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Andrés Aguilera

Factors and mechanisms controlling genome integrity

Genome instability can take place in the form of mutations, recombination and DNA rearrangements as well as of chromosome loss. It can be initiated by different manners, as a consequence of replication failures, DNA repair dysfunction or genotoxic stress, so that a variety of mechanisms can lead to different instability events. These events may be harmful for the cell and the organism and they are usually associated with pathological disorders. In humans it is often associated with premature aging, various cancer predispositions and hereditary diseases. We are trying to define the role of a number of proteins, including endonucleases, Holliday Junction resolvases, DNA repair proteins, DNA helicases, histone-modifying enzymes and other nuclear structural components on the maintenance of genome integrity. Here we will present our work on the origin of genome instability associated with transcription and nucleotide excision repair (NER). Transcription is among the sources of genetic instability caused by high levels of recombination. Mutations in a number of conserved eukaryotic factors with a role in mRNP biogenesis, such as the THO/TREX or THSC/TREX-2 complex confer a strong transcription-associated hyper-recombination, which seems to depend in part on R-loop formation and replication fork progression impairment. On the other hand, Rad3/XPD is a component of the TFIIH eukaryotic complex, involved in transcription initiation and NER. Due to its role in several central cellular processes, mutations in human XPD cause serious diseases such as Xeroderma Pigmentosum (XP), Cockayne Syndrome (CS) and Trichothiodystrophy (TTD). In Saccharomyces cerevisiae, hypomorphic RAD3 mutants display either UV-sensitivity or transcriptional defects. However, specific rad3 alleles, whose deleterious activities compromise genome integrity, confer weak NER deficiencies and full transcription proficiency, but a strong impact on DNA break-mediated instability. The use of the yeast Saccharomyces cerevisiae to understand transcription and NER-associated instability provide us important clues to understand instability in eukaryotes, as we will try to show using yeast as a model system and extending our studies to C. elegans and human cell lines.

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Joaquín Ariño

Identifying multifunctional proteins in yeast: the case of Hal3

Coenzyme A (CoA) is a ubiquitous and essential cofactor that is used by a wide variety of enzymes in reactions where it mainly acts as a carrier and activator of acyl groups. The essential five-step CoA biosynthetic pathway has been characterized from bacteria to human but, strikingly, it has not been fully resolved in yeast until very recently. Specifically, the nature of the gene(s) encoding the phosphopantothenoylcysteine decarboxylase (PPCDC) activity remained elusive. Sequence homology analyses suggested three candidates—Ykl088w, Hal3 and Vhs3—as putative PPCDC enzymes in Saccharomyces cerevisiae. Remarkably, Hal3 and Vhs3 were in the past characterized as negative regulatory subunits of Ppz1, a protein phosphatase involved in salt tolerance. However, a hal3 vhs3 mutant was synthetically lethal in a way that was independent of its Ppz1-regulatory role. We have shown that YKL088w does not encode a third Ppz1 regulatory subunit nor provides by itself PPCDC activity. We also demonstrated that the essential roles of Ykl088w and the Hal3/Vhs3 pair are complementary, cannot be interchanged and can be attributed to PPCDC-related functions. While known eukaryotic PPCDCs are homotrimers, we present a model in which the active yeast enzyme is a heterotrimer that consists of Ykl088w and Hal3/Vhs3 monomers that separately provides two essential catalytic residues at the interface of the monomers. Therefore, our results unveil Hal3 and Vhs3 as moonlighting proteins involved in both CoA biosynthesis and protein phosphatase regulation. Current work in our laboratory, in collaboration with Erick Strauss and coworkers (Stellenbosch University), aims to elucidate the structural basis for such disparate cellular functions.

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Uli Brandt

Yarrowia lipolytica, a powerful yeast genetic system to study structure and function of mitochondrial complex I

Mitochondria from fermenting yeasts like Saccharomyces cerevisiae do not contain proton pumping NADH:ubiquinone oxidoreductase or complex I. This is one of the major reasons why the mechanism of this largest component of the respiratory chain that has been implicated in numerous mitochondrial disorders and neurodegenerative processes is still elusive. Therefore, we developed the strictly aerobic yeast Yarrowia lipolytica as a genetic model to study the structure and function of complex I [1]. Its respiratory chain, as that in most mitochondria from plants and fungi, in addition to complex I comprises a so-called non-proton pumping NADH:ubiquinone oxidoreductase (NDH2). Since the only NDH2 present in Y. lipoytica faces the intermembrane space, functional complex I is essential for survival in wild-type strains. To complement complex I deficiency we added a mitochondrial targeting signal to autologous NDH2 resulting in a strain expressing a functional internal version of this enzyme. Addition of a hexa-histidine-tag to the C-terminus of the nuclear coded 30-kDa subunit of complex I allowed efficient affinity purification of complex I.

As constituents of membrane-integral complex I from Y. lipolytica 41 different subunits with a total molecular mass of about 950 kDa were identified up to now [2]. The seven most hydrophobic subunits are encoded by the mitochondrial genome. Deletion strains that we constructed for many of the nuclear complex I genes provided important insights into the subunit architecture of complex I. Site directed mutations in the vicinity of the prosthetic groups allowed functional analysis and a detailed mapping of the ubiquinone and inhibitor binding sites of complex I [3].

Complex I purified from Y. lipolytica has proven to be very stable and well suited for structural analysis. 3D models of holo-complex I and several subcomplexes were obtained at 20-25 Å resolution by electron microscopic single particle analysis [4]. Recently, we could complete the first X-ray structural analysis of a mitochondrial complex I with crystals from holo-complex I of Y. lipolytica and obtained an electron density map at 6.3 Å resolution [5].

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Carmen Lisset. Flores

Relationships between the trehalose biosynthetic pathway and glycolysis in different yeast species

Trehalose, a non-reducing disaccharide formed by two glucose units, has important and varied functions in different organisms. In yeasts trehalose is synthesized by a two-step pathway: trehalose-6-phosphate (T6P) is formed from glucose-6P and UDP-glucose by the enzyme T6P synthase (Tps1) encoded by the TPS1 gene and then dephosphorylated by a T6P phosphatase (Tps2) encoded by the gene TPS2. These proteins exist in a complex with two other proteins Tps3 and Tsl1 without catalytic activity. In Saccharomyces cerevisiae or Kluyveromyces lactis mutations in the gene TPS1 cause inability to grow in glucose. Previous work of the laboratory traced this effect to the loss of the inhibitory effect of T6P on hexokinase and a mathematical modelization of glycolysis has confirmed the importance of this control in S. cerevisiae. Some yeast species like S. pombe have hexokinases that are not inhibited by T6P and disruption of TPS1 does not inhibit growth in glucose. The inhibition of hexokinase by T6P is widespread among yeasts but its strength is variable. The most inhibited hexokinase reported is that of the yeast Yarrowia lipolytica with a Ki of 3.5 M, therefore we isolated its TPS1 gene and analyzed the effects of its disruption on the

physiology of this yeast. We used the phenotypic complementation of the glucose negative growth of a S.cerevisiae tps1 mutant to isolate the TPS1 gene from a cDNA library of Y. lipolytica. We found only a single isolate complementing the phenotype with sequence homology to Tps1. Publication of the genomic sequence of Y. lipolytica showed another gene with sequence homology but after its isolation we could show that it did not complement the glucose negative phenotype mentiond. Likely this gene corresponds to the TPS3 gene of the trehalose biosynthetic complex. During the attempts to disrupt YlTPS1 we found that in Y. lipolytica the relative position of TPS1 and of the essential TFC1 is particular since they are contiguous and transcriptionally divergent in such a way that part of the TFC1 promoter could overlap with the coding sequence of YlTPS1. In other yeast species TPS1 appears separated of TFC1 by PTC4 and their transcription direction is the same. Disruption of YlTPS1 did not preclude growth in glucose of Y. lipolytica. We hypothesize that the lack of effect of the YlTPS1 disruption on glucose growth is due to the presence of a glucokinase, not inhibited by T6P, as the main glucose phosphorylating activity. This hypothesis could also explain the behaviour of Hansenula anomala in which a similar phenotype has been described. We found that diploids homozygous for the disruption, Yltps1/Yltps1, exhibited a decreased sporulation frequency with relation to a wild type diploid.

Our current vision of trehalose metabolism and its relationship with glycolyis in yeast has been mostly based on results obtained with S. cerevisiae. However, our results show that in other yeast species the trehalose biosynthetic pathway has not, or only minor, influence in the regulation of glycolysis due to different strategies evolved to regulate this pathway.

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Juana María Gancedo

Signalling through cAMP-dependent protein kinases in Saccharomyces cerevisiae

cAMP plays an important regulatory role, in organisms ranging from bacteria to humans, through its control of cAMP-dependent proteins, mainly the cAMP-dependent protein kinase (PKA). However, while in bacteria, as well as in animals, an increase in cAMP levels is a signal for starvation and other stresses, in yeasts cAMP levels are elevated in conditions leading to active growth. To investigate to what extent the cAMP signalling pathway is required for diverse processes taking place in *S. cerevisiae*, we have used mutant strains devoid of PKA. For such strains to be viable they have to carry suppressor mutations such as *msn2 msn4* or *yak1*. The processes we have studied are: changes in global transcription in the transition gluconeogenesis/glycolysis, control of transcription of the glucose regulated gene *SUC2* encoding invertase, and the inactivation/degradation of the gluconeogenic enzyme fructose-1,6-bisphosphatase. We found that PKA activity is not required for the induction by glucose of genes related with glucose metabolism nor for glucose repression of genes involved in the utilization of carbon sources alternative to glucose. We noted, however, that different genes implicated in the biosynthesis of aminoacids respond differently to the absence of PKA. We established that repression of *SUC2* by a high glucose concentration is independent of PKA activity. Induction of *SUC2* by a low concentration of glucose is decreased in strains lacking PKA, while in the absence of glucose the expression of *SUC2* in such strains increases up to 10-fold. The inactivation and degradation of fructose-1,6-bisphosphatase, triggered by glucose, is strongly impaired by the absence of PKA.

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Rafael Giraldo

Beyond natural yeast prions: Engineering a synthetic prionoid from first principles

Protein amyloids arise from the conformational conversion and assembly of a soluble protein into fibrilar aggregates with a crossed □-sheet backbone. Amyloid aggregates are able to replicate by templating the structural transformation in further identical protein molecules. In physicochemical terms, amyloids constitute the simplest self-replicative macromolecular assemblies (1). Since their pioneering discovery by the Wickner and Lindquist laboratories, yeast prions (Sup35p/[PSI^{+}], Ure2p/[URE3^{+}]) have been instrumental in addressing the molecular basis for amyloid conformational templating, structural polymorphism and cell-to-cell transmissibility (2,3). However, the amyloidogenic sequence stretches in yeast prions are consistently Gln/Asn-rich, unlike most proteins involved in amyloid proteinopathies (which bear hydrophobic stretches). In addition, yeast prions are the epigenetic determinants of distinct advantageous phenotypes that provide quick adaptative responses to environmental challenges (4) but, by no means, they are the causative agents of any known proteinopathy. As mammalian PrP does (5), the WH1 domain of the bacterial, plasmid-encoded protein RepA (6) can assemble into amyloid fibers upon binding to short, defined DNA sequences in vitro (7,8). Exploiting our current understanding of ligand (DNA)-induced RepA-WH1 amyloidosis, we have found that, when fused to a red fluorescent protein, causes a synthetic amyloid proteinopathy in Escherichia coli (9), leading to cell aging. The RepA-WH1 prionoid is a minimalist bacterial model system for transmissible amyloid proteinopathies. As a proof of concept for the universal basis for protein amyloidogenesis, we have engineered repeats of the RepA-WH1 amyloid stretch in substitution of the Q/N-rich amyloid repeats in Sup35p to build chimeral [PSI+] prions which are functional as epigenetic determinants in Saccharomyces cerevisiae.

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D. Grahame Hardie

The Yeast Snfi Complex As A Model For The Mammalian Ampk System

The yeast SNF1 complex and mammalian AMPK-activated protein kinase (AMPK) were discovered by independent genetic and biochemical approaches. The SNF1 (also known as CAT1 or CCR1) and SNF4 (CAT3) genes had been identified as mutants that would grow on glucose but not on other carbon sources such as sucrose, glycerol or ethanol. Snf1 and Snf4 form a complex, and this interaction was used to establish the two-hybrid assay[1]. The alternate third subunits of the yeast SNF1 complex (Sip1, Sip2 or Gal83) were subsequently identified by two hybrid screens using Snf1 as bait. AMPK was identified in 1973 as fractions causing time- and ATP-dependent inactivation of enzymes catalyzing key steps in fatty acid and cholesterol biosynthesis, and was named AMPK by the author[2] when we realized that these were functions of the same protein kinase that was activated by AMP. It was purified and shown to be a heterotrimeric complex with □, □ and □ subunits, and when the genes were sequenced it was realized that they were orthologues of Snf1, Snf4 and the Sip1 family. The SNF1 complex is activated during glucose starvation by a mechanism similar to that of mammalian AMPK, involving phosphorylation at a conserved threonine (Thr-210/Thr-172 in yeast Snf1/mammalian □) within the kinase domain by upstream kinases[3].

Since the merging of these two fields, studies of AMPK have led the way in some areas, and of SNF1 in others. One area where studies of yeast made a vital contribution was the identification of upstream kinases. It proved difficult to purify and identify these from mammals, but the kinases that phosphorylate Thr-210, i.e. Pak1 (now Sak1), Tos3 and Elm1, were identified by global protein interaction screens using Snf1 as bait, and by a biochemical screen of yeast kinases that phosphorylated and activated AMPK. This led to identification of LKB1 and CaMKK□ as the kinases phosphorylating Thr-172 on AMPK. The emergence of LKB1 was particularly exciting because it had been previously identified as a tumor suppressor gene that is mutated in a human cancer susceptibility disorder, Peutz-Jeghers syndrome. Its identification as an upstream kinase for AMPK not only solved a long-standing problem in the AMPK field, but also identified the first downstream target for LKB1, and provided an explanation for its function as a tumour suppressor. This has led to clinical trials testing the efficacy of AMPK-activating drugs for treatment of cancer.

Another area where the yeast system led the way was in explaining how the SNF1/AMPK complexes regulated gene expression. The SNF1 complex was shown to directly phosphorylate the transcription factor Mig1, a repressor protein involved in the phenomenon of glucose repression, at multiple sites within the regulatory domain. This phosphorylation promotes binding to the nuclear export protein Msn5 (originally identified as a multicopy suppressor of snf1 mutants), triggering relocation of Mig1

from the nucleus to the cytoplasm. It also abolishes the ability of Mig1 to interact with co-repressors. This provides a model for regulation of the many genes known to be regulated by the AMPK system in mammals.

With respect to other aspects of regulation, studies of the mammalian AMPK system have led the way. AMPK is activated by elevation of the ADP:ATP and AMP:ATP ratios during metabolic stress. The mammalian □ subunits contain two sites that reversibly bind AMP, ADP or ATP. Binding of ADP or AMP (but not ATP) to one site promotes phosphorylation of Thr-172 by inhibiting dephosphorylation, while binding of AMP (but not ADP or ATP) causes further allosteric activation. Although the nucleotide binding sites appear to be partially conserved in the sequence of Snf4, and there are large changes in ADP:ATP and AMP:ATP ratios when yeast cells are starved of glucose[3], effects of AMP on the regulation of the SNF1 complex have not yet been reported. Recent work addressing this problem will be discussed.

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Enrique Herrero

Redox regulation and oxidative stress in Saccharomyces cerevisiae

Glutaredoxins are thiol oxidoreductases acting as one of the main protein redox regulators, employing glutathione as reductant of sulfydryl groups. Although they share a common thioredoxin fold and a number of sequence motifs, there exists a diversity of glutaredoxin classes reflecting different functions in the cell. Studies in Saccharomyces cerevisiae are providing information on such diversity of functions at several cellular organelles. Classical dithiol glutaredoxins (CPYC active site motif) are considered as general regulators of protein redox state upon oxidative stress, although they may have specific targets such as ribonucletide reductase. In yeast, Grx1 and Grx2 are cytosolic, although a minor fraction of Grx2 locates at mitochondria. Our group has shown that Grx1 and Grx2 have redundant functions in protection against necrotic death caused by selenite, and the specific targets of both glutaredoxins are being analyzed using proteomic approaches. We have also shown that selenite elicits a complex response in yeast cells which independently involves the Snf1 protein kinase pathway and the Aft1/Aft2 transcription factors. Mutants in the corresponding SNF1, AFT1 and AFT2 genes are hypersensitive to selenite. Aft1 and Aft2 regulate the expression of genes implicated in iron uptake and compartmentalization in S. cerevisiae. Iron homeostasis is also a functional target of Grx3/4/5, which constitute the monothiol glutaredoxin class (CGFS active site motif) in yeast. Grx5 is involved in the synthesis of Fe/S clusters at the mitochondrial matrix, and its absence results in a diversity of phenotypes including genetic instability. Grx3 and 4 modulate the activity of Aft1 and of diverse iron-requiring proteins, and they themselves are Fe/S proteins. Overall, monothiol glutaredoxins seem to have functions related with their ability to coordinate Fe/S clusters, independently of the thiol oxidoreductase activity. A third class of glutaredoxins in S. cerevisiae is formed by Grx6 and Grx7, structurally similar to Grx1 and 2 although containing a single cysteine residue at the active site. Grx6 and Grx7 are associated at the endoplasmic reticulum and Golgi membranes, and we have provided evidence on functional interactions with the PDI/Ero1 machinery for correct protein folding at the early steps of the secretory pathway.

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Matt Kaeberlein

Using yeast to define conserved mechanisms of aging.

The budding yeast Saccharomyces cerevisiae is one of the premier model organisms used in aging-related research. Two aging paradigms have been established in yeast: replicative and chronological. Replicative aging is a model for aging of mitotically active cells in which the lifespan of a mother cell is defined by the number of daughter cells produced prior to senescence. Replicative lifespan (RLS) is measured by physical removal of daughter cells, which are easily distinguished from mother cells under a light microscope. Chronological aging is a model for aging of post-mitotic cells in which lifespan is defined by how long a yeast cell can survive in a non-dividing, quiescent-like state. Chronological lifespan (CLS) has been most commonly measured by culturing cells in liquid media where they enter a non-dividing state once the carbon source has been exhausted. Here I will describe results from large-scale studies of both replicative and chronological aging using the yeast ORF deletion collection. These studies have provided important insights into the molecular processes that influence aging in yeast and have identified several dozen longevity factors that play a conserved role in the nematode Caenorhabditis elegans. The mechanisms of life span extension from dietary restriction, including reduced TOR signaling, and the genetic variants that influence the response to dietary restriction will be discussed in detail.

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Klaus Natter

Skinny or fat: switches in storage lipid metabolism of yeast

Most eukaryotic organisms use neutral lipids as reserves for the generation of energy and as a pool of building blocks for the synthesis of membrane lipids. These strongly hydrophobic lipids are stored in intracellular cytosolic lipid droplets (LD) or transported between different organs as lipoproteins. Although the principal biochemical pathways for synthesis of complex lipids are well known, we are just beginning to understand the regulatory mechanisms that control these reactions.

S. cerevisiae belongs to the group of non-oleaginous yeasts. Under optimum conditions triacylglycerol and steryl esters are produced only in very low amounts. We use this model system to investigate the mechanisms that regulate lipid homeostasis and the impact of defects in these regulatory mechanisms on lipid storage capacity, cellular growth, and during exposure to lipotoxic conditions.

The lipid droplet plays a central role in the metabolism of neutral lipids. In S. cerevisiae, the LD-associated subset of the proteome is well defined. However, it is still unclear how proteins are targeted to this organelle. Experimental evidence indicates that at least two independent targeting mechanisms are responsible for the sorting of proteins to the LD. Interestingly, these two processes result in a spatial separation of lipid anabolic and catabolic enzymes during or immediately after translation and in different localization patterns on the lipid droplet. Alterations in these localization patterns suggest a participation of the LD in the control of lipid synthesis, turnover and degradation. Our results show that lipase-catalyzed mobilization of storage lipids from the lipid droplet is a tightly controlled process, including changes in localization patterns of lipases as well as kinase and phosphatase activities. These mechanisms regulate lipid homeostasis in a cell cycle dependent manner but also according to changes of environmental conditions.

Surprisingly, lipid storage is dispensable for yeast cells under normal growth conditions. However, robustness and the ability to respond to environmental changes is reduced in mutants lacking LD and leads to a rapid loss of viability under certain conditions. Hence, complete disruption of storage lipid synthesis is deleterious for a cell. On the other hand, storage of large amounts of neutral lipids doesn't seem to have a negative impact. Oleaginous yeasts are able to store triacylglycerol in enormous amounts, often exceeding 50% of lipid in the biomass. For S. cerevisiae it has not yet been shown to which maximum level the flux to neutral lipids can be increased. We applied systems biology and omics-based approaches to identify the pathways and regulatory switches that are limiting lipid accumulation. Flux Balance Analysis was used as a tool to identify changes in metabolic fluxes with a predicted increase in lipid accumulation. Experimental results confirmed that genetic engineering strategies can be used to increase the storage lipid content of baker's

yeast significantly. These studies indicate that, in addition to genetic interventions in lipid metabolism itself, perturbations of central carbon metabolism can have a strong impact on lipid storage. In the future, S. cerevisiae strains with high lipid synthesis rates might be able to serve as platforms for biotechnological processes aiming at the production of lipids or other hydrophobic substances.

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Sara E. Mole

Using Schizosaccaromyces pombe to study lysosomal diseases

My lab uses the fission yeast Schizosaccharomces pombe as a genetically tractable unicellular model organism to study genetic diseases that affect the lysosome, because this yeast has numerous small and dynamic vacuoles, the equivalent organelle to the mammalian lysosome, that are easily visualised. So far we have focused on the neuronal ceroid lipofusinoses (NCL) and Chediak-Higashi syndrome. Deletion of btn1, the S. pombe homologue of human CLN3, or of lvs1, the S. pombe homologue of human LYST, both cause enlargement of yeast vacuoles.

Early studies using light and electron microscopy indicate that deletion of lvs1 directly affects vacuole homeostasis. In contrast, deletion of btn1 also causes pleiotropic phenotypes that are rescued by ectopic expression of Btn1p or H.s. CLN3, indicating that these proteins are functional orthologues. H.s. CLN3 is a multispanning membrane protein that was thought to act at the lysosome. We identified functionally important regions by mimicking disease-causing mutations in Btn1p. Mutations that affect the luminal loops and amphipathic helix have most effect on a set of independent marker phenotypes. Cells deleted for btn1 area affected in many aspects

of cell biology including vacuole homeostasis, cytokinesis, the cell wall, polarized growth, endocytosis, sterol-rich domains and trafficking of vacuolar enzymes. GFP-Btn1p traffics slowly to the vacuole, and its steady-state location is predominantly at the Golgi, not the vacuole. Changes in the expression level of Btn1p affect the number, intracellular location and morphology of Golgi compartments (viewed by high pressure freezing EM). We propose that the primary function of Btn1p, and therefore CLN3, is at the Golgi, and that its loss affects multiple post-Golgi trafficking pathways including the vacuole. This yeast work led us to investigate the common mutation found in juvenile NCL world-wide. We conclude that this is a mutation-specific phenotype in which CLN3 function is not completely lost, and that mutations in CLN3 may cause widely varying disease.

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Maria Molina

Yeast as a tool for PI3K-Akt signalling research and drug discovery

All biological systems use signal transduction pathways to perceive the surrounding environment and undergo a proper response to its changes through the regulation of key cellular processes, such as proliferation, differentiation, motility or apoptosis. Such response, often essential for cell survival, relies on the regulation of gene expression and the post-translational modification of effector proteins. Given the remarkable conservation of signalling mechanisms and cellular processes along the evolutionary scale, the yeast Saccharomyces cerevisiae, a readily manageable unicellular eukaryotic organism, represents an excellent tool for the functional study of proteins of more complex organisms that, upon heterologous expression, are able to couple or interfere with yeast signaling or growth. This sort of approach, often referred as

'humanizing yeast', also allows the development of yeast-based bioassays adaptable to high throughput screening (HTS) technologies, for identifying specific inhibitors of human proteins involved in disease. Growth inhibition caused by overexpressing the human target under controlled conditions provides a valuable platform to screen chemical collections in search for non-toxic selective compounds by their ability to restore yeast growth. Easy mutagenesis analyses can be performed in yeast either to perform structure/function analysis or to validate the specificity of target inhibition and identify drug-resistant mutations.

In this line, our research has resulted in the successful reconstitution in yeast of the mammalian pathway involving class I PI3K (phosphoinositide 3-kinase), PTEN (phosphatidylinositol 3-phosphatase) and protein kinase Akt/PKB, which has roles in multiple processes, like cell proliferation and apoptosis. Its constitutive activation has been implicated in the progression of a wide variety of human tumours and it is considered, therefore, a good target for anticancer drug discovery. Overexpression of a constitutively active membrane-bound allele of the catalytic PI3K subunit p110α in yeast leads to activation of the MAPK-mediated cell integrity pathway and inhibition of cell growth, which is relieved by simultaneous overexpression of PTEN. By combining directed and random mutagenesis with phenotypical analysis we have identified a number of both loss- and gain-of-function mutations in both proteins, proving that yeast serves as a sensitive biological sensor of PI3K oncogenicity and PTEN tumor suppressor activity. Yeast growth inhibition was also achieved by co-expression of wild type p110α and Akt, which leads to increased phosphorylation and re-location of Akt to membranes, and induces the formation of characteristic plasma membrane invaginations. Bioassays based on this yeast growth interference phenotype caused by the reconstituted PI3K-Akt pathway have been set up and validated using commercial specific inhibitors of these kinases.

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Fernando Moreno

Yeast hexokinase 2: At the hub of cell energy metabolism and glucose repression signalling

Hexokinase 2 (Hxk2) from Saccharomyces cerevisiae was one of the first metabolic enzymes described as a multifunctional protein. Hxk2 has a double subcellular localization; it functions as a glycolytic enzyme in the cytoplasm and as a regulator of gene transcription of several Mig1-regulated genes in the nucleus. To get more insights into structure-function relationships of the Hxk2 protein, we followed two different approaches. In the first we deleted the last eight amino acids of Hxk2 and substituted serine 304 with phenylalanine to generate Hxk2wca.

Analysis of this mutant demonstrated that these domains play an essential role in the catalytic activity of yeast Hxk2 but have no effect on the regulatory function of this protein. In the second we analysed whether amino acids from lysine 6 to methionine 15 of Hxk2 (Hxk2wrf) are essential to the regulatory role of Hxk2 and if there is an effect on the hexose kinase activity of this protein. We found that the Hxk2wca mutant protein interacts with the Mig1 transcriptional repressor and the Snf1 protein kinase in the nucleus at the level of the SUC2-Mig1 repressor complex. We will present evidence showing that Hxk2wca maintains full regulatory function because the glucose repression signalling of the wild-type machinery is conserved.

We also found that the Hxk2wrf mutant allele is incapable of glucose repression signalling because it does not interact with Mig1 at the level of the SUC2-Mig1 repressor complex. The two mutants, Hxk2wca and Hxk2wrf, retain single functions as a transcriptional factor or as an enzyme with hexose phosphorylating activity, but have lost the original bifunctionality of Hxk2. We will present a model of glucose repression signalling in which in high-glucose conditions, a fraction of Hxk2 protein enters the nucleus to stabilize the SUC2-Mig1 repressor complex. Conversely, in low-glucose conditions Hxk2 nuclear exit dissociates the repression complex and allows SUC2 expression. An important question is how the nucleocytoplasmic traffic of Hxk2 takes place during the glucose repression signalling cycle. We have found that Hxk2 is an export substrate of the carrier protein Xpo1(Crm1) and that Hxk2 nuclear export and the binding of Hxk2 and Xpo1 involve two leucine-rich nuclear export sequences (NES).

We will also show that the Hxk2 protein is an import substrate of the Kap95/Kap60 transport system. Finally, we describe that the Hxk2 phosphorylation at serine-14 promotes Hxk2 export and inhibits Hxk2 import by facilitating the association of Hxk2 with Xpo1 and the dissociation of Hxk2 from Kap95/60. Thus, our study identifies Hxk2 phosphorylation at serine-14 as a regulatory mechanism that controls the nucleocytoplasmic traffic of Hxk2.

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Jean Marc Nicaud

Yarrowia lipolytica as a model for bio-oil and bioplastic production.

Hgh energy prices and depletion of crude oil supplies unravel the possibility to find alternative sources such as microbial oils and microbial plastics. Among the oleaginous yeasts, Yarrowia lipolytica is the only one for which genomic and efficient molecular genetic tools are currently available. We are using this yeast as a model of lipid metabolism (1-3). Y. lipolytica can efficiently utilize hydrophobic substrates

[alkanes; fatty acids (FA) and triglycerides (TAG)] and osidic substrates. Depending on environmental conditions, intracellular fatty acids can be either degraded by □-oxidation in the peroxisomes or stored as TAG into lipid bodies.

For Bio-lipid production, the glycerol-3-phosphate shuttle is a key step for lipid synthesis. GUT2 inactivation (gene encoding the glycerol-3-phosphate dehydrogenase catalyzing the conversion of G3P to DHAP) improved lipid accumulation (4). GPD1 overexpression (gene encoding the glycerol-3-phosphate dehydrogenase catalyzing the reverse reaction) led to in an increase of G3P level. However, higher total lipid accumulation under GPD overexpression was observed only in strains presenting also an altered □-oxidation (by inactivating POX1-6 or MFE1 genes). A 25 up to 70% increase of total lipid accumulation was observed depending on the genetic background. An innovative fermentation process, preventing citric acid production, allowed obtaining with the wild-type a biomass concentration up to 132 cdwg. L^{-1} with 38 % (w/w) of lipids content in 70 hours with a conversion yield of 0.38 $Cmol_{Lip}.Cmol_{Glc}^{-1}$ near best performances described previously.

For Bio-plastic production, we are using the production of polyhydroxyalkanoates (PHA) as a model. Recombinant strains of Y. lipolytica expressing the PHA synthase (PhaC) from Pseudomonas aeruginosa in the peroxisome were found able to produce PHA. The PHA production yield but not the monomer composition was shown to be dependent to the POX genotype (POX genes coding for acyl-CoA oxidases) (5). In contrast, redirecting of the fatty acid flux towards □-oxidation by deletion of the neutral lipid synthesis competitive pathway (mutant strain Q4 devoid of the acyltransferases encoded by the LRO1, DGA1, DGA2 and ARE1 genes), in combination with variants of the multifunctional enzyme MFE1, lead to both, a significant increase in PHA level (reaching 7.3% of the cell dry weight) and to a modification of the PHA monomers composition. With functional MFE enzyme it was possible to produce hetero-polymers. While, with MFE enzyme devoid of 3-hydroxyacyl-CoA dehydrogenase activity, the homo-polymers were composed mainly of the 3-hydroxyacid monomer (> 80%) of the external fatty acid used for the feeding. Finally, presence of shorter monomers (up to 20% of the monomers) in a mutant strain lacking peroxisomal 3-hydroxyacyl-CoA dehydrogenase domain evidenced the presence of a partial mitochondrial □-oxidation in Y. lipolytica.

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José Manuel Siverio

The yeast Hansenula polymorpha as a model to study how eukaryotic nitrate assimilation is regulated

The genes involved in nitrate assimilation are induced by nitrate and subjected to nitrogen catabolite repression (NCR). Nitrate acts as an inducer once it enters the cell, this entails that nitrate transporters -influx and efflux- play a key role in regulating

these genes. Ynt1 transports nitrate and nitrite with high affinity, deletion of YNT1 led to cells incapable of growing in nitrate. Phosphorylation of Ynt1 by the Ser/Thr protein kinase Npr1 is essential to deliver it to the plasma membrane and constitutive dephosphorylation down-regulates nitrate assimilation gene expression. More recently, we have uncovered two transporters involved in nitrate and nitrite extrusion. The sulfite efflux system Ssu2 participates in nitrate efflux, while Nar1, belonging to the formate–nitrite transporter family (FNT), is also involved in nitrate and nitrite efflux. Our results suggest that these systems are involved in modulating nitrate assimilation gene expression to maintain nitrite homeostasis, since nitrite is very toxic for the cell. Concerning NCR, I will deal with the role of calcineurin and its links with URE2 and the GATA transcriptional factors GAT1 and GAT2. Deletion of URE2 produced a reduction in the levels of nitrate assimilation gene expression and FK506 Na^+ and Li^+ sensibility. A strong parallelism between Δure2 and Δcnb1 (lacking the regulatory subunit of calcineurin) was observed, suggesting a link between Ure2 and the calcineurin pathway. Substantial evidence indicates that calcineurin regulates Gat1 levels and as a result nitrate assimilation gene expression.

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Denis J. Thiele

Using yeast to understand regulatory responses to Iron deficiency: the most common dietary deficiency on earth

Iron (Fe) serves as an essential metal ion cofactor for most life forms on this planet. Due to its ability to exist in an oxidized ($Fe3^{+}$) or reduced ($Fe2^{+}$) state, Fe drives catalytic reactions for enzymes involved in mitochondrial oxidative phosphorylation, oxygen transport, intermediary metabolism, chromatin remodeling and a host of other critical biochemical reactions. Fe deficiency leads to a number of severe health consequences in humans including anemia and cognitive and developmental disorders. Indeed, Fe deficiency is the leading nutritional disorder on earth, thought to impact nearly 2 billion people, with disproportionate effects on pregnant women and children. Although Fe deficiency is so profound, we know very little about how cells dynamically alter their metabolism in response to changes in Fe availability. The baker's yeast S. cerevisiae is an excellent organism for delineating the components involved in Fe acquisition, utilization and regulation. In response to Fe deficiency the Aft1/2 Fe-sensing transcription factors activate an Fe regulon comprising over 90 genes. Two Fe regulon genes, CTH1 and CTH2, encode RNA binding proteins that bind to AU-rich Elements (AREs) located in the 3'-UTR of mRNAs to induce the degradation of over 90 specific mRNAs. While the Cth1 and Cth2 proteins degrade mRNAs encoding Fe utilizing and storage proteins, thereby facilitating the cellular prioritization of limited Fe, they have only partially overlapping target mRNAs. Moreover, Cth1 and Cth2 appear to occupy distinct subcellular geography and are subject to additional regulatory controls that, together, reglulate how yeast cells achieve rheostatic control during periods of Fe limitation and Fe supplementation. Understanding how Cth1 and Cth2 function, and the nature of their regulation and target genes, will provide a cellular wide view of the adaptive responses to a changing Fe landscape.

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Ida van der Klei

A synthetic yeast producing penicillin

The filamentous fungus P. chrysogenum is the industrial producer of the important β-lactam antibiotic penicillin. The initial steps of the penicillin biosynthetic pathway are localized in the cytosol, namely the non-ribosomal peptide synthetase δ-(L-aminoadipyl)-L-cysteinyl-D-valine synthetase (ACVS), which produces the tripeptide ACV, and isopenicillin N synthetase (IPNS), which catalyses the formation of isopenicillin N (IPN). The last steps of penicillin biosynthesis occur in specialized organelles, the microbodies (peroxisomes). These organelles contain the enzymes isopenicillin N:acyl CoA acyltransferase (IAT) and phenylacetyl-CoA ligase (PCL), which catalyze the conversion of IPN into penicillin G.

The peroxisomal localization of IAT and PCL in peroxisomes is essential for efficient penicillin production, because mutants defective in peroxisome formation show reduced penicillin production levels. Moreover, a strong correlation seem to exist between penicillin production and the volume fraction of peroxisomes per cell [1]. Additionally, artificial overproduction of a single peroxisomal membrane protein, Pex11p, resulted in increased penicillin production levels together with massive proliferation of peroxisomes. In this strain the level of the penicillin biosynthetic enzymes was not altered [2]. We introduced the penicillin biosynthesis pathway into the yeast Hansenula polymorpha. In this organism penicillin was produced and efficiently secreted in the medium. Also in this heterologous host peroxisome deficiency resulted in strongly decreased penicillin production levels [3].

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