F009

Characterization of Monothiol Glutaredoxin4 in *Schizosaccharomyces pombe*

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The fission yeast *Schizosaccharomyces pombe* genome contains five genes encoding glutaredoxins. Two of them encode typical dithiol glutaredoxins acting as glutathione-dependent disulfide oxidoreductase with a cysteine pair at their active sites. We identified and characterized another glutaredoxin subfamily (Grx3, Grx4, and Grx5) which contains single cysteine residue at the putative active site. To investigate their functions, we made null mutants and Grx-overproducing strains. Strains deleted of *grx3* or *grx5* were viable, whereas *grx4*-deficient strain was not. Grx4 consists of an N-terminal thioredoxin-like domain and a C-terminal glutaredoxin domain. Fluorescence microscopy revealed that Grx4 resides mainly in the nucleus. Thioredoxin-like domain is required for such location. Grx4 contains two cysteine residues at positions 35 and 172. Both cysteine substitutions were not able to complement the defect of *grx4* conditional lethal mutant, implying the importance of both cysteines. In an effort to find a clue for why Grx4 is absolutely required for the growth of *S. pombe*, multicopy suppressor genes for Grx4 deficiency were screened and being analyzed.

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F010

SSB2 Suppresses the Defective *gcn4* Phenotype through the Increase of Protein Level

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Gcn4p is known to be a master regulator that stimulates the transcription of many amino acid biosynthetic genes under starvation for any several amino acids in *S. cerevisiae*. In this study, defective *gcn4* mutants that have the point mutations in DNA binding domain were isolated to find the unknown proteins to suppress the mutant phenotype under an amino acid depletion condition. As a results, *SSB2*(Stress-Seventy subfamily B2) gene was found to be a suppressor for those mutants though the multi-copy suppression test. *SSB2* is a member of yeast hsp70 family that probably aids the passage of nascent polypeptide chain through ribosomes. This study showed that Ssb2p activated the expression of Gcn4p target genes by the increase of the defective DNA-binding affinity. It seems that the increase of the defective DNA-binding affinity was resulted from the interaction between Ssb2p and Gcn4p and the improvement of the Gcn4p stability. These findings indicate that Gcn4p might be the first reported substrate of yeast chaperone, Ssb2p. Further characterization of this chaperone in the suppression for the defective gcn4p mutants is to be investigated.

F011

Cloning and Expression of a Chitinase from an Antarctic Bacterium, *Sanguibacter* sp. KCTC10714

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We isolated a chitinase-producing bacterium strain KCTC10714 from sea sand that had been collected around the King Sejong Station, King George Island in Antarctica (62° 13′ S, 58° 47′ W). It was identified as *Sanguibacter* sp., based on the biochemical properties and 16S rRNA gene sequence. KCTC10714 chitinase degraded chitin to produce chitinoligomer and chitobiose. KCTC10714 chitinase showed enzyme activity in broad range of pH 4 - 9, and temperature from 0 to 70℃. At 0℃, it showed 70.9% of relative activity in comparison with 100% at 45℃. We cloned the chitinase gene from KCTC10714 using inverse PCR cloning method. KCTC10714 chitinase gene was 1,888 bp in length including 388 bp of 5'-UTR and 51 bp of 3'-UTR, and it was designated as *chi21702*. The ORF of *chi21702* consisted of 1,449 bp (482 amino acid), and contained ChtBD3 (a chitin/cellulose binding domain) and an active site for chitinase family 18. We also confirmed that the recombinant protein of *chi21702* made approximately 56 kDa after overexpression in *E.coli* system. The characterization of the recombinant Chi21702 is under investigation.

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F012

Identification and Characterization of Mycothiol in *Streptomyces coelicolor* A3(2)

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Mycothiol (MSH) is a low-molecular-weight thiol compound produced by a number of actinomycetes, and has been suggested to serve both anti-oxidative and detoxifying roles. To investigate the metabolism and the role of mycothiol in *Streptomyces coelicolor*, the genes for the biosynthesis (*mshA, B, C* and *D*) and the detoxification (*mca*) were predicted based on sequence homology with the mycobacterial genes, and confirmed experimentally. MSH-deficient mutants were constructed, and their phenotypes to oxidants, alkylating agents, heavy metals and antibiotics were analyzed. MSH plays the important roles in maintaining redox potential, protecting against xenobiotics and regulating the response to the oxidative stress.

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