

F005

Characterization of Gpx1, a Mitochondrial Thio-redoxin Peroxidase, in *Schizosaccharomyces Pombe*

Si-Young Lee*, Eun-Soo Kwon, and Jung-Hye Roe
Laboratory of Molecular Microbiology, School of Biological Sciences, and Institute of Microbiology, Seoul National University

We analyzed the gene product and the role of *gpx1*⁺ encoding a putative glutathione peroxidase of 158 aa in *Schizosaccharomyces pombe*. Different from *S cerevisiae* which contains 3 *gpx*-like genes, *S. pombe* contains only one *gpx* gene. The null mutation of *gpx1* caused a retarded growth on glycerol, a non-fermentable carbon source, whereas it grew well as the wild type on glucose. In glycerol media, *Agpx1* exhibited a low respiration rate, suggesting that Gpx1 protein is involved in maintaining optimal respiration ability in *S. pombe*. Western blot analysis revealed that Gpx1 increased as growth phase proceeded and was enriched in organellar fractions. Consistent with protein level, *gpx1*⁺ transcripts increased in the stationary phase. Fractionation of organelles and confocal microscopy demonstrated that Gpx1 is located most likely in mitochondria. Purified Gpx1 exhibited peroxidase activity not with glutathione, but with thioredoxin, suggesting that it is a thioredoxin peroxidase. The essential cysteine residues as well as the redox state of Gpx1 *in vivo* are being investigated.

[Supported by grants from KOSEF]

F006

Cloning of a Laccase Gene from *Ganoderma lucidum*

Ji Kook Park*, Dong Hyun Lee, Sun Hi Lee, Min-A Park, Hye Rin Jeon, Hyoung-Tae Choi, and Kyunghoon Kim
Division of Life Sciences, College of Natural Sciences, Kangwon National University

A laccase gene of the white-rot fungus *Ganoderma lucidum* has been cloned by inverse PCR, sequenced and analyzed. The isolated gene consists of 4361 bp, with the coding region interrupted by nine introns and flanked by an upstream region in which putative CAAT and TATA boxes were identified. The cDNA of laccase gene contains an open reading frame of 1482 bp. The deduced mature laccase protein consisted of 494 amino acids. The laccase gene shares the highest similarity to laccase *lcc3-1* from *Polyporus ciliatus* (about 75%). The deduced amino acid sequence was also similar to those of *Trametes versicolor* laccase (74%) and *Trametes pubescens* laccase 2 (74%). We have also constructed a YEplac based promoter fusion plasmid containing the laccase promoter-*lacZ* fusion in which *lacZ* expression depends on the laccase gene promoter region. Effects of metal ions on the expression of the fused gene in *Saccharomyces cerevisiae* were monitored by ONPG assay.

F007

Proteomic Responses to the Morphological Transition Signals in *Candida albicans*

Kimoon Seong^{1*}, Je-Hyun Baek², Ju Yeon Hwang¹, Jae-Yung Lee³, and Joon Kim¹
¹Laboratory of Biochemistry, School of Life Sciences and Biotechnology, Korea University, ²Functional Proteomics Center, Korea Institute of Science and Technology, ³Department of Biology, Mokpo National University

C. albicans is one of the most commonly encountered human pathogens, causing a wide variety of infections ranging from mucosal infections in generally healthy persons to life-threatening systemic infections in individuals with impaired immunity. This pathogen has the ability to undergo a dimorphic shift from a yeast form to a hyphal growth form in response to the environmental signals such as temperature, pH and nutrients. And this morphological transition is thought to be one of the important virulence factors. Here, to identify the novel factors and to compare the changed proteome in morphogenesis of *C. albicans*, we performed the two-dimensional gel electrophoresis and tandem mass analysis with μ RPLC-ESI-Iontrap and MALDI-TOF/TOF in the induced morphological transition state of SC5314 strain. A total of 1730 protein spots were identified. And 72 of them were increased and 41 of them were decreased in terms of protein levels. These proteins altered in expression levels under hyphae-inducing conditions could supply more information about the mechanism of infection. The relationships between these changed proteins and pathogenesis will be investigated further.

F008

Regulation of Yeast *RPS3* Transcription by Two Major Yeast Transcription Factors, Gcn4p and Rap1p

Yoo Jin Joo^{1*}, Jae Yung Lee², and Joon Kim¹
¹Laboratory of Biochemistry, School of Life Sciences and Biotechnology, Korea University, ²Department of Biology, Mokpo National University

Rps3p is a component of the ribosomal complex, and it also has an extra-ribosomal function, an AP endonuclease activity. There are upstream activating sequences in the promoter of ribosomal protein genes. One of these is the binding site of Rap1p, yeast transcription factor which activates transcriptions of ribosomal protein genes. In the promoter of *RPS3*, five putative UASrpgs were found and serial upstream deletion mutants of promoter region were constructed. By using β -galactosidase reporter system and EMSA, the UASrpg of *RPS3* was identified. Interestingly, *RPS3* promoter region has two putative Gcn4p-responsive elements (UAS_{GCRE}), which are not usually found in the ribosomal protein promoters. This study revealed that Gcn4p and Rap1p bind to the promoter of *RPS3 in vitro*. In addition, the physical interactions between Gcn4p and Rap1p *in vitro* and *in vivo* were confirmed. Gcn4p and Rap1p appear to regulate the gene in a negatively and a positively way, respectively. When an amino acid starvation condition was induced by 3-amino triazole, *ie* 3-AT, or a rapamycin treatment or post-confluent culture condition, the transcriptional level of *RPS3* appears to be controlled by Gcn4p.