Instituto Juan March
de Estudios e Investigaciones

133 CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Stress in Yeast Cell Biology... and Beyond

Organized by

J. Ariño

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H. J. Bohnert
M. Carlson
M. S. Cyert
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Professor Dr. Helmut Ruis

This meeting was dedicated to Helmut Ruis, who organized the scientific program with Joaquin Ariño, but tragically died of a never fully determined illness on September 1st, 2001.

Trained as an organic chemist, Helmut Ruis made many invaluable contributions towards the advancement of Austrian Biosciences. During the 1980s he was one of the driving forces in the founding stages of the Vienna Biocenter. In his research he made significant contributions to the understanding of yeast stress response pathways.

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Introduction
J. Ariño

Living cells must adapt to changes in the environment to survive. In many cases, these changes compromise cell growth or even cell survival, and adaptation requires a fast and complex functional response. By analyzing at the molecular level the characteristics of this response, we can learn about many aspects of the cell biology. Stress responses are particularly relevant in microorganisms, because conditions in their environment are far from constant and they have to face sudden changes in temperature, nutrient availability, osmolarity or exposure to toxic ions. Because of their accessibility to very powerful genetic tools, yeasts (particularly *S. cerevisiae*) represent a very useful model to study stress response mechanisms that are often conserved in more complex organisms.

The aim of this workshop has been to analyze, from different points of view, the most recent findings on the response of yeasts to stress, and to identify relevant aspects that could serve as starting points for research in plants and animals. Many different types of stress have been examined, such as osmotic and saline, oxidative, temperature, pH, nutritional... It has been pointed out how yeast cells integrate different stress signals to provide a general response. The initial step requires sensing of the stress condition. The sensing machinery has been elucidated in some cases, such as osmotic stress, but the problem still remains unsolved in many others. Transduction of the stress signal often involves elements that are fully conserved among eukaryotes: heat shock factors, cAMP-dependent protein kinase (PKA) or MAP kinases modules. A general feeling among the participants was that more examples of conserved mechanisms should be expected to emerge within the next few years. Finally, the response generates in many cases changes in gene transcription, that have been thoroughly examined by DNA microchip analysis in the last few years, although other mechanisms such as changes in RNA stability or even translational control cannot be excluded.

Yeasts are not only important as a research model, but also key elements in food production and biotechnology (clearly the case of *S. cerevisiae*). These aspects were not omitted in the workshop, as they were addressed by different talks and poster presentations, pointing out how better understanding of yeast stress response can not only help us to understand how living cells function, but also lead to improvements in very old (and often pleasant) processes, such as wine making.

Joaquín Ariño

Session 1: Nutritional and oxidative stresses
Chair: Gustav Ammerer

TOR signaling: control of cell growth in response to nutrients

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The *Saccharomyces cerevisiae* targets of rapamycin TOR1 and TOR2 are phosphatidylinositol (PI) kinase-related protein kinases that activate cell growth in response to nutrient availability (1). A TOR deficiency or rapamycin treatment causes a nutrient stress response, including inhibition of translation initiation, arrest in the G_1 phase of the cell cycle, glycogen accumulation, autophagy, down-regulation of glycolysis, rRNA, tRNA, and r-protein genes, and up-regulation of tricarboxylic acid (TCA) cycle genes (1-9). The TORs control cell growth via at least two signaling pathways.

Inhibition of phosphatase activity appears to be a major mechanism of TOR signaling, in both yeast and mammalian cells. In yeast, TOR negatively regulates the type 2A-related phosphatase SIT4 by promoting the association of this phosphatase with the inhibitor protein TAP42 (10). Upon TOR-inactivating conditions, nitrogen starvation or rapamycin treatment, TAP42 dissociates from SIT4, permitting the phosphatase to act on target proteins such as the GATA-binding transcription factor GLN3 or the Ser/Thr kinase NPR1. Dephosphorylated GLN3 dissociates from the cytoplasmic anchor protein URE2 and moves into the nucleus to activate target genes. Dephosphorylated and activated NPR1 controls the ubiquitination and stability of nutrient permeases.

In collaboration with the group of Kim Arndt, we have recently found that TOR controls the association of SIT4 and TAP42 via the conserved, TAP42-interacting protein TIP41 (11). Deletion of the *TIP41* gene confers partial resistance to rapamycin, suppresses the growth defect of a *tap42* mutant, and prevents dissociation of SIT4 from TAP42. Furthermore, a *TIP41* deletion prevents SIT4-dependent events such as dephosphorylation of NPR1 and nuclear translocation of GLN3. Thus, TIP41 negatively regulates the TOR pathway by binding and inhibiting the TOR-effector TAP42. The binding of TIP41 to TAP42 is stimulated upon rapamycin treatment or nitrogen limitation, via SIT4-dependent dephosphorylation of TIP41, suggesting that TIP41 is part of a feedback loop that rapidly amplifies SIT4 phosphatase activity under TOR-inactivating conditions. Our findings in yeast suggest that the as-yet-uncharacterized mammalian counterpart of TIP41 may be a tumor suppressor.

Current work aimed at answering some of the following questions will be presented. How are nutrient levels interpreted by TOR? What are the identities and functions of TOR-associated factors? Is there cross-talk between TOR and other nutrient-responsive signalling cascades, such as the RAS/PKA pathway?

References:

1. Schmelzle, T. & Hall, M. N. (2000) TOR, a central controller of cell growth. *Cell* 103, 253-262.
2. Kunz, J., Henriquez, R., Schneider, U., Deuter-Reinhard, M., Movva, N. R. & Hall, M. N. (1993) Target of rapamycin, TOR2, is an essential phosphatidylinositol kinase homolog required for G1 progression. *Cell* 73, 585-596.

3. Barbet, N. C., Schneider, U., Helliwell, S. B., Stansfield, I., Tuite, M. F. & Hall, M. N. (1996) TOR controls translation initiation and early G1 progression in yeast. *Mol. Biol. Cell* 7, 25-42.
4. Noda, T. & Ohsumi, Y. (1998) TOR, a phosphatidylinositol kinase homolog, controls autophagy in yeast. *J. Biol. Chem.* 273, 3963-3966.
5. Zaragoza, D., Ghavidel, A., Heitman, J. & Schultz, M. C. (1998) Rapamycin induces the G0 program of transcriptional repression in yeast by interfering with the TOR signaling pathway. *Mol. Cell. Biol.* 18, 4463-4470.
6. Powers, T & Walter, P. (1999) Regulation of ribosome biogenesis by the rapamycin-sensitive TOR signaling pathway in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* **10**, 987-1000.
7. Hardwick, J. S., Kuruvilla, F. G., Tong, J. K., Shamji, A. F. & Schreiber, S. L. (1999) Rapamycin-modulated transcription defines the subset of nutrient-sensitive signaling pathways directly controlled by the TOR proteins. *Proc. Natl. Acad. Sci.* 96, 14866-14870.
8. Cardenas, M. E., Cutler, N. S., Lorenz, M. C., Di Como, J. C. & Heitman, J. (1999) The TOR signaling cascade regulates gene expression in response to nutrients. *Genes Dev.* **13**, 3271-3279.
9. Komeili, A., Wedaman, K. P., O'Shea, E. K. & Powers, T. (2000) Mechanism fo metabolic control: Target of rapamycin signaling links nitrogen quality to the activity of the Rtg1 and Rtg3 transcription factors. *J. Cell. Biol.* 151, 863-878.
10. Beck, T. & Hall, M. N. (1999) The TOR signalling pathway controls nuclear localization of nutrient-regulated transcription factors. *Nature* 402, 689-692.
11. Jacinto, E., Guo, B., Arndt, K. T., Schmelzle, T., & Hall, M. N. (2001) TIP41 interacts with TAP42 and negatively regulates the TOR signaling pathway. *Mol. Cell* 8, 1017-1026.

Role of the Snf1 kinase in transcriptional and developmental responses to nutrient stress

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The Snf1 protein kinase has broad roles in transcriptional, metabolic, and developmental regulation in response to stress in fungi, plants, and animals [1, 2]. In *Saccharomyces cerevisiae*, the Snf1 kinase is required for adaptation to glucose limitation and has been implicated in responses to salt stress, starvation for other nutrients, and heat shock. The response to glucose limitation has long been known to entail transcriptional and metabolic changes, and recent work has shown that haploid *S. cerevisiae* cells also undergo a transition between yeast-form growth and filamentous invasive growth, which requires the Snf1 kinase [3].

Invasive growth depends on the *FLO11* gene, which encodes a cell surface flocculin and has a large and complex promoter that is regulated by the PKA and MAP kinase pathways [4]. We have shown that *FLO11* expression is also regulated by the Snf1 kinase. One of the roles of the Snf1 kinase in promoting invasive growth in response to glucose depletion entails antagonism of two zinc-finger repressors, Nrg1 and Nrg2 [5], which inhibit *FLO11* expression.

S. cerevisiae cells contain three isoforms of the Snf1 kinase, each with a different β subunit, Sip1, Sip2 or Gal83. Although the β subunits exhibit significant functional redundancy, they have important roles in regulating specificity and subcellular localization of the kinase. The Gal83 subunit becomes nuclear localized in response to glucose limitation. We present evidence that Snf1 affects invasive growth by a pathway involving the Gal83 isoform of the kinase.

References:

1. Hardie, D.G., D. Carling, and M. Carlson. 1998. Ann. Rev. Biochem. 67: 821-55.
2. Gancedo, J.M. 1998. Microbiol. Mol. Biol. Rev. 62: 334-361.
3. Cullen, P.J. and G.F. Sprague, Jr. 2000. Proc. Natl. Acad. Sci. USA 97: 13619-13624.
4. Rupp, S., E. Summers, H.-J. Lo, H. Madhani, and G. Fink. 1999. EMBO J. 18: 1257-1269.
5. Vyas, V.K., S. Kuchin, and M. Carlson. 2001. Genetics 158: 563-572.

Hydroperoxides and electrophiles sensing by the Yap1 transcription factor

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Yap1 and Skn7 are two yeast transcriptional activators which co-operate to regulate the H_2O_2 response. Deletion of either regulator results in H_2O_2 hypersensitivity and the inability to induce the expression of about 100 proteins in response to this oxidant, including most yeast antioxidants, the thioredoxin and GSH thiol redox control systems, the pentose phosphate pathway and other stress genes. Yap1 fulfils the definition of an oxidative stress sensor since in the presence of low H_2O_2 levels, it is activated by oxidation and disulfide bond formation, which control its nuclear export by the export receptor Crm1. The partial constitutive and sustained Yap1 activity in thioredoxin pathway mutants suggest that thioredoxins catalyze its reduction (deactivation). Yap1 is also activated by the O_2^- generating drug menadione, cadmium and several electrophiles. The question is whether there exist an unifying mechanism for the activation of Yap1 by these different compounds. Surprisingly, in these cases, there is no need of disulfide formation for activation. Instead, one c-terminal cysteine is sufficient for activation, suggesting either the oxidation of one or more cysteine residues to a sulfenate, or formation of an adduct between cysteine residues and these compounds. The more potent Yap1 response to cadmium and diamide in mutants with low GSH levels might indicate a direct competion between GSH and Yap1 sulfhydrils for the binding of these compounds, and is consistent with the idea of adduct formation. Finally, we will also present recent data on the purification of Yap1 and molecular details on its activation by peroxides and electrophiles.

A genetic and genomic dissection of iron metabolism

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Iron is an essential element for all eucaryotes and most procaryotes. Iron is a substrate for heme, the major oxygen binding and sensing molecule in cells. Because of its facile ability to gain and loose electrons, iron has become a required co-factor in oxidation-reduction reactions. The same ease by which iron gains and looses electrons has also made iron a potentially toxic molecule capable of generating reactive oxygen radicals. All organisms tightly control the concentration of iron in biological fluids by regulating the rates of iron uptake and storage.

The facile genetics of yeast have permitted an in depth understanding of the mechanisms of iron transport and storage. A number of different screens have led to the identification of genes for high and low affinity plasma membrane iron transport systems. These screens have also led to the discovery of the iron-sensing transcription factor that regulates the high affinity iron transport system. This system is comprised of Fet3p, a cell surface ferroxidase and Ftr1p a transmembrane permease. Fet3p is a multicopper oxidase that obtains its copper in a trans-Golgi vesicle. The proper targeting of holoFet3p to the cell surface requires both normal copper homeostasis as well as vesicular traffic. Thus, analysis of iron transport mutants has led to the discovery of genes required for copper transport and for vesicular traffic. The same genetic screens and approaches have also uncovered intracellular iron transporters. Genes that affect mitochondrial iron transport and vacuolar iron storage have been identified and in some cases characterized.

The genetic analysis of transition metal metabolism has been greatly enhanced by two recent technological developments, transcript profiling through Microarray analysis and the generation of large-scale genomic screens. Microarray analysis for example has led to the discovery of a family of siderophore transporters. A large-scale genomic screen has uncovered a number of genes involved in the targeting and assembly of holoFet3p.

Studies in yeast have had great impact in understanding iron and transition metal metabolism in other species. The ability to complement specific yeast mutants has to the identification of genes for metal transporters in other species. Many of the genes that affect transition metal homeostasis in *Saccharomyces cerevisiae* have orthologues in other eucaryotes extending from plants to humans. Understanding the function of these orthologues have led to insights into both normal and disease processes.

References:

Babcock M., DeSilva D., Oaks R., Davis-Kaplan S., Jiralerspong S., Montermini L., Pandolfo M. and *Kaplan J.* (1997) Regulation of mitochondrial iron accumulation by Yfh1p, a putative homolog of Frataxin. Science 276:1709-1712.

Radisky D.C. and *Kaplan J.* (1999) Regulation of Transition Metal Transport across the Yeast Plasma Membrane. J. Biol. Chem. 274 :4481-4484.

Li, L, Chen O.S., and Kaplan J. (2001) CCC1 is a transporter that mediates vacuolar iron storage in yeast. J.Biol Chem. 276 :29515-9.

Grx5 is a mitochondrial monothiol glutaredoxin involved in Fe/S cluster assembly and iron homeostasis in yeast

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Yeast cells contain three proteins (Grx3, Grx4 and Grx5) with glutaredoxin signatures and a single cysteine residue at the putative active site (PKCGFSR), the lack of Grx5 leading to a dramatic increase in the sensitivity to oxidative stress caused by external agents, and also resulting in constitutive protein oxidation in the absence of external oxidants and growth defects in media lacking amino acid supplements (1). A number of point mutants have been created that show that the cysteine residue in the above sequence is essential for the biological activity of Grx5. Changes in activity in the mutants have been correlated with the effects on the three-dimensional structure of Grx5, that has been predicted from the conformation of other dithiol glutaredoxin proteins (2).

The growth defects of grx5 mutants are suppressed by overexpression of SSQ1 and ISA2, involved in the biogenesis and assembly of Fe/S clusters at the yeast mitochondria (3). Grx5 localizes at the mitochondrial matrix, as other proteins of the Fe/S cluster assembly complex (3-6). Grx5 derivatives lacking the amino-terminal residues compartimentalize at the cytosol, and yeast cells that exclussively express this truncated form behave as null mutants. Several phenotypes of the grx5 cells are similar to those of other mutants in Fe/S cluster assembly: high frequency of petite cells, inactivation of mitochondrial enzymes containing Fe/S clusters and iron accumulation. The inactivation of Fe/S enzymes is the primary defect due to the absence of Grx5, which would lead to the accumulation of iron, constitutive oxidation of cell proteins and lack of activity of Fe/S enzymes required for the synthesis of three amino acids (leucine, lysine and glutamic acid). Grx5 would act on protein mixed disulphides formed during Fe/S assembly.

References:

(1) Rodríguez-Manzaneque, M.T. et al. (1999) Mol. Cell. Biol. 19:8180. (2) Martin JL (1995) Structure 3:245. (3) Lill, R. and Kispal, G. (2000) Trends Biochem. Sci. 25:352. (4) Jensen, L.T. and Culotta, V. (2000) Mol. Cell. Biol. 20:3918. (5) Lange, H. et al. (2000) Proc. Natl. Acad. Sci. USA 97:1050. (6) Voisine,C. et al. (2001) Proc. Natl. Acad. Sci. USA 98:1483.

Session 2: Saline, osmotic and other forms of stress
Chair: Paul Russell

Control of transport proteins in yeast osmoadaptation and their effects on osmoshock-induced signalling

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Osmotic stress is associated with the flux of water out of the cell (hyperosmotic stress) or into the cell (hypo-osmotic stress). In mammals and plants it is well established that water flux through cellular membranes is controlled by a multitude of specialised water channels, aquaporins (4). For instance, human has 10 and *Arabidopsis* 35 members of the aquaporin family (also called MIP family). The yeast *Saccharomyces cerevisiae* has two. Curiously, all but one (Σ1278) of the commonly used laboratory strains carry alleles of the genes *AQY1* and *AQY2* that encode inactive gene products (3, 5). Hence, under laboratory conditions, aquaporins do not play an essential role in yeast physiology.

Aqy2p is a plasma membrane water channel. Expression of *AQY2* is controlled by growth and osmotic signals. The gene is specifically expressed during rapid proliferation. Hyper-osmotic shock leads to downregulation of the expression of *AQY2* and this effect requires the HOG osmosensing MAP kinase pathway. Subsequent shift to low osmolarity stimulates expression of *AQY2*. Together with the observation that ectopic expression of human AQP1 in yeast causes sensitivity to high osmolarity these observations indicate that Aqy2p plays a role in water export and assists in the control of turgor during growth.

Expression of *AQY1* is controlled by developmental signals. The protein is detectable only in yeast spores suggesting that it participates either in spore maturation or spore germination.The aquaporin family is divided into two main branches, the aquaporins *sensu stricto* and the glycerol facilitators. Yeast Fps1p is such a glycerol transport protein and it plays a crucial role in yeast osmoadaptation by controlling the intracellular glycerol level (8). Glycerol is well known as the major osmolyte in yeast cells and it is produced and accumulated to high levels under hyperosmotic conditions (1, 2). Mutants lacking Fps1p (*fps1Δ*) fail to rapidly export glycerol after a hypo-osmotic shock and show a decreased survival. Hence, yeast cells show a response related to regulated volume decrease (RVD) known from mammalian cells. In addition *fps1Δ* mutants show several additional phenotypes related to glycerol export and osmotic regulation (7, 8, 10).

Glycerol transport through Fps1p is rapidly regulated upon osmotic shock and probably Fps1p senses osmotic changes itself. We have characterised in detail a small regulatory domain within the N-terminal extension of Fps1p that is required for channel closing. Molecular modelling suggests that this domain may provide a membrane anchor, thereby possibly sensing osmoshock-induced changes in the membrane, or fold back to close the channel. Also sequences within the C-terminal extension seem to be required for proper channel regulation.Mutants expressing Fps1p that is unable to rapidly close upon a hyperosmotic shock loose most of the glycerol they produce and are sensitive to high osmolarity (6, 8). *FPS1* deletion mutants and strains expressing hyperactive alleles can be used to manipulate the osmotic response in order to study aspects of the signal transduction. This is illustrated in two examples.

Adding high glycerol levels to the growth medium activates the HOG pathway. This even leads to transcriptional responses, such as elevated expression of *GPD1*, which encodes an enzyme in glycerol biosynthesis. However, no such response is observed in a strain expressing truncated Fps1p, which does not close upon osmotic shock. This is probably due to glycerol rapidly equilibrating across the membrane in that strain, illustrating that the HOG pathway is stimulated by turgor or volume changes and not by water stress *per se*. The latter is in accordance with the observation that ethanol does not stimulate the HOG pathway (9).

We also use different alleles of Fps1p to study feedback regulation of the HOG pathway. The parallel monitoring of different events in osmotic adaptation, Hog1p phosphorylation, mRNA levels, protein production, glycerol accumulation and proliferation, demonstrate that mutants unable to rapidly accumulate glycerol show a much prolonged and more pronounced response. Specifically, there seems to be a good correlation between the decline in Hog1p phosphorylation and the mRNA level of HOG target genes and the onset of glycerol accumulation. These observations suggest that feedback regulation of the pathway requires successful execution of the central aspect of the adaptation programme, the accumulation of glycerol. Indeed, the HOG pathway can be stimulated with a second osmotic shock at any time during the adaptation from a first shock, illustrating that the pathway is not desensitised or shut off via an autonomous feedback loop.

References:

1. **Albertyn, J., S. Hohmann, J. M. Thevelein, and B. A. Prior.** 1994b. *GPD1*, which encodes glycerol-3-phosphate dehydrogenase is essential for growth under osmotic stress in *Saccharomyces cerevisiae* and its expression is regulated by the high-osmolarity glycerol response pathway. Mol. Cell. Biol. **14:**4135-4144.

2.**Ansell, R., K. Granath, S. Hohmann, J. M. Thevelein, and L. Adler.** 1997. The two isoenzymes for yeast NAD+-dependent glycerol 3-phosphate dehydrogenase encoded by *GPD1* and *GPD2* have distinct roles in osmoadaptation and redox regulation. EMBO J. **16:**2179-2187.

3.**Bonhivers, M., J. M. Carbrey, S. J. Gould, and P. Agre.** 1998. Aquaporins in *Saccharomyces*. Genetic and functional distinctions between laboratory and wild-type strains. J. Biol. Chem. **273:**27565-27572.

4.**Hohmann, S., S. Nielsen, and P. Agre.** 2001. Aquaporins, vol. 51. Academic Press, San Diego, CA.

5.**Laizé, V., F. Tacnet, P. Ripoche, and S. Hohmann.** 2000. Polymorphism of *Saccharomyces cerevisiae* aquaporins. Yeast **16:**897-903.

6.**Luyten, K., J. Albertyn, W. F. Skibbe, B. A. Prior, J. Ramos, J. M. Thevelein, and S. Hohmann.** 1995. Fps1, a yeast member of the MIP family of channel proteins, is a facilitator for glycerol uptake and efflux and is inactive under osmotic stress. EMBO J. **14:**1360-1371.

7.**Philips, J., and I. Herskowitz.** 1997. Osmotic balance regulates cell fusion during mating in *Saccharomyces cerevisiae*. J. Cell Biol. **138:**961-974.

8.**Tamás, M. J., K. Luyten, F. C. W. Sutherland, A. Hernandez, J. Albertyn, H. Valadi, H. Li, B. A. Prior, S. G. Kilian, J. Ramos, L. Gustafsson, J. M. Thevelein, and S. Hohmann.** 1999. Fps1p controls the accumulation and release of the compatible solute glycerol in yeast osmoregulation. Mol. Microbiol. **31:**1087-1104.

9.**Tamás, M. J., M. Rep, J. M. Thevelein, and S. Hohmann.** 2000. Stimulation of the yeast high osmolarity glycerol (HOG) pathway: evidence for a signal generated by a change in turgor rather than by water stress. FEBS Lett. **472:**159-165.

10.**Tao, W., R. J. Deschenes, and J. S. Fassler.** 1999. Intracellular glycerol levels modulate the activity of Sln1p, a *Saccharomyces cerevisiae* two-component regulator. J. Biol. Chem. **274:**360-367.

Downstream components of the osmotic stress response

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Exposure of yeast cells to increases in extracellular osmolarity activates the Hog1 MAP kinase. Activation of Hog1 MAPK results in induction of a set of osmoadaptive responses, which allow cells to survive in high osmolarity environments. Cells respond to saline stress by inducing the expression of a very large number of genes, and suggest that stress adaptation requires regulation of many cellular aspects. The transcriptional induction of most genes that are strongly responsive to salt stress was highly or fully dependent on the presence of the MAP kinase Hog1, indicating that the Hog1-mediated signaling pathway plays a key role in global gene regulation under osmotic stress conditions. Some transcription factors under the control of the MAP kinase have been described recently such as Sko1, Hot1, Msn2/Msn4. We have characterized some of the effects of the MAPK over the transcription factor Sko1 and futhermore identified a new transcription factor, Smp1, regulated by the MAPK. Smp1, a transcription factor of the MEF2 family, is targeted by Hog1 upon stress and seem to be part of the responses to osmotic shock because *smp1Δ* cells are more sensitive to osmotic shock than wild type cells. Apart from the control of gene expression, the Hog1 MAPK is also responsible for cell cycle regulation upon osmotic stress. We have characterized a G2/M arrest caused by sustained activation of the MAPK.

The HOG pathway is essential for osmo-stress adaptation.

Cells respond to saline stress by inducing the expression of a very large number of genes, and suggest that stress adaptation requires regulation of many cellular aspects. The transcriptional induction of most genes that are strongly responsive to salt stress was highly or fully dependent on the presence of the MAP kinase Hog1. Some transcription factors under the control of the MAP kinase have been described recently (such as Sko1, Hot1, Msn2 and Msn4) but they can not account for the complex gene regulation regulated by Hog1

Smp1 transcription factor acts downstream of the Hog1 MAPK.

To identify new transcription factors under the control of the MAPK, we designed a genetic screen to isolate clones whose overexpression were able to induce *STL1* gene transcription. *STL1* is a gene strongly regulated upon osmotic stress in a *HOG1* dependent manner

A) Overexpression of the MEF2-like transcription factor, *SMP1*, induced transcription of the *STL1::LacZ* gene expression even in the absence of osmotic stress.

B) Induction of *STL1::LacZ* expression was enhanced by overexpression of *SMP1* under normal conditions and upon osmotic stress and reduced in *smp1Δ* cells.

Hog1 phosphorylates Smp1 in response to osmotic stress.

A) Smp1 was phosphorylated upon a brief osmotic shock (15 min 0,4M NaCl) and its phosphorylation was dependent on the presence of Hog1.

B) Time course of Hog1 and Smp1 phosphorylation upon osmotic stress.

The Smp1 transcription factor interacts with Hog1.
Both, two hybrid analysis (A) and in vivo binding assays (B) showed that the C-terminal domain of Smp1 interacts with Hog1.

Smp1 is a direct substrate for the Hog1 MAPK.
In vitro kinase assays demonstrated that Hog1 is able to phosphorylate directly Smp1. Moreover, this phosphorylation occurs basically in the C-terminal region of Smp1

Cell survival upon osmotic stress is reduced in a *smp1Δ* strain.
Upon osmotic shock cell viability is compromised. To analyze the role of Smp1 in osmotic stress adaptation we measured the incorporation of PI as a measure of cell viability. *Hog1Δ* cells were highly sensitive to an osmotic shock (60 min, 1M NaCl) as compared to the wild type. *smp1Δ* strain was over three times more sensitive than a wild type strain indicating that Smp1 is involved in omo-stress stress adaptation.

Smp1 nuclear localization depends on Hog1.
To analyze the mechanisms of regulation of Smp1 by Hog1 we investigate Smp1 localization. No big differences on Smp1 localization were observed upon osmotic stress, but interestingly, Smp1 nuclear localization was dependent on the presence of Hog1. This suggests that Hog1 could be regulating Smp1 activity through several mechanisms.

Integration of nutrient signals and osmostress signals for a coordinated transcriptional response

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The general stress response in yeast is defined by transcriptional changes in a large set of genes regularly induced either by diverse stress exposures or by acute nutrient starvation. Beyond this common response, a particular stress situation such as osmotic stress will also target smaller groups of genes that help cells to cope with stress specific damage. Finally, the overall growth conditions have a distinct influence on the extent and duration of stress responses as optimal nutrient supplies suppress the expression of stress protecting factors whereas nutrient limitations will increase it. Our aim has been to understand how these different external conditions might be integrated into a defined cellular response. We have focussed thereby on studying signals transduced via the high osmolarity glycerol response pathway and the cAMP dependent kinase system.

The transcription factor Msn2 is a key regulator in the coordination of the general stress response. The function of Msn2 seems to be regulated at several levels such as intracellular distribution, DNA binding, co-factor recruitment and stability. A functional dissection of Msn2 allowed us to define regulatory domains including its nuclear transport signals. Accordingly, the nuclear import signal of Msn2 is a direct and exclusive target of cAMP-dependent protein kinase (cAPK). Msn2-NLS inhibition by phosphorylation is highly sensitive to carbohydrate fluctuations during fermentative growth but not to nitrogen starvation or any number of stress treatments including osmotic stress. The rapid decrease in phosphorylation observed under acute glucose deprivation appears to be effected by changes in cAPK activity rather than an increase in protein phosphatase activity. Interestingly, the Bcy1 subunit appears to be the main target of regulation during acute glucose starvation even if cAMP concentrations are kept constant. Our data support the notion that carbohydrate signals via cAPK will only provide a theshold needed to be overcome by other acute stress signals while cAPK itself is not modulated during acute stress.

Since full length Msn2 accumulates in the nucleus during osmotic stress, this effect must be due to mechanisms independent of nuclear import control. Indeed, we could define a region in Msn2 that responds to osmotic stress and also contains a nuclear export signal. Moreover, using chromatin immuno-precpitation assays, we found that the osmostress activated MAP kinase Hog is recruited to general stress responding promoters in an Msn2 dependent manner. We propose that the observed interaction between Hog1 and Msn2 serves two purposes. On one hand, Hog1 severes the interactions of Msn2 with the nuclear export machinery. On the other hand, activated Hog1 functions as a co-activator in the assembly of the transcription initiation complex. Recruitment and function of Hog1 kinase as transcriptional co-activator at all osmostress induced promoters could explain the highly coordinated response of specific and general sets of stress protecting genes. We will present data in support of this model.

Yeast response to alkaline pH stress

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The yeast *S. cerevisiae* grows preferently in an acid environment, and therefore growth even in a moderately alkaline media involves an important stress. Exposure of different fungi to an alkaline environment results in relevant phenotypic changes that can lead, as in the case of *C. albicans*, to pathogenesis (1). Most of the available information on the response mechanisms derives from work using *Aspergillus nidulans* as a model. In this organism, a transcription factor encoded by the gene *pacC* was identified as a key component of the transcriptional response. Surprisingly, little is known about the behaviour of *S. cerevisiae* under alkaline conditions, although current evidence suggest that the molecular architecture for the signal transduction pathway involved in the alkaline response could be relatively well conserved (2,3).

A project aiming to the characterization of the alkaline response in *S. cerevisiae* was recently initiated in our laboratory. By using genomic DNA microarrrays we have analysed the global transcriptional response to mild alkaline conditions. About 150 genes were induced at least 2-fold and 230 were repressed at least 3-fold within 45 minutes after exposure to pH 7.6. In most cases, genes induced by alkaline pH did not fall within the *common environmental response* gene family, suggesting a relatively specific response.

The response of several induced genes, such as *ENA1* (encoding a Na+-ATPase), and *PHO84* and *PHO89* (encoding H+/phosphate and Na+/phosphate permeases) was further characterized. *ENA1* and *PHO84* respond very rapidly to alkaline stress, whereas the response of *PHO89* is somewhat delayed. These genes show differences in their sensitivity to alkaline stress and, in fact, induction of *PHO84* is already observed near neutrality. The mechanism for *ENA1* alkaline response is being investigated by using different mutants in key components of known stress signalling pathways. In our hands, the *ENA1* response is not impaired by mutation of *HOG1*, *CNB1*, *MSN2/MSN4* or *YAP1*. In addition, it is essentially independent of the presence of Rim101, the *S. cerevisiae* homologue of *A. nidulans pacC*. The *ENA1* promoter was functionally mapped and two defined regions required for alkaline pH response have been identified.

References:

1.- De Bernardis *et al.* (1988) Infect. Immun. 66, 3317-3325.
2.- Denison *et al.* (1998) Mol. Microbiol. 30, 259-264.
3.- Denison, SH. (2000) Fungal Genet. Biol. 29, 61-71

Global proteomic adaptation for optimal sulfur economy in response to cadmium toxicity

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Cadmium is very toxic at low concentrations. In yeast, the metal is detoxified by chelation to glutathione and the complex is transported into the vacuole. However, the basis for its toxicity are not clearly understood. To gain insight into the biological effects of cadmium, we analyzed the proteomic response of yeast cells to the metal. We identified 54 induced and 43 repressed proteins. Among the induced proteins, 9 of them were enzymes of the sulfur amino acid biosynthetic pathway. Consistent with the induction of this pathway, we observed that glutathione synthesis is strongly increased in response to cadmium treatment.

Moreover, 60% of the sulfur assimilated by the cells is converted into glutathione, resulting in a decreased availability of sulfur amino acids for protein synthesis. Strikingly, cells adapt to this situation by modifying their proteome to reduce the production of sulfur-rich proteins. Cadmium induced proteins have a particularly low frequency of methionine and cysteine residues. Moreover, some abundant enzymes of the glycolysis are replaced by sulfur depleted isozymes. The global change in protein expression under Cd2+ condition allows an over-all sulfur amino acid economy of 30%. A comparative analysis of the transcriptome and the proteome showed that this global proteome response is essentially regulated at the transcriptional level.

Session 3: The transduction of the signal, transcription factors and stress response
Chair: Marian Carlson

Ca2+/calcineurin-dependent signal transduction in yeast

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In Saccharomyces cerevisiae, one function of the Ca^{2+}/calmodulin regulated protein phosphatase, calcineurin, is to activate transcription in response to specific types of environmental stress. The genes turned on under these conditions were identified using DNA microarrays, and encode proteins that participate in many functions including cell wall synthesis, ion homeostasis, membrane trafficking and signal transduction. Calcineurin regulates gene expression by dephosphorylating the transcription factor Crz1p/Tcn1p and effecting its rapid translocation from the cytosol to the nucleus. We characterized the mechanism by which Crz1p enters the nucleus, and found that dephosphorylation of Crz1p promotes its association with the importin, Crz1p. We also examined Crz1p nuclear export and determined that the Crz1p nuclear export signal (NES) is inactivated by calcineurin-mediated dephosphorylation. Thus, calcineurin promotes nuclear accumulation of Crz1p both by increasing its nuclear import and down-regulating its nuclear export. A small motif in Crz1p, PIISIQ, is required for its regulation by calcineurin. This motif mediates the *in vivo* interaction between calcineurin and Crz1p and is related to the PxIxIT docking site for calcineurin on the mammalian transcription factor, NFAT. Thus in both yeast and mammals, calcineurin must associate with its substrate to dephosphorylate it efficiently.

Other studies have focused on the mechanism by which yeast cells regulate cytosolic Ca^{2+}. We showed that Ca^{2+} rises transiently after exposure of yeast to hypertonic stress. This signal results from release of Ca^{2+} from the vacuole and is mediated by Yvc1p, an ion channel that is part of a newly defined fungal branch of the TRP family of ion channels.

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Functional analysis of the general stress responsive factor Msn2p

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in memoriam Helmut Ruis

Saccharomyces cerevisiae cells respond and adapt to various stress conditions with complex shifts of transcriptional regulation. Our understanding of many aspects of stress sensing, signalling and integration is far from complete. The transcription factor Msn2 and its homologue Msn4 are involved in the induction of many genes in response to a variety of cell damaging and starvation conditions. The aim of our work was to define and dissect the mechanisms mediating diverse signals to Msn2 and lead to the nuclear import and activation of this transcription factor.

Stress regulates Msn2 at several levels including intracellular localisation, stability and DNA-binding. Domain analysis resulted in the isolation of two domains important for intracellular localisation. The nuclear import signal is located at the C-terminus, which includes the Zn-finger DNA-binding domain. A separate region was found to be required for efficient re-localisation from the nucleus to the cytoplasm and thus works like a nuclear export or retention domain. However, the general nuclear export factor Crm1 is not required for Msn2p nuclear export but rather the presumed exportin Msn5. Stress and PKA have a strong effect on the nuclear export domain whereas the nuclear import domain is only regulated by PKA in response to glucose starvation. A further layer of regulation is imposed by the stress and PKA regulated binding to promoters as demonstrated by in vivo footprint experiments. Stability of Msn2, on the other hand, seems to be a consequence of its localisation but may contribute to efficient shut down of a stress or starvation signal.

PKA has a general influence on the activity of Msn2. High PKA levels can reduce Msn2 activity even under stress conditions while in situations of low PKA activity Msn2 seems to be constitutively active. Strong genetic evidence places Msn2 downstream of PKA. Assuming a direct regulation of Msn2 through phosphorylation by PKA we generated a series of mutants in all the six putative PKA phosphorylation sites. Mutation of all six PKA site serines to alanine (Msn2-Ala6) indeed resulted in a highly active version of Msn2, which is toxic to cells when expressed from a conditional promoter. Cells lacking PKA are not viable but can be rescued by the simultaneous deletion of Msn2 and Msn4. Therefore part of this phenotype is caused by misexpression of genes under the control of Msn2/4. Using microarrays we confirmed that the Msn2-Ala6 mutant indeed simulates the absence of PKA since a large number of genes are induced which are also upregulated under sudden deprivation of PKA activity. Both Msn2-Ala6 expression and inactivation of PKA leads to a G1 arrest, which can be overcome in the absence of the Yak1 kinase. Therefore, Msn2 which together with Yak1 is required for the PKA dependent G1 arrest induces a yet unidentified factor.

Our results confirm and partly explain the strong relationship between the general stress response and PKA in S.cerevisiae. Furthermore, we conclude that PKA is not the main stress sensor for Msn2 apart from being involved in the signalling of acute glucose starvation. We further conclude that low PKA activity does probably not directly cause a G1 arrest but rather achieves this effect via Msn2 dependent transcriptional regulation.

A Rho1p specific GAP protein negatively regulates the cell integrity pathway and cell wall (1,3)β-glucan biosynthesis in *Schizosaccharomyces pombe*

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Rho proteins switch between an inactive GDP-bound form and an active GTP-bound form. The rate of conversion between both states is modulated by three kinds of proteins: guanine nucleotide exchange factors (GEFs) which favour the GTP-bound conformation; guanine nucleotide dissociation inhibitors (GDIs), and GTPase activating proteins (GAPs), which favour the GDP-bound state. All these regulators may play important roles in the specificity of Rho functions.

The gene named gpr1+ encodes a Rho1-GAP that shares similarity to *S. cerevisiae* Sac7p. Overexpression of rho1+ in gpr1D cells was lethal and caused a higher increase in the (1,3)β-D-glucan synthase activity than that detected by rho1+ overexpression in wild type cells. Gpr1p co-immunoprecipitates with Rho1p and shows specific GAP activity for Rho1p as detected in vitro and in vivo. Gpr1p participates in the regulation of Pck1p and Pck2p stability by Rho1p. It also plays a role in actin cytoskeleton regulation.

Overexpression of gpr1+ caused a rapid cell lysis and the (1,3) β-D-glucan synthase activity was rapidly reduced to 40% that of the wild type. By contrast, gpr1D cells are viable and show a mild morphological phenotype at 37°C. the (1,3)β-D-glucan synthase activity of this cells is considerably increased. Elimination of the first gpr1p 25 aminoacids considerably impaired the lethal effect of Gpr1p overexpression. By contrast, elimination of the C-terminal amino acids, that form a putative F-actin capping domain, increases the lethal effect of Gpr1p.

Function of heat shock transcription factors from yeast to humans

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It is critical that cells mount appropriate responses to a number of stress conditions that include heat, metals, reactive oxygen species, pharmacological agents, infection and inflammation. One such system for eukaryotic stress responses involves a transcription factor called Heat Shock Factor (HSF), and its cognate DNA binding site, the Heat Shock Element (HSE). Indeed, HSF and the HSE represent one of the most evolutionarily conserved pair of transcription factors and corresponding cis-acting elements from yeast to humans, since many aspects of the basic structure of HSF, and the sequence of the HSE are maintained throughout evolution. HSEs are composed of inverted repeats of the sequence nGAAn, with a typical high affinity HSF binding site composed of three tandem pentameric repeats.

In *S. cerevisiae*, HSF plays an important role in the heat shock induction of many genes encoding protein chaperones such as Hsp70 and Hsp90, and also serves to regulate basal levels of expression of subsets of chaperone genes. *S. cerevisiae* cells harbor a single gene encoding HSF and cells lacking this gene are inviable at all temperatures tested. Yeast HSF harbors both an amino- and carboxyl-terminal domain capable of trans-activation, with distinct target genes exhibiting differential requirements for each trans-activation domain. For example, while activation of the Hsp70 gene requires the amino-terminal domain, the CUP1 metallothionein gene exhibits a rather strict requirement for the carboxyl-terminal trans-activation domain of HSF. Furthermore, the Hsp70 promoter HSEs are spaced in the consensus organization, while the CUP1 promoter HSE is composed of an imperfect HSE. Therefore, yeast HSF is able to recognize distinct promoter sequences to activate target genes through the use of two independent trans-activation domains.

S. cerevisiae harbors a single HSF gene yet mammals harbor genes encoding three distinct HSF proteins, HSF1, HSF2 and HSF4. Although the functions of HSF2 and HSF4 are poorly understood at present, HSF1 is a stress responsive transcription factor which, upon exposure to stress, is converted from a monomer to a homotrimer that binds to HSEs with high affinity. Our recent experiments have made use of both yeast and mammalian cells deficient in HSF genes to understand the molecular basis for HSF target gene specificity and the mechanisms by which HSF proteins sense stress and activate gene expression.

References:

1. Wu, C. *Ann. Rev. Cell Biol.* (1995) 11: 441-469.

2. Liu, X., Liu, P.C.C., Santoro, N. and Thiele, D.J. (1997) *EMBO J.* 16: 6466-6477.

3. Ahn, S-G., Liu, P.C.C., Klyachko, K., Morimoto, R., and Thiele, D.J. (2001) *Genes and Development* 15: 2134-2145.

Negative and positive control of stress genes

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The expression of a great number of genes is stimulated by hyperosmotic or salt stress in the yeast Saccharomyces cerevisiae (1, 2). The High Osmolarity Glycerol (HOG) MAP kinase pathway with its central Hog1 MAP kinase is dedicated to rapidly induce the expression of most of these stress genes. The Hog1 MAP kinase is imported upon stress into the nucleus where it associates with various transcription factors.

One of the downstream effectors of Hog1 is the ATF/CREB transcription factor Sko1 (=Acr1) which binds to cAMP responsive promoter elements (CRE) (3, 4, 5, 6). Sko1 represses transcription by recruiting the general corepressor complex Ssn6-Tup1 to osmostress inducible genes like ENA1 (Na+ extrusion ATPase) (5), HAL1 (ion homeostasis determinant) (7), and genes whose expression is also stimulated by oxidative stress like AHP1 (alkylhydroperoxide reductase), GRE2 (yeast homolog of plant isoflavonoid reductases) and GLR1 (glutathione reductase) (8, 9). The Hog1 MAP kinase interacts directly with Sko1 in vivo and regulates its function by multiple phosphorylations (6). Hog1 phosphorylations decrease the interaction between Sko1 and Ssn6-Tup1 (6). However, recent chromatin immunoprecipitation analyses show that the Sko1-Ssn6-Tup1 complex is bound to promoters in vivo even under activating conditions. Moreover, Sko1 is required to recruit Hog1 as well as the chromatin modifying coactivator complexes SAGA and SWI/SNF upon stress. This suggests that HOG actually switches Sko1 from a repressor (recruiting Ssn6-Tup1) to an activator (recruiting SAGA and SWI/SNF) upon stress (10).

Additionally to the regulation by HOG, Sko1 is multiply phosphorylated by protein kinase A (6). We show that normal PKA activity is needed for nuclear accumulation of Sko1 and efficient repression (11), representing yet another layer of control.

Finally, under severe salt stress conditions the general amino acid control pathway becomes involved in the stress response. Under these conditions Gcn4, the key transcriptional activator of amino acid biosynthesis, becomes essential and contributes to stress gene activation as found for the regulation of HAL1 (7).

References:

(1) Posas, F., Chambers, J.R., Heyman, J.A., Hoeffler, J.P., de Nadal, E., and Arino, J. (2000) J. Biol. Chem., 275:17249-17255.
(2) Rep, M., Krantz, M., Thevelein, J.M., and Hohmann, S. (2000) J. Biol. Chem., 275:8290-8300.
(3) Vincent, A.C. and Struhl, K. (1992) Mol. Cell. Biol. 12:5394-5405.
(4) Nehlin, J.O., Carlberg, M., and Ronne, H. (1992) Nucleic Acids Res. 20:5271-5278.

(5) Proft, M. and Serrano, R. (1999) Mol. Cell. Biol. 19:537-546.
(6) Proft, M., Pascual-Ahuir, A., de Nadal, E., Arino, J., Serrano, R., and Posas, F. (2001) EMBO J. 20:1123-1133.
(7) Pascual-Ahuir, A., Serrano, R., and Proft, M. (2001) Mol. Cell. Biol. 21:16-25.
(8) Rep, M., Proft, M., Remize, F., Tamas, M., Serrano, R., Thevelein, J.M., and Hohmann, S. (2001) Mol. Microbiol. 40:1067-1083.
(9) Garcia-Gimeno, M.A. and Struhl, K. (2000) Mol. Cell. Biol. 20:4340-4349.
(10) Proft, M., Serrano, R., and Struhl, K. (manuscript in preparation).
(11) Pascual-Ahuir, A., Posas, F., Serrano, R., and Proft, M. (2001) J. Biol. Chem. 276:37373-37378.

Use of *Saccharomyces cerevisae* as a model for studing the effect of *Salmonella typhimurium* pathogenic proteins on the GTPase Cdc42 and MAP Kinase mediated signal transduction pathways

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Rho-type GTPases (Cdc42, Rac, Rho) play essential roles in cell polarity by regulating actin organization, and in stress responses by signaling to MAP kinase pathways, from yeast to mammalian organisms. Taking advantage of the cellular function of these proteins, some bacterial pathogens have evolved to induce their internalization into non-phagocytic host cells by modulating specific signaling pathways. *Salmonella typhimurium* directs the delivery of effector proteins, that modulates the activity of the Rho GTPases Cdc42 and Rac-1. The Salmonella GEF protein SopE2 stimulate Cdc42 and Rac-1 function leading to bacteria internalization, whereas SptP, a protein tyrorine phosphatase with GAP activity, reverses the activation of these GTPases leading to cell recovery (1). We have used the yeast *S. cerevisiae* as a model to study the effect of these proteins on the eukaryotic signalling machinery. Expression of SopE2 promoted filamentous growth of *S. cerevisiae* and the activation of the Kss1 MAPK mediated filamentation pathway, suggesting that Cdc42 is indeed a target of SopE2. Furthermore, the Slt2 MAPK cell integrity pathway is activated following expression of SopE2.

In order to analyze whether Cdc42 affects the cell integrity pathway, we studied the amount of activated Slt2 in cells bearing the Cdc42-GAPs RGA1, RGA2 and BEM3 deletions. Double and triple mutants in these GAP encoding genes showed an increased Slt2 phosphorylation. This activation was abolished when the STE20 or STE7 genes were also deleted, indicating the essential role of the pheromone response/invasive growth pathway in the Cdc42 induced Slt2 activation. Furthermore, expression of SptP was able to reduce the level of Kss1 phosphorylation in double mutants affected in these Cdc42-GAPs. Interestingly, Slt2 activation by Cdc42 has been observed not to be mediated by Rho1.

References:

(1). Galan JE and Zhou D. 2000. Striking a balance: modulation of the actin cytoskeleton by Salmonella. PNAS 97:8754-8761.

Session 4: Stress responses in yeasts others than *S. cerevisiae*
Chair: Dennis J. Thiele

pH regulation in fungi

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Regulation by gene expression by ambient pH is widely found among ascomycete fungi, where it controls de synthesis of extracellular enzymes, permeases ands exported metabolites exposed to environmental pH. This regulatory circuit has been most extensively studied in the model filamentous fungus *Aspergillus nidulans*.

The *A. nidulans* PacC zinc finger transcription factor mediating regulation of gene expression by ambient pH, in common with a number of other transcription factors, notably the transducer of the Hedgegog signal Cubitus interruptus,, undergoes proteolytic processing activation. The ease with which *A. nidulans* can be manipulated genetically makes PacC ideally placed for understanding how a transcription factor prevents its activation in the absence of appropriate signal transduction.

In response to a signal transduced at alkaline ambient pH by the products of the six *pal* genes, the 674 residue translation product of the *A. nidulans pac*C gene is processed to a functional form containing the ~248-250 N-terminal residues. The PacC DNA binding domain (DBD), containing three Cys_2His_2 zinc fingers and binding to GCCARG promoter sites is located centrally within the processed form. The pH-sensitive step in the regulatory cascade is the accessibility of the PacC primary translation product to the processing protease, as PacC alternates between a protease-resistant ('closed' and a protease-sensitive ('open') form in response to ambient pH. Interactions between two PacC regions which overlap the processing site and the ~ 150 C-terminal residues hold the 'closed' protein conformation. These interactions are destroyed by the ambient pH signal, which therefore irreversibly sensitises PacC to the processing protease. In the absence of pH signal, the translation product is cytosolic. The processed PacC form is localised in the nucleus, Therefore, the proteolytic activation of PacC results in commitment to nuclear localisation.

References:

Tilburn, J., S. Sarkar, D.A. Widdick, E.A. Espeso, M. Orejas, J. Mungroo, M.A. Peñalva, and H.N. Arst, Jr. The Aspergillus PacC zinc finger transcription factor mediates regulation of both acid- and alkaline-expressed genes by ambient pH. EMBO J. 14: 779-790 (1995).

Orejas, M., Espeso, E., Tilburn, J., Sarkar, S., Arst, H.N., Jr. and Peñalva, M.A. Activation of the *Aspergillus* PacC transcription factor in response to alkaline ambient pH requires proteolysis of the carboxy-terminal moiety. Genes & Devel. 9: 1622-1632 (1995).

J.-M. Mingot, J. Tilburn, E. Díez, E. Bignell, M. Orejas, D.A. Widdick, S. Sarkar, C.V. Brown, M.X. Caddick, E.A. Espeso, H.N. Arst, Jr. and M.A. Peñalva. Specificity determinants of proteolytic processing of the *Aspergillus* PacC transcription factor are remote from the processing site and processing occurs in yeast provided pH signalling is bypassed. Mol. Cell. Biol. 19: 1390-1400 (1999)

E.A. Espeso, T. Roncal, E. Díez, L. Rainbow, E. Bignell, J. Álvaro, T. Suárez, S.H. Denison, J. Tilburn, H.N. Arst, Jr. and M.A. Peñalva. On how a transcription factor can avoid its proteolytic activation in the absence of signal transduction.
EMBO J. 19: 719-728 (2000).

E. A. Espeso and H. N. Arst. On the mechanism by which alkaline pH prevents expression of an acid-expressed gene. Mol.Cell.Biol. 20:3355-3363 (2000).

J.M. Mingot, E.A. Espeso, E. Díez and M.A. Peñalva. Ambient pH signaling regulates nuclear localization of the *Aspergillus nidulans* PacC transcription factor. Mol. Cell. Biol. 21: 1688-1699 (2001).

Roles of protein kinase A isoforms in morphogenesis and stress response of *Candida albicans*

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TPK1 and TPK2 encode both isoforms of protein kinase A (PKA) catalytic subunits in Candida albicans. Mutants lacking both TPK1 alleles showed defective hyphal morphogenesis on solid inducing media, while in liquid media hypha formation was affected only slightly. In contrast, tpk2 mutants were only partially morphogenesis-defective on solid media, while a strong block was observed in liquid. In addition, the yeast forms of tpk2- but not tpk1-mutants were completely deficient to invade agar. Because Tpk1p and Tpk2p differ in their N-terminal domains of approximately 80-90 amino acids, while the catalytic portions are highly homologous, the functions of hybrid Tpk-proteins with exchanged N-terminal domains were tested. The results demonstrate that the catalytic portions mediate Tpk protein-specificities with regard to filamentation, while agar invasion is mediated by the N-terminal domain of Tpk2p. Homozygous tpk1 and tpk2 mutants grew normally; however, a tpk2 mutant strain containing a single regulatable TPK1 allele (PCK1p-TPK1) at low expression levels was severely growth-defective, it was completely blocked in hyphal morphogenesis and it was stress-resistant to high osmolarities or temperatures. Thus, both Tpk isoforms in C. albicans share growth functions including roles in stress response, but unlike Saccharomyces cerevisiae isoforms, they have only positive and specific roles in filament formation in different environments. Downstream targets of PKA isoforms appear to include the Efg1p morphogenetic regulator, since a Efg1p mutein lacking a potential PKA-phosphorylation site is morphogenesis-defective. On the other hand, overproduction of PKA isoforms leads to filamentous growth, but surprisingly, downregulates expression of the EFG1 gene. Combined with the finding that EFG1 is an autoregulated gene these results suggest a model, in which induction conditions activate PKA and Efg1p, but that this activation simultaneously lowers the responsiveness to inducers, i.e. leads to adaptation.

References:

Sonneborn, A., Bockmühl, D. P., Gerads; M., Kurpanek, K., Sanglard, D. and Ernst,J.F. (2000) Protein kinase A encoded by TPK2 regulates dimorphism of the human pathogen Candida albicans. Mol. Microbiol. 35,386-396.

Bockmühl, D. P. and Ernst, J. F. (2001) A potential phosphorylation site for an A-type kinase in the Efg1 regulator protein contributes to hyphal morphogenesis of Candida albicans. Genetics 157, 1555-1567.

Bockmühl, D. P., Krishnamurthy, S., Gerads, M., Sonneborn, A. and Ernst, J. F. (2001) Distinct and redundant roles of the two protein kinase A isoforms Tpk1p and Tpk2p in morphogenesis and growth of Candida albicans. Molecular Microbiology, in press

Stress signaling in *S. pombe*

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Unicellular organisms have evolved strategies for dealing with variable and often-harsh environments. A key aspect of these stress responses is the transcriptional activation of genes encoding defense and repair proteins. In this talk I will summarize recent work describing factors controlling stress-induced gene expression in the fission yeast, *Schizosaccharomyces pombe*.

In *S. pombe* the Sty1 (also known as Spc1, Phh1) MAP kinase pathway is activated by a wide range of adverse stimuli. Accordingly, inactivation of *sty1* results in hypersensitivity to UV and other DNA damaging agents, osmotic stress, oxidative stress, heat stress and heavy metal toxicity. The components of the Sty1 pathway are homologous to kinases in the *Saccharomyces cerevisiae* HOG1 osmosensing MAPK pathway and to the mammalian JNK and p38 stress-activated protein kinase cascades.

Upstream of the Sty1 MAPK module a 'two-component'-like signaling pathway controls activation of Sty1 in response to hydrogen peroxide (Buck *et al.*, 2001) but not other stresses. This pathway is most similar to the 'two-component'-like pathway that lies upstream of the osmosensing HOG1 MAPK cascade in *S. cerevisiae* (Posas *et al.*, 1996). Stress-dependent activation of Sty1 results in increased activity of two transcription factors, Pap1 and Atf1. These are both bZip-containing proteins, which share sequence similarity with the mammalian factors cJun and ATF2, respectively. Analysis of mutant phenotypes has suggested that Pap1 controls the response to oxidative stress, cadmium stress and a range of environmental toxins (Toone *et al.*, 1998). Whereas Atf1 has been shown to play a role in regulating the response to osmotic stress and nutrient deprivation as well as the oxidative stress response (Wilkinson *et al.*, 1996; Nguyen *et al.*, 2000). A third transcription factor, Prr1, is also important for hydrogen peroxide-dependent gene expression. Prr1 is a response regulator-like transcription factor suggesting that two-component signaling may have a role, in addition to Sty1 activation, in regulating oxidative stress.

We have characterized the role of the Sty1 (Spc1) stress-activated MAPK pathway, and the Pap1 and Atf1 transcription factors, in regulating the response to H_2O_2 as well as other stresses in the fission yeast, *S. pombe*. We find that H_2O_2 activates the Sty1 pathway in a dose-dependent manner via at least two sensing mechanisms. At relatively low levels of H_2O_2, the two component-signaling pathway, regulates Sty1 phosphorylation, while, at high levels of H_2O_2, Sty1 activation is controlled predominantly by a two-component independent mechanism. Individual transcription factors were also found to function within a limited range of H_2O_2 concentrations. Pap1 activates target genes primarily in response to low levels of H_2O_2 while Atf1 primarily controls the transcriptional response to high concentrations of H_2O_2. Our results demonstrate that *S. pombe* uses a combination of stress responsive

regulatory proteins to gauge and effect the appropriate transcriptional response to increasing concentrations of H_2O_2 (Quinn *et al.*, 2001).

Until recently our analysis of the transcriptional response to stress has relied on a relatively small group of stress response genes. With the completion of the *S. pombe* sequencing project we now have the opportunity to extend these observations with whole genome expression studies. Using microarrays, we have examined the transcriptional response to different intensities of H_2O_2 stress in wild-type, *sty1*, *pap1* and *atf1* mutant strains. These studies provide us with a comprehensive list of genes induced by different levels of oxidative stress and the factors required for their induction. To determine whether these gene expression profiles are specific to H_2O_2 stress we have also conducted microarray analysis of wild-type and mutant strains of *S. pombe* exposed to cadmium induced stress, osmotic stress, heat stress and MMS treatment. Analysis of these results will provide us with a foundation for toxicogenomic studies and for comparing *S. pombe* stress responses with responses in other organisms.

References:

Buck, V., Quinn, J., Soto Pino, T., Martin, H., Saldanha, J., Makino, K., Morgan, B.A. and Millar, J.B.A. (2001) Peroxide sensors for the fission yeast stress-activated mitogen-activated protein kinasepathway. *Mol. Biol. Cell*, **12**, 407-419.

Nguyen, A.N., Lee, A., Place, W. and Shiozaki, K. (2000) Multistep phosphorelay proteins transmit oxidative stress signals to the fission yeast stress-activated protein kinase. *Mol. Biol. Cell*, **14**, 1169-1181.

Posas, F., Wurgler-Murphy, S.M., Maeda, T. Witten, E.A., Thai, T.C., and Saito, H. (1996) Yeast HOG1 MAP kinase cascade is regulated by a multi-step phospho-relay mechanism in the SLN1-YPD1-SSK1 'two-component' system. *Cell*, **86**, 865-875.

Quinn, J., Findlay, V., Dawson, K., Millar, J.B.A, Jones, N., Morgan, B. A. and Toone, W. M. (2001) Distinct regulatory proteins control the graded transcriptional response to increasing H2O2 levels in the fission yeast, *S. pombe Mol. Biol. Cell* (in press).

Toone, W. M., Kuge, S., Samuels, M., Morgan, B.A., Toda T. and Jones, N. (1998) Regulation of the fission yeast transcription factor Pap1 by oxidative stress: requirement for the nuclear export factor Crm1 (Exportin) and the stress-activated MAP kinase Sty1/Spc1. *Genes and Dev.*, **12**, 1453-1463.

Wilkinson, M.G., Samuels, M., Takeda, T., Toone, W.M., Shieh, J.C., Toda, T., Millar, J.B. and Jones, N. (1996) The Atf1 transcription factor is a target for the Sty1 stress-activated MAP kinase pathway in fission yeast. *Genes and Dev.*, **10**, 2289-2301.

Control of the oxidative stress response in fission yeast

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Oxidative stress and osmotic stress produce different gene expression responses in fission yeast, and yet both stress signals are transmitted via the stress activated protein kinase (SAPK) Spc1. We want to understand how stress responses are tailored to different types of stress. To this end we have carried out a screen for genes that modulate transcriptional responses controlled by Spc1. We have identified one such gene that specifically modulates the gene expression response induced by oxidative stress. We will present our studies of this gene and its protein product.

Session 5: Yeast as a model for other organisms
Chair: Michael N. Hall

Nutrient signalling and loss of stress resistance during the initiation of yeast fermentation

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The initiation of fermentation in the yeast *Saccharomyces cerevisiae* by addition of glucose to cells pregrown on nonfermentable carbon sources or to stationary phase cells triggers a rapid loss of general stress resistance. This is inconvenient for industrial applications of yeast in which actively fermenting cells are subject to specific stress conditions. Typical examples are the preparation of freeze doughs and high-sugar doughs. During the preparation of freeze doughs a minimal perfermentation time of the dough is important to obtain a good texture of the bread. However, the rapid loss of freeze tolerance during this prefermentation causes loss of yeast viability during the freezing process and requires the use of higher amounts of yeast. This often imparts an undesirable 'yeasty' taste to bakery products. The presence of high amounts of sugar in doughs causes osmostress and significantly reduces the fermentation capacity of the yeast.

The initiation of fermentation in yeast is associated with a rapid mobilization of the trehalose content of the cells. Because of the strong correlation between trehalose content and general stress resistance of yeast cells, glucose-induced trehalose mobilization has long been thought to be a major cause of glucose-induced loss of stress resistance. We have shown, however, that deletion of the *NTH1* gene, encoding neutral trehalase, largely abolishes glucose-induced trehalose mobilization without preventing loss of stress resistance (Van Dijck et al. 1995). Before glucose addition, on the other hand, there was a clear correlation between stress resistance and trehalose content. These results indicated that glucose causes the disappearance of other factors that are required for heat shock resistance and that trehalose is unable to support high stress resistance in their absence.

Subsequently, we have isolated mutants deficient in fermentation-induced loss of stress resistance (*'fil'* mutants). These mutants were selected by applying a heat shock to a culture of UV-mutagenised cells shortly after the initiation of fermentation by addition of glucose. The *fil1* mutant contained a specific mutation in adenylate cyclase (E1682K) which apparently reduced the activity to such an extent that glucose-induced loss of stress resistance was significantly prevented but without negative effect on the fermentation or growth rate of the cells (Van Dijck et al. 2000). These results showed that it was possible to enhance stress resistance during active fermentation without reducing the fermentation rate. The identification of the *fil1* mutant confirmed the important role of glucose activation of the cAMP-PKA pathway for the loss of stress resistance during the initiation of fermentation.

The *fil2* mutant contained a nonsense mutation in the *GPR1* gene, which encodes a G-protein coupled receptor (GPCR) upstream in the cAMP-PKA pathway (Kraakman et al. 1999). The identification of the *fil2* mutant revealed for the first time the connection between

the Gpr1 receptor and glucose activation of the cAMP pathway. Both the *fil2* mutant and the *gpr1Δ* mutant are deficient in glucose-induced activation of cAMP synthesis. Apparently, a GPCR system composed of the Gpr1 receptor, Gpa2 Gα protein and Rgs2 RGS protein, mediates activation of cAMP synthesis by glucose and sucrose (Versele et al. 2001). Remarkably, this GPCR system is unable to activate adenylate cyclase in the absence of active glucose phosphorylation (Rolland et al. 2000). Recent work has provided strong evidence that glucose and sucrose directly bind to the Gpr1 receptor as ligands. Site-directed mutagenesis of TMD6 residues into cysteine has resulted in the identification of several Gpr1 mutant alleles which are deficient in glucose signaling but not affected in sucrose signaling. Moreover, addition of the polar sulfhydryl-binding compound MTSEA completely blocked the remaining sucrose signaling in two of these alleles whereas it was without any effect on signaling by the wild type Gpr1 allele. These results indicate that binding of MTSEA to the modified receptor blocks access for sucrose to its binding site. They strongly suggest that glucose and sucrose directly bind to the Gpr1 receptor. Since the apparent Ka for sucrose was much smaller (1-2 mM) than for glucose (50-70 mM) Gpr1 appears to have an important function in sensing low concentrations of sucrose in addition to high concentrations of glucose.

We have also isolated *fil* mutants directly in a commercial bakers' yeast strain. This was done by UV-mutagenesis of the tetraploid/aneuploid strain S47, preparation of small doughs with the mutagenised culture and subjecting the doughs to multiple rounds (up to 200) of freeze/thaw treatments. In this way we isolated strain AT25, which showed a better freeze tolerance than the parent strain S47 and behaved in a very similar way as S47 for most other properties in lab scale experiments, except that it showed a 10% lower initial fermentation capacity (Teunissen et al. 2001). Remarkably, after pilot scale cultivation the fermentation capacity of the strain was 10% higher than that of the parent strain and the yeast maintained a better viability also in frozen doughs. Unexpectedly, further examination showed that AT25 is a diploid strain. Control experiments indicated that UV mutagenesis of S47 resulted in many strains with reduced DNA content. However, since random spore analysis of S47 also yielded several diploid segregants with a comparable freeze tolerance as AT25 (but inferior performance for other properties), it remains unclear whether AT25 is a UV-induced mutant of S47, which has lost part of its DNA, or whether it originates from a spontaneously formed spore of S47. AT25 has recently been converted into a tetraploid strain, TAT25, using the HO system. Evaluation of this strain after pilot scale production is ongoing.

Genome-wide gene expression analysis of several strains derived from the AT25 strain has revealed a consistent correlation between freeze resistance and up- or down-regulation of several genes, including the aquaporin genes *AQY1* and *AQY2*. Of all the genes examined further, only deletion of the aquaporin genes in a laboratory strain reduced freeze tolerance while overexpression of the aquaporin genes significantly enhanced freeze tolerance (Tanghe et al. 2001). Also heterologous expression in yeast of the human aquaporin gene, *hAQP1*, improved freeze resistance. These findings support a role for aquaporin-mediated plasma membrane water transport activity in determination of freeze tolerance in yeast. This appears to be the first clear physiological function identified for microbial aquaporins. We suggest that rapid osmotically driven efflux of water during the freezing process reduces intracellular ice crystal formation and resulting cell damage.

References:

Kraakman L., K. Lemaire, P. Ma, A.W.R.H. Teunissen, M.C.V. Donaton, P. Van Dijck, J. Winderickx, J.H. de Winde and J.M. Thevelein (1999) A *Saccharomyces cerevisiae* G-protein coupled receptor, Gpr1, is

specifically required for glucose activation of the cAMP pathway during the transition to growth on glucose. Molecular Microbiology 32, 1002-1012

Rolland F., J.H. de Winde, K. Lemaire, E. Boles, J.M. Thevelein and J. Winderickx (2000) Glucose-induced cAMP signalling in yeast requires both a G-protein coupled receptor system for extracellular glucose detection and a separable hexose kinase-dependent sensing process. Molecular Microbiology 38, 348-358

Tanghe A., P. Van Dijck, F. Dumortier, A. Teunissen, S. Hohmann and J.M. Thevelein (2001) Improvement of freeze tolerance in industrial baker's yeast by overexpression of aquaporin genes. Submitted for publication.

Teunissen A., F. Dumortier, M.-F. Gorwa, J. Bauer, A. Tanghe, A. Loïez, P. Smet, P. Van Dijck and J.M. Thevelein (2001) A freeze-tolerant diploid derivative of an industrial baker's yeast strain: isolation, characterization and use in frozen doughs. Submitted for publication.

Van Dijck P., D. Colavizza, P. Smet and J.M. Thevelein (1995) Differential importance of trehalose in stress resistance in fermenting and nonfermenting *Saccharomyces cerevisiae* cells. Applied and Environmental Microbiology 61, 109-115

Van Dijck P., P. Ma, M. Versele, M.-F. Gorwa, S. Colombo, K. Lemaire, D. Bossi, A. Loïez and J.M. Thevelein (2000) A baker's yeast mutant (*fil1*) with a specific, partially inactivating mutation in adenylate cyclase maintains a high stress resistance during active fermentation and growth. Journal of Molecular Microbiology and Biotechnology 2, 521-530

Versele M., K. Lemaire and J.M. Thevelein (2001) Sex and sugar in yeast: two distinct GPCR systems. EMBO reports 2, 574-579

Transcript profiling of the adenylate cyclase mutant fil1 reveals novel ORF's essential in stress resistance during fermentation

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Baker's yeast is highly resistant to certain stress conditions. It can be stored dry for at least two years without significant loss of its fermentation power. However, there is an opposite relationship between fermentation activity and stress resistance. When S. cerevisiae is mixed with the sugar-containing ingredients to prepare the dough, it starts to ferment immediately resulting in a rapid loss of its stress tolerance. Subsequent freezing of the dough causes a drastic loss in yeast viability and fermentation power. Our aim is to identify genes that upon overexpression improve stress resistance during initiation of fermentation without compromising the yeast fermentation power. Initially, we screened for mutants in a laboratory strain with greatly improved resistance to stress after the addition of glucose to non-fermenting cells in stationary phase. These mutants were called `fil` for deficient in fermentation-induced loss of stress resistance. Fil1 was identified as a point mutation in the CYR1 gene encoding adenylate cyclase. The mutation in fil1 caused reduced activity of the enzyme leading to a strong reduction of glucose-induced cAMP synthesis and hence a decrease in glucose-induced loss of stress resistance. Interestingly, fil1 showed the same growth - and fermentation rate as the corresponding wild type strain. In order to identify genes correlated with the enhanced stress resistance phenotype of fil1, we performed a differential hybridization experiment using micro-array filters. 47 genes with altered expression in fil1 versus wild type were identified, of which 27 were confirmed by Northern blot analysis. The effect of these genes on stress resistance was verified by deleting them in fil1. Deletions in 10 genes each caused a loss of the fil1 phenotype and hence a faster loss of stress resistance during fermentation similar to the wild type. Interestingly, 6 of these ORF's (SRF: stress resistance during fermentation) encode for proteins without previously assigned function. Overexpression of some of the gene products in a wild type strain and fil1 improves heat stress resistance. Currently we are overexpressing those genes in industrial strains and testing freeze resistance.

Expression in yeast as acid test to identify plant stress genes

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A great part of stress processes occur at the cellular level. Accordingly, we have been using yeast cells to express plant cDNA libraries and functionally identify plant stress genes. A library was constructed from mRNAs isolated from salt-stressed sugar beet plants. The vector was phage lYPGE15, which can be excised in vivo as a shuttle E.coli-S. cerevisiae plasmid by the cre-lox recombinase system. The recipient yeast strain was a double mutant *ena1-4 nha1*, devoid of the two most important sodium efflux systems of yeast (the Ena1 ATPase and the Nha1 antiporter). After transforming 100.000indiivual yeast cells, colonies were pooled and selected for their ability to grow in the presence of 150 mM NaCl, which completely blocks the growth of the mutant yeast strain. From 22 positive clones we characterized 12 independent genes which corresponded to the following families of proteins:

a) RNA binding proteins: 8 different genes
b) casein kinase II (CK2, isolated twice)
c) dihydroorotase (homolog to yeast Ura4)
d) eIF-1A (isolated 3 times)
e) one gene without homologies in data banks

CK2 does not affect ion homeostasis and seems to modify a salt toxicity target. eIF-1A seems to protect ribosomal protein synthesis against salt toxicity. The multiple RNA binding proteins isolated do not seem to alter ion homeostasis, suggesting that RNA metabolism is an important target of salt toxicity. Finally, uracil biosynthesis seems to constitute another target of salt toxicity.

Yeast salinity stress responses - a progression of programs

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Transcript abundance and changes in the transcript profile in response to hyper-salinity of *Saccharomyces cerevisiae* was determined using microarrays that included 6,144 open reading frames. We see our analysis as an extension of similar studies that describe genome-wide expression profiles for salt-stressed yeast (Posas et al., 2000; Rep et al., 2000) and as a practice session for similar studies in plants. Following incubation of logarithmically growing cells in 1M NaCl for different time intervals, normalized changes in transcript abundance, more than 2-fold relative to controls, were classified. Salinity-induced ORFs increased over time: 107 (10'), 243 (30'), and 354 (90') with the number of upregulated, functionally unknown ORFs increasing from 17 to 149. The expression patterns were similar during short time periods of stress, with 67% of the upregulated transcripts after 10' identical to those induced after 30'. In contrast, the expression profile after 90' revealed a set of up-regulated transcripts different from the early patterns (identities of 13% [10' time point] and 22% [30' time point], respectively). Nucleotide and amino acid metabolism exemplified the earliest responses to NaCl stress, followed by ORFs with functions related to intracellular transport, protein synthesis and protein destination. Transcripts related to energy production were upregulated throughout the time course with respiration-associated transcripts strongly induced after 30'. Prevalent at 90' were ORFs specifying canonical stress responses, such as HSPs, including the known salinity stress-induced genes, genes determining detoxification functions, transporters of the major facilitator superfamily, metabolism of energy reserves, nitrogen and sulfur compounds, and lipid, fatty acid and isoprenoid biosynthesis. We have chosen severe stress conditions for most analyses to reveal responses of cells in biochemical pathways necessary for survival. Under our conditions, the most strongly upregulated early transcript, probably appearing within less than one minute, is that for PMP3 (Yale & Bohnert, 2001), a small protein that seems to alter the specificity of the plasma membrane proton ATPase or possibly act by generating pores (Navarre & Goffeau, 2000). This was further probed by utilizing a strain in the analysis that lacks the osmotic adjustment component because *GPD1* and *GPD2* are mutated thus abolishing the pathway leading to glycerol biosynthesis controlled by the HOG1 pathway (Albertyn et al., 1994; Ansell et al., 1997). Requiring much milder stress conditions (0.4M NaCl), the stress response is very much different from that of wild type cells. A most conspicuous response of the Δgpd1/gpd2 cells is that function related to retrotransposon activation, which are not affected in wild type cells, are strongly upregulated under these mild stress conditions.

Three signal transduction pathways in yeast, which control response to changes in osmolarity and the ionic environment, are known by the terms HOG (High Osmolarity Glycerol), calcineurin (CaN) and HAL4. Activation of HOG results in glycerol biosynthesis and partial activation of *ENA1* (sodium ATPase). *CaN* induction also leads to increased *ENA1* but this pathway additionally involves the global activation of ion transport processes.

CaN, together with *HAL4*, regulates Na+-exclusion by post-transcriptional activation of the K+-influx transporters, *TRK1/2*. We have generated gene deletion mutants of key components in each of these pathways to monitor changes in steady-state RNA amounts relative to the isogenic wild type. Genome-wide expression profiles revealed differences between wild type and mutants for signaling components and downstream reactions under high salt conditions, which were categorized as either lower or higher than wild type. Lower levels of RNA induction in mutants confirmed the regulation of known genetic components and implicated additional determinants in signaling. Higher levels relative to wild type suggest compensation and cross talk between different signaling pathways and extend the genetic network beyond the known elements of stress perception and response (Matsumoto et al., 2002).

The dissimilar ecological conditions found at the "Evolution Canyon" in Israel provided an opportunity for the analysis of different wild yeast strains. Evolution Canyon is the name of a valley where the south-facing slope in full sunlight is characterized by hot dry conditions while the lightly forested north-facing slope enjoys a mild Mediterranean climate (Nevo, 2001). We have compared a number of strains from different locations on both slopes and the valley floor with respect to stress responses, elevated levels of peroxide in particular. RNA from cells in the presence of 100 mM H_2O_2, in comparison to the unstressed condition, was used in genome-wide microarray experiments. An analysis with respect to transcript abundance and stress-dependent changes in transcript amount will be presented. The data point towards variations in sensitivity among the different strains with strains collected on the south-facing slope characterized by higher tolerance to peroxide stress.

Yeast up-regulated ORFs, including many functionally unknown transcripts, have counterparts in transcripts that are upregulated in salt-stressed plants (Bohnert et al., 2001; Kawasaki et al., 2001; Öztürk et al., 2002). It will be attempted to distill trend and meaning out of a large number of plant microarray data that are contained in our OSMID (osmotic stress microarray information database; http://www.genomica.agmarley.arizona.edu) and stress databases (http://www.stress-genomics.org). .

References:

Albertyn J, Hohmann S, Thevelein JM, Prior BA. 1994. GPD1, which encodes glycerol-3-phosphate dehydrogenase, is essential for growth under osmotic stress in *Saccharomyces cerevisiae*, and its expression is regulated by the high-osmolarity glycerol response pathway. Mol Cell Biol. 14: 4135-4144.

Ansell R, Granath K, Hohmann S, Thevelein JM, Adler L. 1997. The two isoenzymes for yeast NAD+-dependent glycerol 3-phosphate dehydrogenase encoded by GPD1 and GPD2 have distinct roles in osmoadaptation and redox regulation. EMBO J. 16: 2179-2187.

Bohnert HJ & members of the plant stress consortium. 2001. A genomics approach towards salt stress tolerance. Plant Physiol. Biochem. 39: 295-311.

Kawasaki S, Borchert C, Deyholos M, Wang H, Brazille S, Kawai K, Galbraith DW, Bohnert HJ. 2001. Gene expression profiles during the initial phase of salt stress in rice. Plant Cell 13: 889-906.

Matsumoto T, Yale J., Hasegawa PM, Bressan RA, Bohnert HJ. 2002. Gene expression changes in yeast *Hog1*, *Cnb*, and *Spk1* mutants in response to salinity stress. manuscript in preparation.

Navarre C, Goffeau A. 2000. Membrane hyperpolarization and salt sensitivity induced by deletion of PMP3, a highly conserved small protein of yeast plasma membrane. EMBO J 19: 2515-2524.

Nevo E. 2001. Evolution of genome-phenome diversity under environmental stress. Proc Natl Acad Sci USA 98: 6233-6240.

Öztürk ZN, Talamé V, Michalowski CB, Gozukirmizu N, Tuberosa R, Bohnert HJ 2002. Monitoring large-scale changes in transcript abundance in drought- and salt-stressed barley. Plant Mol. Biol., in press.

Yale J, Bohnert HJ. 2001. Transcript expression in *Saccharomyces cerevisiae* at high salinity. .J Biol. Chem. 276: 15996-16007.

The AMP-activated/SNF1 protein kinases – guardians of the cell's energy status

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The AMP-activated/SNF1-related protein kinases are a family of protein kinases that are highly conserved across the eukaryotic domain, the archetypal examples being the AMP-activated protein kinase in mammals and the SNF1 complex in Saccharomyces cerevisiae [1,2]. They are heterotrimeric complexes comprising a catalytic α subunit associated with regulatory β and γ subunits. In mammals there are multiple isoforms of each subunit encoded by distinct genes (α1, α2, β1, β2, γ1, γ2, γ3), yielding at least 12 possible heterotrimeric combinations [3]. In S. cerevisiae there are three β subunit isoforms (Sip1, Sip2, Gal83) that can associate with the single α (Snf1) and γ (Snf4) subunits to form three alternate SNF1 complexes. In Drosophila melanogaster there are single genes encoding α, β and γ subunits. In all species, the kinase appears to be completely inactive unless it is phosphorylated at an equivalent site within the "activation loop" of the α subunit kinase domain. The identities of the upstream kinase(s) responsible for these phosphorylation events remain a major unsolved problem.

In mammals, the primary signal that activates the AMP-activated protein kinase (AMPK) system is a rise in the AMP:ATP ratio, which activates the downstream kinase via a multi-step mechanism results in an ultrasensitive response [4]. Due to the enzyme adenylate kinase, a rise in the ADP:ATP ratio (signifying falling cellular energy status) is transmitted into an even larger rise in AMP:ATP ratio. Thus, AMPK is activated by cellular stresses that inhibit ATP production (e.g. hypoxia, glucose starvation, metabolic poisons), as well as by stresses that accelerate ATP consumption (e.g. exercise in skeletal muscle). Once activated, the system switches on ATP-producing catabolic pathways (e.g. glucose/fatty acid uptake and oxidation) while switching off ATP-consuming processes (e.g. protein, polysaccharide and lipid synthesis). It achieves this both by direct phosphorylation of target proteins, and also by regulation of transcription factors that modulate their expression.

In S. cerevisiae, a puzzling feature is that the SNF1 complex is not directly activated by AMP in cell-free assays, although in the known situation where the kinase is switched on (starvation for glucose) there is a dramatic increase in the cellular AMP:ATP ratio. Genetic evidence suggests that the SNF1 system may respond directly to glucose availability (rather than indirectly through changes in AMP/ATP) via a mechanism involving the Reg1-Glc7 complex, although exactly how this operates remains unclear. It is also unclear to what extent the glucose-sensing mechanism is conserved in mammals, and the energy charge-sensing mechanism in yeast.

As well as providing an overview of these systems, some recent developments from my own laboratory will be presented. These may include studies on the D. melanogaster system, and studies on the subcellular localization of the mammalian kinase, its effects on progress through the cell cycle, and the effects of mutations in the γ subunits.

References:

[1] Hardie, D.G. and Hawley, S.A. (2001) BioEssays, in press.
[2] Hardie, D.G., Carling, D. and Carlson, M. (1998) Ann. Rev. Biochem. 67, 821-855.
[3] Cheung, P.C.F., Salt, I.P., Davies, S.P., Hardie, D.G. and Carling, D. (2000) Biochem. J. 346, 659-669.
[4] Hardie, D.G., Salt, I.P., Hawley, S.A. and Davies, S.P. (1999) Biochem J 338, 717-722.

POSTERS

Isolation and characterization of constitutively active forms of the MAPK p38/Hog1

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Mitogen activated protein kinases (MAPKs) play pivotal roles in growth, development, differentiation and apoptosis. These kinases are conserved from yeast to mammals. Yeast cells contain several MAPK pathways, controlling crucial cell responses. Although extensively studied, the exact role of a given MAPK in these processes is not fully understood. Constitutively active MAPKs could be an unequivocal strong tool to address this issue, but such molecules are not available. MAPKs are unique enzymes, whose activation requires dual Tyr/Thr phosphorylation, catalyzed by MAPKKs. It is not known how to mimic this type of phosphorylation by mutagenesis. This complexity explains why constitutively active MAPK molecules are not available.

Here we describe the first series of constitutively active variants of the MAPK Hog1. Each of the active molecules contains just a single point mutation. Interestingly, most of the mutations occurred in the L16 domain, which may play a role in dimerization. The active mutants were obtained through a novel genetic screen that could be applied for isolation of constitutively active MAPKs of other families. Each of the mutants is catalytically and biologically active, independent of MAPKK phosphorylation. Combining several mutations on the same molecule was found to be lethal, indicating that such molecules may have an even stronger catalytic activity. Equivalent single mutations, introduced to the human p38a rendered the enzyme constitutively active. Thus, the mutations described here could be readily applied for production of active forms of MAPKs from yeast to human and finally open the way to revealing their precise biological functions.

Characterisation of an N-terminal activation domain of Msn2 which is activated by heat shock in a PKA independent way in *S. cerevisiae*

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The transcription factors Msn2 and Msn4 (Msn2/4) of *S. cerevisiae* are activated by various stresses. Their nuclear localisation is controlled by PKA. Hyperphosphorylation of Msn2/4 is correlated with their activation by stress. In order to analyse the regulation of Msn2, different parts of this protein have been fused to the LexA DNA binding domain. These fusion proteins have been tested for their ability to activate the expression of a *lacZ* reporter gene under the control of the lexA operator in yeast. The effect of heat shock and of the PKA pathway on their transcriptional activity has been studied. Various regions tested behave as transactivators when fused to the LexA DNA binding domain. The Msn2 DNA binding domain is not required for the activation by stresses. We have identified a transcriptional activation domain in the N-terminal part of Msn2 which is activated upon heat shock in a PKA independent way. This domain is not hyper phosphorylated uponheat shock contrarily to the whole lenght Msn2, thus hyper-phosphorylation is not essential for activation by heat shock.

Hyper-phosphorylation in response to heat shock and diauxic transition is only observed with the fusion protein containing the largest part of Msn2 (amino acid 1 to 642) but missing the Msn2 DNA binding domain. Thus these phosphorylations are not localized in the DNA binding domain and should require a large part of the protein to occur.

Yeast mitochondrial HSP60 prevents protein damage under oxidative stress

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Many stress protein, including members of the Hsp60 and Hsp70 families are constitutively expressed and fulfill essential functions as "molecular chaperones" under normal cellular conditions (1).

In previous studies (2) we showed that when a yeast culture was submitted to H2O2 stress several proteins became specifically oxidized, as measured by carbonyl formation. Identification of such proteins revealed the presence of several key enzymes involved in energy metabolism (pyruvate dehydrogenase, a-keto-glutarate dehydrogenase, aconitase, gliceraldehyde 3P dehydrogenase and enolase), an elongation factor (EF-2), fatty acid synthase, and the mitochondrial Hsp60. In a parallel study (3), when an E. coli culture was stressed by H2O2, we identified DnaK (the prokaryotic member of the Hsp70 family) as a major target.

S. cerevisiae Hsp60 is an ATP-dependent mitochondrial chaperone (which, in cooperation with Hsp10) promotes folding and assembly of newly imported proteins, binds heat-denatured mitochondrial proteins and prevents aggregation. The concentration of mitochondrial Hsp60 increases 2 to 3 fold at 42°C.

E. coli DnaK (the prokaryote member of the Hsp70 family), in cooperation with DnaJ stabilizes newly made proteins, stimulates protein export, facilitates degradation of abnormal proteins and control heat-shock responses.

The role of these chaperones protecting cells against stress conditions has been well documented. Nevertheless, could this protection be exerted through preventing protein oxidative damage?

To address this question, a yeast strain with the hsp60 gene under the doxycycline-regulatable tet promoter was constructed. Cultures of cells expressing Hsp60 protein levels ranging from 300-400% to 10-20% of those of the wild-type (DHsp60 is not viable) were treated with menadione (a superoxide generator) or H2O2, and carbonyl groups were detected by western-blot. Cell viability was also measured. Experimental data indicated that increasing the levels of Hsp60 resulted in a decreased protein oxidative damage, and in accordance, cell viability was higher.

In a parallel study, an *E. coli* strain bearing a PIPTGdnaKJ was constructed. Addition of IPTG resulted in a five-fold increase in the DnaK levels. Aerobic cultures (30°C) were treated with H2O2 for 1 hour. Cultures without IPTG presented a 4-fold increase in carbonyl content after the stress, meanwhile in IPTG-induced cells only a 2-fold increase was obtained. Cell viability was also increased in IPTG-treated cultures.

References:

1. Hendrick, J.P., Hartl, F.U. (1993) Annu. Rev. Biochem. 62: 349-384
2. Cabiscol, E., Piulats, E., Echave, P., Herrero, E., Ros, J. (2000) J. Biol. Chem. 275: 27393-27398.
3. Tamarit, J., Cabiscol, E., Ros, J. (1998) J. Biol. Chem. 273: 3027-3032.

Cold shock induces a SAPK-mediated response in *S. pombe*

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The *Schizosaccharomyces pombe* Sty1/Phh1/Sph1 MAP kinase pathway is structurally related to the mammalian stress activated protein kinases (SAPKs) and also activated by a similar range of environmental stresses, including osmotic stress, heat shock, oxidative stress and UV radiation. This suggests that the yeast and mammalian pathways are highly conserved. Cold shock is another type of stress that induces a variety of responses in both prokariotes and eukaryotes that are mainly based in the induction of cold shock proteins (Csp´s). However, little or nothing is known in eukaryotes about the existence of SAPK-mediated response to a thermal downshift. In this work we have dissected the MAP kinase cascade in *S. pombe* cells subjected to a cold shock. Results so far obtained indicate that:
(i) The fission yeast Sty1 MAP kinase is transiently activated by phosphorylation when the cultures are subjected to a thermal downshift from 30ºC to 10-15 ºC. However the kinetics of Sty1 phosphorylation is clearly delayed as compared to other types of stress, like heat shock and osmotic or oxidative stresses. (ii) Wis1 is the MAPK kinase (MEK) responsible for Sty1 activation during cold shock. (iii) Wis1 activation under the above conditions is almost fully dependent on phosphorylation mediated by Wak1 (MEKK). (iv) The bZIP transcription factor Atf1 becomes phosphorylated in a Sty1-dependent way during a cold shock and is responsible for the expression of gpd1+ (glycerol-3-phosphate dehydrogenase) under these conditions. (v) *S. pombe* strains deleted in transcription factors Atf1 or Pcr1 (another bZIP protein that forms heterodimers with Atf1) are unable to grow at low temperatures, whereas disruptants in any of the members of the SAPK pathway (Wis1, Sty1,...) are able to do so, although with slower growth than the wild type strains. All these data strongly suggest that S. pombe responds to low temperatures by inducing the SAPK pathway, although its function is dispensable during yeast grow at these conditions. However, the presence of unphosphorylated Atf1/Pcr1 heterodimers is critical to ensure yeast growth at low temperatures by an as yet undefined mechanism/s.

References:

-Millar, J.B.A., Buck, V., and Wilkinson, M.G. 1995. Genes and Development. 9: 2117-2130.
-Phadatare, S., Alsina, J., and Inouye, M. 1999. Current Opinion in Microbiology. 2: 175.180.
-Shiozaki, K., and Rusell, P. 1995. Nature. 378: 739-743.
-Shiozaki, K., and Russell, P. 1996. Genes and Development. 10: 2276-2288
-Wilkinson, M.G., Samuels, M., Takeda, T., Toda, T., Toone, M.W., Shieh, J-C., Millar, J.B.A., and Jones, N.C. 1996. Genes and Development. 10: 2289-2301.

The GATA transcription factors GLN3 and GAT1 link TOR to salt stress in *Saccharomyces cerevisiae*

José L. Crespo

One of the most recent functions assigned to the TOR signaling pathway in yeast is the coordination of the transcription of genes involved in nutrient utilization. Here we show that transcription of ENA1, a gene encoding a lithium and sodium ion transporter, essential for salt tolerance in yeast, is controlled by the TOR signaling pathway. First, ENA1 expression is strongly induced under TOR-inactivating conditions. Second, the absence of the TOR-controlled GATA transcription factors GLN3 and GAT1 results in reduced basal and salt-induced expression of ENA1. Third, a gln3 gat1 mutant displays a pronounced sensitivity to high concentrations of lithium and sodium. Fourth, TOR1, similar to ENA1, is required for growth under saline stress conditions. In summary, our results suggest that TOR plays a role in the general response to saline stress by regulating the transcription of ENA1 via GLN3 and GAT1.

The TOR-controlled transcription activators GLN3, RTG1 and RTG3 are regulated in response to intracellular levels of glutamine

José L. Crespo, Ted Powers, Brian Fowler, and Michael N. Hall

The essential, rapamycin-sensitive TOR kinases regulate a diverse set of cell growth-related readouts in response to nutrients. Thus, the yeast TOR proteins function as nutrient sensors, in particular as sensors of nitrogen and carbon. However, the specific nitrogenous or carbon metabolites that act upstream of TOR are unknown. We investigated the role of glutamine, a preferred nitrogen source and a key intermediate in yeast nitrogen metabolism, as a possible regulator of TOR. We show that the glutamine synthetase inhibitor L-methionine sulfoximine (MSX) specifically provokes glutamine depletion in yeast cells. MSX-induced glutamine starvation caused nuclear localization and activation of the TOR-inhibited transcription factors GLN3, RTG1, and RTG3, all of which mediate glutamine synthesis. The MSX-induced nuclear localization of GLN3 required the TOR-controlled, type 2A-related phosphatase SIT4. Other TOR-controlled transcription factors, GAT1/NIL1, MSN2, MSN4 and an unknown factor involved in the expression of ribosomal protein genes, were not affected by glutamine starvation. These findings suggest that TOR senses glutamine. Furthermore, as glutamine starvation affects only a subset of TOR-controlled transcription factors, TOR appears to discriminate between different nutrient conditions to elicit a response appropriate to a given condition.

The Sit4 protein phosphatase of *Saccharomyces cerevisiae* plays a role in the regulation of the PKC1-MAP kinase activity

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Maintenance of cellular integrity in *Saccharomyces cerevisiae* is carried out by the activation of the protein kinase C- mediated MAP kinase pathway. However, little is known about the mechanisms that ensure inactivation of this signalling cascade. Here we report a novel role for the protein phosphatase Sit4 in the downregulation of both basal and induced activity of the PKC1-MAPK pathway. This new Sit4 function is not cell cycle-dependent. The Ppz1 and Ppz2 protein phosphatases have a contrary effect to Sit4 in the modulation of Mpk1 activity that is similar to that previously described in cell cycle progression. The regulatory effect on Mpk1 phosphorylation is independent of the Msg5 and Ptp2 protein phosphatase function and requires an intact PKC1-MAPK module. Our data indicate that Sit4 operates upstream of Rho1, downregulating Pkc1 kinase activity and affecting all known biological functions involving Pkc1. Further analyses are being carried out in order to study possible interactions between Sit4 and cell-membrane receptors. Thus, Sit4 influences Mpk1 activity and cell wall integrity, organisation of the actin cytoskeleton, and ribosomal gene transcription.

Transcription factors under the control of the yeast Hog1 MAPK

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Exposure of yeast cells to increases in extracellular osmolarity activates the Hog1 mitogen-activated protein (MAP) kinase. Activation of Hog1 MAPK results in induction of a set of osmoadaptive responses, which allow cells to survive in high osmolarity environments. Cells respond to saline stress by inducing the expression of a very large number of genes, and suggest that stress adaptation requires regulation of many cellular aspects. The transcriptional induction of most genes that are strongly responsive to salt stress was highly or fully dependent on the presence of the MAP kinase Hog1, indicating that the Hog1-mediated signaling pathway plays a key role in global gene regulation under osmotic stress conditions. Some transcription factors under the control of the MAP kinase have been described recently (such as Sko1, Hot1, Msn2 and Msn4) but they cannot account for the complex gene regulation regulated by Hog1. To identify new transcription factors under the control of the MAPK, we designed a genetic screen to isolate clones whose overexpressions were able to induce STL1 gene transcription. Overexpression of the MEF2-like transcription factor, SMP1, induced transcription of the STL1::LacZ gene expression even in the absence of osmotic stress. Smp1 was phosphorylated in response to osmotic stress in a Hog1-dependent manner and furthermore, Smp1 interacted with Hog1 and was phosphorylated in vitro by the MAPK. Taken together our data suggest that Smp1 acts downstream of Hog1 controlling a subset of the responses induced by the MAP kinase upon osmotic stress.

Response of yeast cells under stress conditions taking place during the vinification process

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Yeast production, alcoholic fermentation and, in some cases, biological aging are steps required for winemaking nowadays. During these processes yeast cells are subjected to several stress conditions that have been reviewed by Attfield (1997). Due to the high sugar content in the must, osmotic stress is affecting yeast cells after inoculation into this medium to carry out alcoholic fermentation. Nitrogen starvation stress could take place during the last days of vinification, when nitrogen compounds are exhausted or can not be taken by yeast cells from the growth medium. Ethanol stress is due to the progressive production of this alcohol, that have effects toxics on yeast cells, being the membranes its primary action place. Ethanol and acetaldehyde stresses are also affecting yeast cells during the biological aging of certain wines such sherry wines. This process of biological aging is carried out by yeast strains different to those carrying out the alcoholic fermentation (Esteve-Zarzoso et al., 2001). These yeasts ("flor yeasts") grow on the surface of the wine producing the so called velum. Due to their respiratory metabolism, very high concentrations of acetaldehyde (up to 1000 mg/L, or even more under certain circumstances, Martínez et al., 1998), also a very toxic agent, are produced from ethanol (which is also maintained at concentrations around 15.5% (v/v)). In our laboratory we have analysed the response of yeast cells under these stress conditions. To study the osmotic stress, we have followed the expression of several genes induced by stress (*GPD1*, *HSP12* and *HSP104*), during the first hours of vinifications carried out with synthetic must and we have found a significant induction of the *GPD1* gene but also a repression of *HSP104* and, specially, *HSP12* genes. To understand the response to nitrogen starvation stress we have followed expression of *CAR1*, *YGP1* and *YVH1* genes -for which induction by this condition has been described in the literature- along vinification and we have not identified a clear pattern of expression related to the consumption of nitrogen compounds. However we have identified arginase activity (Middlehoven, 1964) as a good marker for detecting nitrogen defficiencies along vinification. Finally for the analysis of ethanol and acetaldehyde responses we have carried out an analysis of the expression of several *HSP* genes under laboratory conditions in strains isolated during alcoholic fermentation and biological aging. We have found a significant induction of these genes and a correlation between high levels of induction, resistance to these stress conditions and isolation from steps of biological aging.

References:

Attfield, P.V. (1997) Nat. Biotechnol. 15, 1351-1357.
Esteve-Zarzoso, B., Peris-Torán, M.J., García-Maiquez, E., Uruburu, F., Querol, A. (2001) Appl. Environ. Microbiol. 67, 2056-2061.
Martínez, P., Valcárcel., M.J., Pérez, L., Benítez, T. (1998) Am. J. Enol. Vitic. 49, 240-250.
Middlehoven, W.J. (1964) Biochim. Biophys. Acta 93, 650-652.

Identification of MAPK pathway components in the fungal pathogen *Fusarium oxysporum*

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Filamentous fungi are the causal agents of devastating diseases in crop plants around the world, and are becoming increasingly important as infective agents of humans. Cellular signaling between host and pathogen is the first and most critical step in defining the outcome of fungal infection. During the early steps of interaction, fungal pathogens must perceive stimuli from the plant or human host and respond with appropriate morphogenetic and biochemical changes such as directed hyphal growth, adhesion to the host surface, differentation of specialized infection structures and secretion of enzymes and toxins. The molecular mechanisms by which this perception and adaptation occurs remain largely unknown (1).

Our group uses the vascular wilt fungus *Fusarium oxysporum* as a model to address the role of signal transduction in fungal pathogenesis. Recently, we have identified Fmk1, a mitogen-activated protein kinase (MAPK) of *F. oxysporum* orthologous to Fus3 from *Saccharomyces cerevisiae*. Knockout mutants in the Fmk1 gene show normal vegetative growth and conidiation in culture but are non-pathogenic on tomato plants (2). The mutants fail to differentiate penetration hyphae resulting in strongly reduced root attachment. Additional defects include impaired abilities to breach the liquid-air interface and to grow invasively on living plant tissue. The fmk1 mutants also show strongly reduced transcript levels of pl1 encoding the cell wall-degrading enzyme pectate lyase (3). We conclude that Fmk1 controls several key steps in pathogenesis of F. oxysporum and is part of a conserved signaling cascade in fungal pathogens of humans and plants (4).

Our next objective is to identify the upstream and downstream components of the Fmk1 pathway. These include the signal inputs such as host surface molecules and cell receptors as well as the effector genes essential for infection. Both classes of genes are largely unknown in fungal pathogens (4). Therefore, we will use defined mutants in two well-characterized yeast MAPK signalling cascades, the mating and the filamentation pathways (5), as tools to identify *F. oxysporum* Fmk1 pathway components. Appropriately engineered yeast mutants will be transformed with *F. oxysporum* cDNA libraries and screened for functional complementation of signaling. The role of the identified genes in signaling and pathogenesis will be determined by producing *F. oxysporum* knockout mutants. If successful, this approach is expected to identify new pathogenicity determinants that may serve as targets for novel antifungals.

References:

1. Knogge, W. (1996) Plant Cell 8:1711-1722
2. Di Pietro, A. et al. (2001) Mol. Microbiol. 39:1140-1152
3. Huertas-González, M.D. et al. (1999) Curr. Genet. 35:36-40
4. Lengeler, K.B. et al. (2000) Microbiol. Mol. Biol.. Rev. 64:746-785
5. Madhani, H.D. and Fink, G.R. (1998) Trends Genet. 14:151-155

Expression of GUP1 and GUP2, *S. cerevisiae* glycerol active transport genes

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Two highly homologous genes related to a phenotype of salt stress tolerance were identified in S. cerevisiae 1. These were named GUP1 and GUP2 from Glycerol Uptake. In ethanol-grown cells the activity of glycerol proton symport2 could be attributed to the activity of Gup1p1, while on glucose-grown cells no glycerol uptake2 was ever detectable. The double mutant gpd1*gpd2*, as well as the other deletion combinations defective on either or both GPD genes, once cultivated on glucose in the presence of salt, revealed a surprisingly high transport activity. Further deletion of GUP1 reduced this activity to approximately 50%. The remaining uptake has been attributed to the activity of GUP21. The expression of these two genes was determined by RT-PCR in wt and GPD deleted strains. Surprisingly2, results revealed significant levels of expression of both GUP genes in derepressed as well as on glucose-grown cells. Expression of GUP1 was strongly enhanced by GPD deletions, in particular in cells cultivated with salt and small amounts of glycerol1, whileGUP2 strongest expression enhancement was observed in salt-grown cells without glycerol supply. These results suggest that (1) these genes expression is differently regulated and that (2) further regulation steps of glycerol transport activity downstream transcription of GUP genes might exist.

References:
1 Hölst, B. et al. (2000) Mol. Microbiol, 37: 98-107
2 Lages, F., Lucas, C (1997) Bioch.Biophys Acta, 1322: 8-18

Msg5 as a negative regulator of the cell integrity pathway in *S. cerevisiae*

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The mitogen-activated protein kinase (MAPK) signaling pathways are critical for the response of cells to stress conditions. The magnitude and duration of MAPK signaling must be properly regulated, since they are critical determinants of the response. However, in contrast to the activation of MAPKs, the inactivation of these proteins is poorly understood.

In the yeast *Saccharomyces cerevisiae*, the Slt2 MAPK mediated pathway is essential for the maintenance of the cell integrity. It has been shown to be activated in response to different stress conditions such a high temperature, hypotonic shock and the exposure to agents that alter the cell wall (1).

We have shown that disruption of the dual-specificity protein phosphatase MSG5 (2) in wild type cells results in increased phospho-Slt2 levels. In order to gain insight into the mechanisms that regulate the function of Msg5 on the cell integrity pathway, we have studied the interaction of Msg5 and Slt2. Two hybrid experiments have shown that both proteins interact. Furthermore, this interaction is weaker at high temperatures, as revealed by copurification experiments. We have also found that activation of the cell integrity pathway results in the appearance of several Msg5 species of higher electrophoretic mobility. Treatment with phosphatase alkaline caused Msg5 to migrate as a band of low electrophoretic mobility, indicating that Msg5 is phosphorylated following the stress. This phosphorylation event depends on Slt2. A model of co-regulation of Msg5 and Slt2 will be discussed.

References:

1.- Martin H, Rodriguez-Pachon JM, Ruiz C, Nombela C, Molina M.Regulatory mechanisms for modulation of signaling through the cell integrity Slt2-mediated pathway in Saccharomyces cerevisiae. J Biol Chem. 2000 Jan 14;275(2):1511-9.

2.- Doi K, Gartner A, Ammerer G, Errede B, Shinkawa H, Sugimoto K, Matsumoto K MSG5, a novel protein phosphatase promotes adaptation to pheromone response in S. cerevisiae. EMBO J. 1994 Jan 1;13(1):61-70.

Effect of methylmetane sulfonate in the transcription of DNA repair elements in *Saccharomyces cerevisae*

Michán, C., Monje-Casas, F. and Pueyo, C.

We have studied the response to methylmetanesulfonate (MMS) of the mechanisms of DNA repair in the microorganism *Saccharomyces cerevisae*. We have measured by retrotranscription combined with multiplex PCR (RT-MPCR) the mRNA levels of the genes coding for enzymes that are involved in (i) base excision repair (BER) as Apn1, Apn2, Ntg1, Ntg2 and Ogg1, (ii) mismatch repair (MMR) as Msh2 and Msh6, and (iii) catalytic subunits of DNA polymerases participating in this process as Rev3 and Rad30.

The effect of this DNA damaging agent was recorded at different exposure times. We found that most of the genes in study were induced only after 60 minutes exposure although to different extenses.

References:

Pueyo, C., Jurado, J., Prieto-Álamo, M.J., Monje-Casas, F. and López-Barea, J (2001) Transcriptional regulation of thioredoxin and glutaredoxin pathways in response to oxidative stress. Quantification by multiplex RT-PCR. Method. Enzymol., in press

Expression analysis of thioredoxin and glutathione systems in *Saccharomyces cerevisiae*

Monje-Casas F, Michán C. and Pueyo C.

The intracellular redox state of *Saccharomyces cerevisiae* is maintained by glutathione- and thioredoxin-dependent reduction systems. We examined the in vivo expression of up to 16 genes encoding for components of both systems under different oxidative stress conditions, using a methodology based on multiplex RT-PCR, followed by analysis using the Genescan software. In this way, wild type cells were exposed to different concentrations of H2O2 and at different times, and the expression patterns of the genes under study were elaborated. The sensitivity of the multiplex RT-PCR method allowed us to detect the increase in the expression levels of several genes at low concentrations and short times. The results, in agreement with previous studies of these genes, demonstrate the reliability and sensitivity of the method, and bring up new interesting data for understanding the response of *Saccharomyces cerevisiae* to oxidative stress conditions.

Resistance to oxidative stress in *Candida albicans* depends on hog1 and cap1 in an independent fashion

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Candida albicans hog1 mutants are avirulent in a mouse model of infection. Nevertheless, these mutants are derepressed in the hyphal formation, one of the characteristics usually linked to virulence. In order to understand why a hyperfilamentous *C. albicans* strain is avirulent, we analysed other phenotypic traits. We have found that the MAP kinase Hog1 is necessary in *Candida albicans* to survive to enhanced oxidative stress. Mutants lacking this gene are unable to survive to high concentrations of different substances producing oxidative radicals such as hydrogen peroxyde, menadione or KO_2. In addition, Hog1 is phosphorylated in the presence of hydrogen peroxide.

Other *Candida albicans* proteins involved in resistance to oxidative stress have been described previously. One of them, Cap1, is a putative transcription factor homolog to *Saccharomyces cerevisiae* Yap1. In order to evaluate the relationship between both proteins in sensing oxidative stress, we have constructed *cap1/cap1* and *hog1/hog1 cap1/cap1* *C. albicans* mutants (*C. albicans* is a diploid fungus). Testing the sensitivity of both mutants to hydrogen peroxide, our conclusions are that both signaling events are independent being the double mutant more sensitive to this compound than the single knockouts. Single homozygous *cap1* or *hog1* mutants behave also differently in response to other stimuli (menadione, $CdSO_4$) confirming our hypothesis of a different regulation of the response to oxidative stress. In addition, in response to hydrogen peroxide the phosphorylation of Hog1 in a *cap1* mutant was undistinguishable from the one found in a wild type strain.

On the role of the *Saccharomyces cerevisiae* Sit4 phosphatase in salt tolerance

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The *Saccharomyces cerevisiae* Sit4 phosphatase plays an important role in cell cycle regulation because it is required for progression into S phase (1,2). In addition, Sit4p has been involved in the regulation of ion homeostasis and it has been proposed that, when overexpressed, confers lithium tolerance in galactose medium (3).

By using sets of *S. cerevisiae* strains with different genetic backgrounds, we have studied the role of the SIT4 gene product on the tolerance to different alkali cations (K^+, Na^+, Rb^+, and Li^+). Our results indicate that deletion of SIT4 significantly affected growth in the presence of high Li^+ and K^+ concentrations. Cation contents and fluxes have been determined and the results will be presented.

References:

1.- Sutton et al (1991) Mol. Cell. Biol. 11:2133-2148
2.- Sutton et al (1991) Cold Spring Harbor Symp. Quant. Biol.56:75-81
3.- Masuda et al (2000) J. Biol. Chem. 275:30957-30961

Novel regulators of the oxidative stress response in *S. pombe*

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The response to oxidative stress in eukaryotic cells requires a highly coordinated response and an enormous precision in the triggering of different biochemical switches. In general, is considered that the main pathway involved in the response to oxidative stress is the Spc1 pathway.

Spc1 is a MAP kinase highly homologous to p38 in mammalian cells, that is activated in response to several types of stress and in particular after oxidation. However, is known that many pieces of the puzzle are missing. Genetic evidences suggests the presence of several activation steps that have not been biochemically characterized.

In order to have such information, we decided to perform genetics screenings looking for mutant yeasts that showed increased sensitivity to oxidative stress. The identification of the mutated gene would give us a possible regulator of the pathway, and a more clear picture of the activation of this pathway.

We have identified such a positive regulator. Mutants in this new gene showed increased sensitivity to oxidative stress but not to other types of stress, indicating a high specificity. Moreover, we have obtained biochemical and genetic evidences that demonstrate the role of this protein in the Spc1 pathway.

The presence of highly homologous sequences to this gene in human and mice, indicate a highly conserved mechanism, and a possible starting step for future experiments in mammalian cells.

The role of HOG and PKC pathways in yeast cell wall architecture

César Roncero

Yeast cell wall constitutes a dynamic structure that warrants cell survival in a very fluctuating environment. Cell wall architecture is the result of a delicate balance between synthesis and degradation, a process that have been study at the molecular level during the last years (1). Our group has been involved lately in the study of the influence of the HOG and PKC pathways in the construction of yeast cell wall. We observed that both pathways function under non-induction conditions, and that it is in this state where they work antagonically in the regulation of cell wall plasticity (2). We will describe our results showing the epistatic effect of these routes in signalling, but also their effect on the expression of several cell wall related genes.

Although the molecular mechanism that allows the interaction between both routes is not jet known, our recent results indicates that this interaction could be produced at the level of sensors. These results will be discussed in the frame of our knowledge about cell wall architecture.

In addition we will show how the regulation of chitin biosynthesis, as a compensatory mechanism against cell wall damage, is the result of posttranslational events only partially mediated by the PKC signalling transduction pathway.

References:

1- Smits, G.J., van den Ende, H. and Klis, F.M. (2001) Differential regulation of cell wall biogenesis during growth and development in yeast. Microbiology, 147, 781-784.

2- Garcia-Rodriguez, L.J.., Duran, A., Roncero, C.(2000) Calcofluor antifungal action depends on chitin and a functional High-osmolarity glycerol response (HOG) pathway: Evidence for a physiological role of Saccharomyces cerevisiae HOG pathway under noninducing conditions. J. Bacteriol.182: 2428-2437.

Interconnection between the TOR and the SNF1 protein kinase pathways in the regulation of the subcellular localization of MSN2

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In this study we show that Reg1, the regulatory subunit of the Reg1/Glc7 protein phosphatase (PP1) complex, interacts physically with the two yeast members of the 14-3-3 protein family, Bmh1 and Bmh2. By using different fragments of the Reg1 protein we map the interaction domain at the N-terminal part of the protein. Two-hybrid experiments indicate that Glc7 (the catalytic subunit of the phosphatase complex) also interacts with Bmh2. Bmh2 is involved in the regulation of the subcellular localization of Msn2, a transcriptional activator of STRE regulated genes. In reg1D mutants this regulation is absent, being Msn2 constitutively present in the cytosol. This localization defect was specific of carbon starvation stress. This effect is due to an active Snf1 protein kinase that inhibits the nuclear localization of Msn2 upon carbon starvation. Active Snf1 kinase is also able to avoid the effects of rapamycin, a drug that by inhibiting the TOR kinase pathway leads to a nuclear localization of Msn2 in wild type cells. Therefore, active Snf1 and the TOR kinase pathway may affect the same cytosolic step in the regulation of the subcellular localization of Msn2.

Function and regulation of yeast FPS1 under osmotic and metalloid stress

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The controlled movement of solutes and molecules across the plasma membrane is of critical importance for cells to adapt to environmental stress conditions. The Fps1p glycerol channel plays a central role in yeast osmo-adaptation by controlling the intracellular level of the osmolyte glycerol. Fps1p is rapidly gated by osmolarity changes and may be an osmosensor itself. Channel closure ensures glycerol accumulation under hyper-osmotic stress whereas a drop in osmolarity triggers the opening of Fps1p to allow glycerol efflux. Cells lacking Fps1p are sensitive to hypo-osmotic shock while cells expressing an Fps1p-channel that cannot close grow poorly under hyper-osmotic stress. Closing of Fps1p requires a regulatory domain located close to the first transmembrane domain. Alanine-scanning mutagenesis of this domain has identified specific residues that are critical for the closing mechanism.

Interestingly, Fps1p also plays a role in metalloid tolerance. Inactivation of Fps1p improves growth of yeast cells in the presence of the toxic metalloids arsenite and antimonite. Physiological data as well as direct transport assays show that the metalloids enter yeast cells via Fps1p. This entry pathway appears to be regulated since expression of the FPS1 gene is repressed in the presence of the metalloids.

Hence, cells are able to control Fps1p-dependent transport (glycerol efflux / metalloid influx) according to the environmental stress condition they experience: channel regulation is exerted at the protein level during osmotic stress whereas regulation is exerted at the expression level during metalloid stress.

References:

Tamás, M.J., Luyten, K., Sutherland, C., Hernandez, A., Albertyn, J., Valadi, H., Li, H., Prior, B.A., Kilian, S., Ramos, J., Gustafsson, L., Thevelein, J.M. and Hohmann, S. (1999) Fps1p controls the accumulation and release of the compatible solute glycerol in yeast osmoregulation. Mol Microbiol, 31:1087-1104

Wysocki, R., Chéry, C.C., Wawrzycka, D., Van Hulle, M., Cornelis, R., Thevelein, J.M. and Tamás, M.J. (2001) The glycerol channel Fps1p mediates the uptake of arsenite and antimonite in Saccharomyces cerevisiae. Mol Microbiol, 40: 1391-1401

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