it was proposed that Prp43p, implicated in several steps of ribosome biogenesis, is specifically regulated by Gno1p in the pre-60S particle maturation pathway. Late pre-40S biogenesis in humans was broached by Kamila

Baumas (LBME), who identified RioK3 as a new member of the RIO kinase family involved in rRNA 18S production. She showed that RioK3 is associated in the cytoplasm with pre-40S particles and seems to display a cytoplasmic function required for correct maturation of the 21S pre-rRNA (Baumas et al., 2012).

Finally, Yuri Motorin (AREMS, Nancy, France) presented a structure–function analysis of the Nop2 protein, a putative m⁵C:methyltransferase implicated in maturation of the 25S rRNA. Using various functional complementation experiments, he showed that the N-terminal part of Nop2p is important for viability and nucleolar localisation. Thanks to bisulfite RNA sequencing he discovered new methylation sites of the 25S rRNA that he directly linked to Nop2p activity.

In this ‘RNA, Ribosome and Translation’ session, another important aspect of ribosome life was presented: the regulation of ribosome function, and more particularly translational frameshifting. Oliver Namy and Isabelle Hatin (IGM, Orsay, France) reported their recent results on the [PSI(+)] prion protein corresponding to an aggregated conformation of the translation release factor eRF3. They showed that expression of [PSI(+)] stimulates a +1 frameshift event during translation of OAZ1 mRNA, which encodes a negative regulator of cellular polyamines. This leads to enhancement of antizyme production and, as a direct consequence, a decrease in the polyamine level. To go further, genome-wide approaches such as ribosome profiling were employed to identify a more general role for [PSI(+)] in translation regulation. This ongoing work possibly points out new and unexpected functions of the prion [PSI(+)]. In turn, Sandra Blanchet (IGM, Orsay, France) showed that the [PSI(+)] strain can be used as a tool to study how natural tRNA suppressors are selected during stop codon readthrough. Using a reporter gene harbouring a premature stop codon expressed in the [PSI(+)] strain, she was able to precisely measure the selection rate of each possible tRNA suppressor and incorporation of the corresponding amino acid. This study is of particular importance for the treatment of human pathologies caused by the presence of stop codons in critical genes such as the P53 gene in cancers and the DMD gene in Duchenne muscular dystrophy. Indeed, depending on its nature, the incorporated amino acid may differentially affect the function of the mutated protein.

mRNA level regulation does not always come from where it is expected. Indeed Damien Hermand (LGM, Namur, Belgium) explained that mRNA abundance could come from the codon content that affects translation efficiency. The elongator complex is responsible for the 5-methoxycarbonylmethyl-2-thiouridine modification of the wobble uridine present in the tRNA. The lack of tRNA modification due to elongator mutants leads to various phenotypes such as mitosis delay or cytokinesis failure due to inefficient translation. D. Hermand found that mRNAs that contain more AAA codons than AAG are less translated in mutants of the elongator complex. He highlighted a new mechanism by which the codon content, coupled with the tRNA modifications by elongator, regulates the protein level through translation efficiency.

6. It's all about control: key elements in cell cycle progression and establishment of cell polarity

Several new and fascinating results were presented during the sessions devoted to 'Cell cycle, cytoskeleton and polarity'. The importance of the fission yeast *S. pombe* as a model for cell division was here again demonstrated, with two talks on microtubule organisation and one on the contractile ring. First, Phong Tran (Institut Curie, Paris, France) in his introductory lecture attempted to answer the question: "How to build a bipolar mitotic spindle?" He described a screen for spindle-assembly-defective mutants and the identification of a new protein, Sad2p, involved in initiation of mitotic spindle bipolarity. Then, Yannick Gachet (LBCMCP, Toulouse, France) described the development of a powerful mathematical model of chromosome segregation which enables predicting defects in segregation and chromosome attachment to microtubules in different mutants and conditions. Finally, Anne Paoletti (Institut Curie, Paris, France) gave a presentation on contractile ring assembly. A previous work from the author showed that Pom1p kinase concentrates at cell ends, so its concentration decreases in the middle of the growing cell, hence allowing cell division (Moseley et al., 2009). A. Paoletti now reported that Pom1p inhibits Mid1p/Anilin, a major factor in division plane definition and cell size control during division. Pom1p also inhibits kinases Nim1p, Cdr1p and Cdr2p, three inhibitors of Wee1p. Pom1p phosphorylation sites on Cdr2p have been mapped and it is suggested that they influence Cdr2p–Mid1p interactions and Cdr2p kinase activity.

The budding yeast *S. cerevisiae* was nevertheless the model used in several other cell-cycle-related presentations. In his talk, Damien Laporte (IBGC, Bordeaux, France) demonstrated that *S. cerevisiae* quiescent cells are distinct from G1-arrested cells. Indeed, they identified G₂ budding cells that are *bona fide* quiescent and that display characteristic structures of quiescent cells, such as actin bodies and proteasome storage granules (PSGs). Remarkably, carbon starvation is sufficient to trigger entry into quiescence, including for cells in G₂/M phase (Laporte et al., 2011). Subsequently, glucose alone can activate mobilisation of the actin bodies and re-entry into proliferation, but it has to be internalised, enter glycolysis and be metabolised at least until pyruvate. The subtlety of proliferation and growth control by nutritional signals was also illustrated by Gregory Bonfils (University of Fribourg, Switzerland). He showed that leucyl-tRNA synthetase Cdc60p controls TORC1, an essential regulator of eukaryotic cell growth, via the EGO complex (Bonfils et al., 2012). In this work, a leucine-dependent physical interaction between Cdc60p and the TORC1 regulators GTPase Gtr1p was identified. This interaction, necessary and sufficient for TORC1 activation by leucine, is mediated by the amino-acid-editing domain CP1 (connective peptide 1) of Cdc60p, and does not require functionally independent amino acylation activity of Cdc60p. The authors proposed a model in which the conformational change in CP1, resulting from its engagement in editing mischarged

tRNA^{Leu}, disrupts the Cdc60p–Gtr1p interaction and consequently downregulates TORC1.

Another elegant study conducted on *S. cerevisiae* was presented by Fabrice Caudron (IBC, ETH Zürich, Switzerland), who consensually received the jury's Nikon prize for the quality of his photographs. In the absence of an actual mating partner, haploid cells adapt to low concentrations of pheromones and restart budding. Once they have escaped from pheromone arrest, mother cells maintain this memory and go on budding. The molecular details of this behaviour have been described and are shown to depend on a new mechanism of cytoplasmic epigenetics.

Acronyms of the laboratories

AREMS	ARN-RNP, structure fonction-maturation, enzymologie moléculaire et structurale
CGM	Centre de Génétique Moléculaire
CRBM	Centre de Recherche de Biochimie Macromoléculaire
IBC	Institute of Biochemistry
IBGC	Institut de Biochimie et Génétique Cellulaires
IBPC	Institut de Biologie Physico-Chimique
IGH	Institut de Génétique Humaine
IGMM	Institut de Génétique Moléculaire de Montpellier
IGM, Orsay	Institut de Génétique et Microbiologie
IJM	Institut Jacques Monod
IRCAN	Institut de Recherche sur le Cancer et le Vieillessement
ISVV	Institut Supérieur de la Vigne et du Vin
LABRI	Laboratoire Bordelais de Recherche en Informatique
LBCMCP	Laboratoire de Biologie Cellulaire et Moléculaire du Contrôle de la Prolifération
LBME	Laboratoire de Biologie Moléculaire Eucaryote
LGM	Laboratoire de Génétique Moléculaire
LISBP	Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés
MICALIS	Microbiologie de l'Alimentation au Service de la Santé humaine
SPO	Sciences Pour l'Oenologie

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