Yeast population genomics: origin and evolution of a classic model organism

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The baker's yeast *S. cerevisiae* has had a long association with human activity, leading to the idea that its use in fermentation leads to its domestication. However, recent studies revealed that *S. cerevisiae* has a rather ubiquitous distribution in the wild, not limited to human-associated environments, showing that its history goes far beyond its association with humans. In the past decade, we applied population-level sequencing to thousands of *S. cerevisiae* strains to further illuminate the population structure and the impact of human activity. Many breeds associated to specific human process (e.g. wine, sake, beer, bioethanol) have specific genomic signatures likely driven by adaptation to industrial environments. In parallel, we discovered many wild lineages and detected substantial genomic differences when compared with the human associated strains. Interestingly, genome analysis of highly diverged wild lineages that predate domestication is consistent with south East Asia as the geographic origin of *S. cerevisiae*.

From the sequenced strains collection, we assembled the *S. cerevisiae* pangenome and characterised ~8000 ORFs that are available to the species. A core set of ~5000 ORFs are invariably present in all sequenced strains, whereas an additional ~3000 ORFs are detected in a subset of strains at varying frequency in the population. We used phylogenetic approaches to infer the origin of the ORF pangenome. We detected pervasive introgressions (~1000 ORFs) from the closest related species, *S. paradoxus*, and horizontal gene transfers (~300 ORFs) from distantly related yeast species that coexist in fermentation settings. The variation in genome content and copy number is highly enriched in the subtelomeres. Long reads sequencing technologies allow accurately assembling these regions and studying their dynamic. Quantitative trait loci mapping show that gene content and copy number variation shape individual quantitative phenotypic variation. Exploiting the natural variation of *S. cerevisiae* using genetics and genomics methodologies can have important implications in the context of biotechnological traits.

Moonlighting proteins in yeasts. A putative moonlighting protein in the N-acetyl glucosamine utilization pathway

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Moonlighting proteins are multifunctional proteins that participate in unrelated biological processes and are not the result of gene fusion or alternative splicing. Many such proteins have been uncovered in yeasts and we will discuss the usefulness of yeasts to study homologous and heterologous moonlighting proteins.

During a study of the N-acetylglucosamine (NAGA) utilization pathway in the non-conventional yeast *Yarrowia lipolytica* we have found that NAGA-kinase, the enzyme that initiates the intracellular metabolism of this sugar , participates in other processes unrelated with its metabolic role. We will highlight the following ones: Influence on gene transcription/ Participation in sporulation/ Alteration of morphology.

The expression of the genes encoding the enzymes of the NAGA utilization pathway is induced by this sugar. Deletion of the gene *YlNAG5* encoding NAGA kinase renders that expression independent of the presence of NAGA. Expression of *YlNAG5* increases during sporulation and homozygous *Ylnag5*/*Ylnag5* diploid strains sporulate very poorly as compared with a wild type control. Overexpression of *YlNAG5* produces aberrant morphologies in different media.
These results suggest that the protein *Yl*Nag5 might act as a moonlighting protein. We will present different experiments to test this idea and will discuss the results obtained and/or the problems faced.

Yeast physiological diversity and interspecies interactions under industrially relevant conditions

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ABSTRACTS

Saccharomyces cerevisiae is the main yeast species involved in traditional or industrial fermentation of sugar rich substrates, including the production of fermented foods and bioethanol. However, other yeast, as well as bacteria, play a relevant role in these fermentation processes, especially fermented foods prepared with non-sterilized raw materials. For example, *Hanseniaspora*/*Kloeckera*, *Pichia*, *Candida* or *Metschnikowia* strains predominate during the initial stages of spontaneous grape wine fermentation. Some decades ago oenologists learned to master wine fermentation processes by using *S. cerevisiae* starter yeasts. Despite the gain in reliability of the fermentation process and overall quality of wines, the rather quick replacement of natural microbiota during the first stages of grape juice fermentation have leaded to a relative uniformity of the sensorial profile of commercial wines. Evidence has also been accumulated during the last twenty years on the positive contribution to wine quality of some of these indigenous yeast species during spontaneous fermentation.

In this context, one of the late trends in wine microbiology is gathering together the advantages of microbiological control and those of the metabolic diversity of natural fermentation, by developing yeast starters from wine yeast species alternative to *S. cerevisiae*. In addition to their contribution to the aromatic profile, the metabolic features of some of these yeasts can eventually be managed to reduce alcohol content of wines through aerobic respiration.

Industrial use of non-conventional yeasts and/or non-conventional fermentation conditions, including aerated fermentation (to allow for partial respiration of sugars) and multispecies starters (either in sequential or simultaneous inoculation), opens new questions whose answers will be interesting both from an academic or an applied perspective. This talk will deal with some of these topics, including the impact of oxygenation on the metabolism of some yeast species, beyond the direct impact on respiration; the relationship between fermentation under micro-oxygenated conditions and acetic acid production; or the early responses of yeast strains to the presence of potential competitors in the same culture medium.

Yeasts and stress responses: learning how to leverage cellular potential for matching industrial requirements

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When exploited as cell factories, microbial cells are exposed to harsh conditions impairing titer, yield and productivity of the fermentative processes. The development of robust strains therefore represents a pivotal but not easy task to accomplish for the implementation of cost-effective bioprocesses. Remarkably, in natural environment microorganisms are very often exposed to stresses, mainly deriving from the constant fluctuation of chemical and physical parameters. They can very well cope with those stresses, triggering specific mechanisms and reorganizing their cellular metabolism and physiology. These mechanisms are still largely unknown, and the cellular rewiring is often leading to metabolic reduction or inactivation. Therefore, it is crucial to develop accurate strategies of strain engineering and design, based on the knowledge of single determinants as well as of the network of signaling and response, to turn cellular capabilities into traits that can be useful for industrial needs. Here we show examples of how stress and stress responses guided our researches on *Saccharomyces cerevisiae* and other non-saccharomyces yeasts, to make them more suitable for industrial application, with a specific attention to the constraints deriving from the use of renewable substrates and from the accumulation of the desired products.

Degradation/synthesis cross talk during mRNA turnover

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The central dogma of molecular biology states that genetic information flows from DNA, through mRNA to protein. Proteins and mRNAs are synthesized and degraded by molecular machineries subject to complex regulatory mechanisms. In many instances both molecules are in steady state, in which the synthesis and degradation rates are identical. This chemical equilibrium, however, is

asymmetrical as degradation, but not synthesis, depends on the concentration of the macromolecules. When analyzing the respective roles on every step along the genetic flow in gene expression regulation three properties should be taken into account: i) the different function and chemical reactivity of mRNA and protein; ii) the fact that protein, not mRNA, is the ultimate goal of gene expression, and, iii) the already explained kinetic asymmetry of the steady state equilibrium.

We have used the model yeast *Saccharomyces cerevisiae* to explore the importance of the various parameters in gene expression. Using global data from our laboratory and from other sources, we conclude that there is a homeostatic control of the global mRNA concentration within relatively narrow limits (ribostasis). These limits seem to be wider than the homeostatic limits for global protein concentration (proteostasis). We have found that to achieve ribostasis, the cell uses cross talk pathways between nuclear transcription and cytoplasmic mRNA degradation. We also found that ribostasis is maintained in spite of changes in cell volume, growth rate (GR) or during stress responses. The global ribostasis, however, does not apply to individual mRNAs or to groups of functionally-related mRNAs. Genes related to translation increase their mRNA concentration in a GR-dependent manner whereas mitochondria and stress-induced genes decrease their mRNAs. Interestingly, each group of genes differentially uses transcription and mRNA degradation to vary mRNA concentrations whereas global mRNA turnover increases with GR and decreases with cell volume.

On the other hand, translation for particular gene groups is much less regulated but, given that it accounts for a large part of the energy expenditure in an exponentially growing yeast cell, the level of protein-biosynthetic machinery is tightly regulated at the transcriptional level.

CRISPR/Cas9: a molecular Swiss army knife. From gene to pathway to genome engineering.

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For decades, *Saccharomyces cerevisiae* has been successfully used as a model organism to decipher biological processes in higher eukaryotes and as a popular metabolic engineering platform. Optimization of its core machinery and expression of heterologous product pathways in *S. cerevisiae*, requires introduction of multiple (successive) genetic modifications . Introduction of the required modifications has so far remained a time-consuming and labour-intensive process, as each individual alteration requires a cycle of transformation, selection and confirmation.

In the past four years a variety of methods have been developed not only enabling faster *S. cerevisiae* strains construction, but also turning this yeast in a molecular biology workhorse for the cloning of whole bacterial genomes by exploiting its unique recombination potential. Combined with genome editing methods using homing endonucleases or CRISPR, yeast biologists have been able to introduce simultaneously several gene deletions and complete metabolic pathway resulting in complex strain engineering. The present paper will review these recent advances and focus on two ground-breaking examples describing the replacement of the ATP-requiring cytosolic acetyl-CoA supply by an ATP-independent route and the exchange of the complete *S. cerevisiae* glycolysis by that of *S. krudiazevii* and by a mosaic pathway comprising yeast and human genes.

Saccharomyces cerevisiae as a tool-box for protein engineering by directed evolution

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Directed molecular evolution is a robust, fast and reliable method to design enzymes. Through iterative rounds of random mutation, recombination and screening, improved versions of enzymes can be generated that act on new substrates, in novel reactions, in non-natural environments, or even to assist the cell to achieve new metabolic goals. Among the hosts used in directed evolution, the brewer's yeast *Saccharomyces cerevisiae* offers a repertoire of solutions for the functional expression of complex eukaryotic proteins that are not otherwise available in prokaryotic counterparts.

Used exhaustively in cell biology studies, this small eukaryotic model has many advantages in terms of post-translational modifications, ease of manipulation and transformation efficiency, all of which are important traits to engineer enzymes by directed evolution. Moreover, the high frequency of homologous DNA recombination in *S. cerevisiae* coupled to its efficient proof-reading apparatus opens a wide array of possibilities for library creation and gene assembly *in vivo*, fostering the

evolution of different systems from single enzymes to complex artificial pathways. This communication summarizes the tools and strategies that we have developed during last decade for the molecular evolution of different ligninases in yeast (oxidoreductases involved in the degradation of lignin during natural wood decay).

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Reconstructing metabolism by high-throughput mass spectrometry

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Cellular metabolism plays a pivotal role in many areas ranging from biotechnology to nutrition, cancer biology, diagnostics, toxicology, etc. Decades of biochemical characterization and, more recently, genomic sequences and in silico reconstructions produced large and yet curated maps of

metabolic networks. Unfortunately, this dense knowledge on the constituents of metabolism does not automatically translates into a better understanding of metabolic responses because we largely lack information on dynamics.

In our lab, we largely rely on mass spectrometry to characterize the in vivo response to internal and environmental perturbations and eventually unravel the complex regulation governing cellular metabolism and growth. In spite of our proficiency in quantitative metabolomics and 13C metabolic flux analysis, over the years we have drifted our strategy in favor of possibly qualitative and non-targeted measurements. To our benefit, our workhorse platform currently allows to profile thousands of compounds in thousands of samples per day. In polar fractions of the intracellular metabolome, we obtain dense coverage of primary metabolism and can thus detect subtle changes in most biosynthetic, anabolic, and energy-generation pathways. The platform is in productive use for e.g. functional genomics, genome-wide association studies, mapping regulatory networks, drug screens, drug target identification, the discovery of allosteric regulation, and even online analysis of cell suspensions at high temporal resolution. More recently, we extended our platform to allow real-time analysis of living cells. The methods was proved to work for microbial and mammalian cells, and can provide full metabolomics profile as fast as all 15 sec.

I'll present two illustrative applications with yeast. First, a proteome-scale enzyme discovery screen with non-targeted metabolomics. Second, a real-time metabolomics analysis of the yeast cell cycle.

Candida albicans-macrophage interaction: insights from proteomics

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Candida albicans is a commensal dimorphic yeast being an assiduous component of the human microbiota, colonizing mucosal surfaces, in up to 50% of the population. *C. albicans* does not normally cause disease in immunocompetent hosts; however, as opportunistic pathogen, it is capable of creating challenging systemic infections in immunocompromised persons such as cancer and HIV patients, as well as patients in postoperative intensive-care units due to the limited antifungal arsenal. Moreover, a lack of efficient diagnostic procedures exacerbates the problem. Understandably, extensive time and effort have been invested in studying the interactions between *C. albicans* and the host.

The innate mammalian immune system plays a key role in fighting *Candida infections*. Phagocytic cells, such as macrophages and neutrophils, are the primary line of defense against microbial infections and are critical to preventing invasive candidiasis. Macrophages are phagocytic cells that play an essential role in the primary response to pathogens, in the maintenance of tissue homeostasis, in the promotion and resolution of inflammation, and in tissue-repair processes. Their relevance for *C. albicans* infections is widely studied because they are capable of engulfing the yeast and eliminating them and, where required, triggering the recruitment and activation of other immune cells. Studies of the interaction between *Candida* and macrophages could provide insights into putative new virulence factors that may aid the development of new treatments. Furthermore, the study of the macrophage response to the yeast may further help to understand the mechanisms of action by which the body can develop an immune response to *Candida* infections.

Macrophages may induce fungal apoptosis to fight against *C. albicans*, as previously hypothesized by our group. To confirm this hypothesis, we analyzed proteins from *C. albicans* cells after 3 h of interaction with macrophages using two quantitative proteomic approaches. A total of 51 and 97 proteins were identified as differentially expressed by DIGE and iTRAQ, respectively. The proteins identified and quantified were different, with only seven in common, but classified in the same functional categories. The analyses of their functions indicated that an increase in the metabolism of amino acids and purine nucleotides were taking place, while the glycolysis and translation levels dropped after 3 h of interaction. Also, the response to oxidative stress and protein translation were reduced. In addition, seven substrates of metacaspase (Mca1) were identified (Cdc48, Fba1, Gpm1, Pmm1, Rct1, Ssb1, and Tal1) as decreased in abundance, plus 12 proteins previously described as related to apoptosis. Besides, the monitoring of apoptotic markers along 24 h of interaction (caspase-like activity, TUNEL assay, and the measurement of ROS and cell examination by transmission electron microscopy) revealed that apoptotic processes took place for 30% of the fungal cells, thus supporting the proteomic results and the hypothesis of macrophages killing *C. albicans* by apoptosis. Targeted proteomic assays of 32 *Candida* proteins related with apoptosis is underway. Furthermore, we developed a quantitative proteomic approach for the study of human macrophage kinases after interaction with *C. albicans* cells that allowed the quantification of the proteins involved in the regulation of different signaling pathways.

In addition, to provide new and expanded proteome documentation of the opportunistically pathogen *C. albicans,* we have developed new protein extraction and analysis routines to provide a new, extended and enhanced version of the *C. albicans* PeptideAtlas. Two new datasets, resulting from experiments consisting of exhaustive subcellular fractionations and different growing conditions, plus two additional datasets from previous experiments on the surface and the secreted proteomes, have been incorporated to increase the coverage of the proteome. High resolution precursor mass spectrometry (MS) and ion trap tandem MS spectra were analyzed with three different search engines using a database containing allele-specific sequences. This novel approach combined with the post-processing and filtering implemented in the Trans Proteomic Pipeline consistently used in the PeptideAtlas project resulted in 49372 additional peptides and 1630 more proteins identified in the new *C. albicans* PeptideAtlas with respect to the previous build. A total of 71310 peptides and 4174 canonical (minimal non-redundant set) proteins (4115 if one protein per pair of alleles is considered) were identified representing 66% of the 6218 proteins in the predicted proteome. This *C. albicans* PeptideAtlas will provide a number of useful features, like comprehensive protein and peptide-centered data exploration tools, and it will stand as a valuable resource to assist targeted proteomic experiments aiding in the selection of candidate proteotypic peptides.

Yeast-based cloning & functional analysis of a candidate ceramide sensor from mammals

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Cells synthesize ceramides in the endoplasmic reticulum (ER) as precursors for sphingolipids to form an impermeable plasma membrane. As ceramides are engaged in apoptotic pathways, cells would need to monitor their levels closely to avoid killing themselves during sphingolipid biosynthesis. How this is accomplished is not known. Work in our lab focuses on homeostatic mechanisms of ceramide biosynthesis in the ER and how these mechanisms affect cellular life-death decisions. Ceramides are synthesized de novo by N-acylation of sphingoid long-chain bases on the cytosolic surface of the ER. The bulk of newly synthesized ceramides in mammals is converted to sphingomyelin in the lumen of the Golgi complex. Using a functional cloning strategy

in budding yeast, we previously identified a mammalian family of sphingomyelin synthases: the SMS family. Subsequent work revealed that one family member, SMSr, lacks SMS activity but synthesizes trace amounts of ceramide phosphoethanolamine in the ER. Acute disruption of SMSr catalytic activity in cancer cells triggers apoptosis due to a rise in ER ceramides and their mislocalization to mitochondria. Strikingly, removal of the enzyme's *N*-terminal sterile alpha motif or SAM domain did not affect SMSr-mediated catalytic activity but abolished its ability to control ER ceramides and prevent apoptosis. We postulate that SMSr is not a conventional sphingolipid synthase but serves a primary role in monitoring ceramide levels in the ER to prevent inappropriate cell death during sphingolipid biosynthesis. Ongoing work is aimed at addressing key aspects of this hypothesis, using budding yeast as experimental model.

From mTOR to mitochondria: how aging yeast cells are providing insights in translational geroscience

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The budding yeast has served as a premier model organism in geroscience for more than twenty years. Despite initial skepticism regarding the utility of yeast for understanding basic mechanisms of aging, several conserved genetic and environmental factors that modulate lifespan were first identified in yeast. Among these, inhibition of nutrient and growth factor responsive Target of Rapamycin (TOR) kinase has emerged as the most effective strategy for slowing aging and improving healthspan in laboratory organisms including yeast, nematodes, fruit flies, and mammals. Here I will describe our ongoing studies of basic mechanisms of aging in yeast as well as our initial attempts toward translational geroscience, which follow directly from these yeast-based studies. In particular, we are implementing clinical strategies to improve outcome in patients with severe mitochondrial diseases and to promote healthy longevity by delaying aging in companion dogs.

Protein folding diseases: Lessons learned from yeast

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The budding yeast *Saccharomyces cerevisiae* has contributed significantly to our current understanding of eukaryotic cell biology. It served as a tool and model to elucidate the molecular basis of a wide variety of cellular phenomena, which appeared to be conserved in other organisms as well. Because of this conservation, the budding yeast became an attractive cellular and biological relevant model to investigate disease-associated proteins, even when the yeast genome does not encode for an apparent homologous counterpart. These so-called humanized yeast models hold great promise for the dissection of disease-related molecular processes and the discovery of novel medicinal compounds. A good example are the yeast models used to clarify the biochemical and cytotoxic properties of proteins linked to neurodegenerative disorders like Parkinson's, Huntington's and Alzheimer's disease, which are commonly classified as protein folding disorders. Studies with these models not only provided fundamental insight on the interplay of protein quality control mechanisms and how failure of these systems result in cytotoxicity and eventually cell death, but also led to the identification of novel players in disease etiology and the validation of prognostic biomarkers that formed the basis for the development of improved diagnostic assays. In addition, these humanized yeast models offered an unsurpassed performance in phenotypic and chemo-genetic screenings aiming to select and study the mode-of-action of lead compounds with promising therapeutic activity.

A new approach to inhibit cancer cell proliferation based on yeast cell cycle studies

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Exposure of cells to increases in extracellular osmolarity results in the activation of the Hog1/p38 family of stress-activated protein kinases (SAPKs) highly conserved among eukaryotic cells from yeast to mammals. Activation of these MAP kinases is required to generate a set of osmoadaptive responses essential for survival under high osmolarity. Adaptation to osmostress requires the modulation of several aspects of cell physiology, such as the control of gene expression and cell cycle progression. In yeast, the Hog1 SAPK is able to delay the G1/S transition of the cell cycle by targeting a CDK inhibitor and through the down-regulation of cyclin expression, thus preventing cells to enter into S phase and maximizing cell survival upon stress. Remarkably, in mammals, the same control of the G1/S transition is implemented by the p38 SAPK. Upon stress, p38 is able to control the p57 CDK inhibitor and also it down-regulates cyclin expression to block G1. The down-

regulation of cyclin expression is achieved by the control of p38 of the RB tumour suppressor, a known transcriptional repressor. N-terminal phosphorylation of RB induces its activity and blocks cell cycle gene expression. This increase on RB activity prevents cell proliferation even in the presence of high CDK activity in cancer cells. Thus, the knowledge of cell cycle control exerted by SAPKs in yeast has served to unravel a new regulatory mechanism for RB in mammals.

Budding yeast as a model system to study the causes and consequences of anaphase bridges

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DNA anaphase bridges comprise a set of cytological phenotypes whereby part of the genetic material still interconnects the nuclear masses while they are being pulled apart towards the centrosomes at the end of the cell division. These phenotypes result from the failure to properly segregate chromosomes due the presence of (i) multicentric chromosomes, (ii) unattached (lagging) chromosomes, (iii) unresolved sister chromatids (in mitosis and meiosis II) or (iv) paired homologous chromosomes (in meiosis I). Anaphase bridges pose a risk for the survival and genetic integrity of the cell progeny and are thought to be of utmost importance in carcinogenesis and tumour genetic instability. Paradoxically, drug-induced anaphase bridges can also be an effective approach to trigger mitotic catastrophe, a major mechanism of cell death in p53 negative tumour cells.

Our group has been working for around eleven years on the genetic causes of anaphase bridges in the budding yeast, as well as the short- and long-term consequences for the cell progeny of such deleterious phenotype. We have specially focused on those anaphase bridges which involved unresolved sister chromatids during the mitotic division. As a postdoctoral in Aragon's lab, we were pioneers in characterizing the master cell cycle phosphatase Cdc14 as a major regulator of sister chromatid resolution through its action on ribosomal DNA array (rDNA) transcription.[1] Recently, we exploited this specificity for the rDNA to address the consequences for the progeny of this anaphase bridge in haploid and diploid cells.[2,3]

Unresolved rDNA upon Cdc14 loss is thought to be due to persistent catenations and, perhaps, unfinished replication. We have also recently studied whether persistent recombination intermediates are also a cause of anaphase bridges, and found that a double knockout for the main nucleases that resolved Holliday junctions, Mus81-Mms4 and Yen1, indeed lead to anaphase bridges.[4]

Finally, we are now revisiting the prototypical example of anaphase bridges formed by catenated sister chromatids, the topoisomerase II (Top2) mutants. We will present unpublished novel data on the cytology of these bridges, the physical nature that keeps the sister chromatids together, and the short-and long-term consequences of Top2 loss for the progeny.

All these works have been supported by Instituto de Salud Carlos III through research projects to F.M. co-financed with the European Commission's ERDF structural funds (06/1211, 09/00106 and 12/00280).

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Why does (sometimes) meiosis fail? Lessons from yeast to prevent gamete aneuploidy

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Meiosis is the specialized cell division that produces haploid gametes from diploid parental cells (gametogenesis). During meiosis, a single phase of DNA replication is followed by two consecutive rounds of chromosome segregation, resulting in the reduction of the ploidy by half. After fertilization, the normal chromosome complement is restored; therefore, the accuracy in the distribution of chromosomes to the gametes is critical for a healthy offspring and species survival. Meiotic errors result in aneuploidy and, in humans, they are the main cause of pathologies associated to reproduction, leading to fertility disorders, spontaneous abortions or genetic birth defects, such as, for example, Down's syndrome. During meioisis, the processes of pairing, synapsis and recombination are crucial to establish physical connections between homologous chromosomes that orchestrate their segregation during the first meiotic division. Given the importance of these events, meiotic cells have developed surveillance mechanisms (checkpoints) that monitor proper distribution of genetic material to the progeny. This meiotic checkpoint network blocks meiotic cell cycle progression in response to defects in recombination and/or chromosome synapsis, thus preventing aberrant chromosome segregation and the formation of aneuploid gametes. Importantly, the incidence of aneuploidy in humans is higher than in other organisms; in fact, about 10% of natural pregnancies harbor monosomic or trisomic embryos; this proportion significantly increases with the maternal age. Although several factors are involved, a weaker checkpoint response to face meiotic errors is one of the causes contributing to this high frequency of human aneuploidy. Therefore, investigating the molecular mechanisms underlying the control of meiotic chromosome dynamics by checkpoints is fundamental to understand the causes of pregnancy losses, infertility and genetic birth diseases.

The budding yeast *Saccharomyces cerevisiae* possesses robust meiotic quality control systems. Given the evolutionary conservation of checkpoint pathways and the proteins involved, as well as the ample array of research tools available, this is an excellent model system to make progress in the understanding of the molecular basis of fertility problems. We will present

our advances in the knowledge of the checkpoint mechanisms employed by yeast meiotic cells to respond to recombination and chromosome synapsis defects.

Synthetic biology platforms for natural product biosynthesis and discovery

Christina D. Smolke

Plants are a rich source of unique scaffolds, including 25% of natural-product-derived drugs. However, the discovery, synthesis, and overall material supply chains for sourcing plant natural products and their derivatives remain ad hoc, biased, and tedious. While microbial biosynthesis presents compelling alternatives to traditional approaches based on extraction from natural plant hosts, many challenges exist in the reconstruction of plant specialized metabolic pathways in microbial hosts. My laboratory has developed approaches to address the challenges that arise in the reconstruction of complex biosynthesis schemes, including spatial engineering strategies to direct the activities and specificities of pathway enzymes and recoding strategies to address folding, processing, and stability issues that may arise with the expression of plant enzymes in heterologous microbial hosts. We have utilized these strategies to develop yeast-based production platforms for an important class of plant alkaloids, the benzylisoquinoline alkaloids, including the medicinal opioids. These synthetic biology platforms will lead to transformative advances in natural product discovery, drug development, and production.