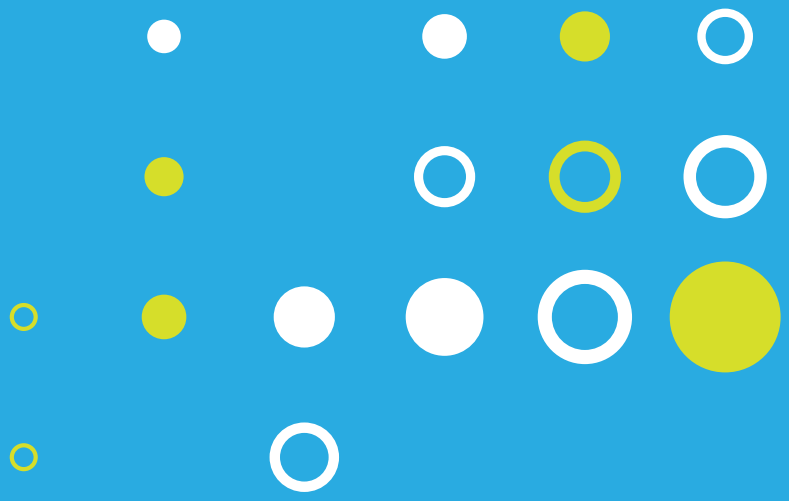
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ST-P13.
PKA-CHROMATIN ASSOCIATION IN STRESS RESPONSIVE TARGET GENES FROM *Saccharomyces cerevisiae*

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Regulation of gene expression by intracellular stimulus-activated protein kinases is essential for cell adaptation to environmental changes. There are three PKA catalytic subunits in *S.cerevisiae*:Tpk1,Tpk2 and Tpk3 and one regulatory subunit:Bcy1. Previously, we had demonstrated that both catalytic and regulatory subunits of PKA were associated to transcribed regions and promoters of target genes in a stress dependent manner. Now, using ChIP-real time assay we analyze the requirement of kinase activity and the role of Bcy1 protein on Tpk chromatin association. Inactive versions of Tpk1 and Tpk2 do not associate to chromatin. Deletion of BCY1 promotes higher Tpk1 association, whereas it abolished Tpk2 association. We then analyse the possible role of Tpk on chromatin remodelling in response to stress conditions. Deletion of Tpk1 or Tpk2 affects the density of H3 on defined gene regions. Additionally, we analyse the kinetics of binding chromatin remodellers in the regions bound by Tpk1 and Tpk2 in response to stress. During stress stimulus there is a transient binding to chromatin of Arp8, Rsc1 and Snf2-chromatin remodellers followed temporarily by Tpk association. We also observe co-occupancy of Tpk2 and DNA-binding transcription factor Rap1p. These results suggest that PKA could participate in gene expression by *in situ* phosphorylation of transcriptional or chromatin regulators.

ST-P14.
IDENTIFICATION OF YEAST PKA SUBUNITS TRANSCRIPTION REGULATORS USING TWO-COLOR CELL ARRAY SCREENING

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Transcriptional regulation is a key mechanism that controls the fate and response of cells to diverse signals. Therefore, the identification of the DNA-binding proteins, which mediate these signals, is a crucial step in elucidating how cell fate is regulated. We applied a functional genomic approach to scrutinize the promoters of the yeast PKA subunits: TPKs and BCY1 to contribute to the understanding of the specificity of cAMP-PKA pathway. We have used a two-color GFP-RFP reporter system: Reporter-Synthetic Genetic Array to systematically assess the effects of gene deletions on the TPK1, TPK2 TPK3 and BCY1 promoter activities. The screen allows measurement of a promoter–GFP reporter gene and a control promoter–RFP reporter gene in an array of yeast deletion mutants and provides quantitative measures of promoter gene activity in each mutant background. We identified potential transcription factors, many of which have no perfect consensus site within the promoters and also that transcription of these genes are repressed by the PKA activity itself, and also in presence of glucose, but not in presence of a nonfermentable carbon source. Our analysis also supports the view that although comparative analysis can provide a useful guide, functional assays are required for accurate identification of TF-binding site interactions in complex promoters

ST-P15.
DIFFERENTIAL ROLES OF PROTEIN KINASE A REGULATORY SUBUNIT ISOFORMS IN *Mucor circinelloides*

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PKA is a tetramer composed of two Regulatory (R) and two Catalytic subunits (C). We have demonstrated the existence of 4 genes for R and 10 genes for C in *M. circinelloides*. In a previous work we had disrupted the gene *pkaR1* (ΔR1) and described its role in growth and morphology. ΔR1 showed a reduction in radial growth, alterations in germination rate, cell volume, germ tube length, and asexual sporulation. In the present work, we have disrupted the genes *pkaR2* (ΔR2), *pkaR3 (ΔR3)*, or *pkaR4* (ΔR4) with the aim of analyzing if each PKAR isoform has a different role in the differentiation process. The germ tube emission in ΔR2 is earlier than in the wild-type strain. PKAR4 appears to be essential for the fungus survival. The homokaryotic ΔR4 strain was not stable; with the successive cultures it became heterokaryotic. ΔR4 strain had an impediment to emit the germ tube under aerobic conditions and after the transition from anaerobic to aerobic conditions; it also has an increased cell volume. ΔR3 shows slight differences from wt. The expression of the pkaR genes was assessed in Δr1, ΔR2 and ΔR3 by semiquantitative RT-PCR. Different mRNA expression was detected in the knockout strains for each pkaR gene. The results indicate that each PKAR isoform has a different role in *M. circinelloides* physiology, thus contributing to the specificity of cAMP-PKA pathway.

ST-P16.
PI3K-DEPENDENT INACTIVATION OF FOXO TRANSCRIPTION FACTORS IN A HIPPOCAMPAL MODEL OF OXIDATIVE STRESS

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We have previously demonstrated the alteration of cell viability, morphology and lipid peroxidation as well as the activation of PI3K/Akt/GSK3ß pathway in a model of iron-induced neurodegeneration. The exposure of a mouse hippocampal neuronal cell line (HT22) to different concentrations of Fe^{2+} (25-200 μM) for 24 h led us to define a mild oxidative injury status (25-50 μM Fe^{2+}). The aim of this work was to investigate the involvement of FoxO transcription factors, known downstream effectors of PI3K, during iron-triggered mild oxidative stress. Under this experimental condition, reactive oxygen species measured by fluorescence microscopy increased with small changes in cell viability, and SOD1 levels decreased whereas catalase levels showed no changes with respect to controls. The localization of phosphorylated and non-phosphorylated FoxO3a was studied in nuclear and cytosolic fractions. Levels of phospho-FoxO3a (the inactive form) increased in the cytosolic fraction of cells treated with iron in a PI3K-dependent manner. Consistent with this, total FoxO3a content decreased in the nuclear fraction. FoxO1 phosphorylation also increased in the cytosolic fraction. Our results show that iron-induced neurotoxicity activates PI3K/Akt/GSK3ß promoting FoxO3a/FoxO1 phosphorylation, their inactivation and their cytosolic localization under mild oxidative injury in hippocampal neurons.