region by P.C.R., eight internal deletion mutants and four 5′ upstream deletion mutants were constructed and confirmed by DNA sequencing. The promoter region of *RPS3* gene was identified by the transcriptional activity using the β-galactosidase reporter system. Interestingly, this promoter region has few putative cis-acting elements compared with the promoters of other yeast RP genes. To investigate which putative cis-acting element has a critical role in the transcription activation of *RPS3*, we assayed the reporter gene activity under various conditions. We conclude that *RPS3* is transcribed actively when cells were supplied sufficient nutrients.

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Characterization of SSB2 with Respect to the Suppression of Transcription Defective Gcn4p in Yeast

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The transcriptional factor Gcn4p in yeast *Saccharomycescerevisiae* is necessary for the transcriptional induction of many amino acid biosynthetic genes in response to conditions of amino acid starvation. In order to identify amino acids in the DNA binding domain of Gcn4p which are involved in protein-protein interaction, we performed saturation mutagenesis with one or two base changes in the DNA binding domain of Gcn4p using oligonucleotides containing randomized codon bases and statistics of Poisson Distribution. These mutants were assayed for their ability to support transcriptional activation by checking the sensitivity with 3-aminotriazole (3-AT). These mutants were also assayed for *invivo* and *invitro* binding activities by b-gal assay using the reporter plasmid and EMSA respectively. Several of these mutants have a normal DNA b ding activities with decreased transcriptional activation abilities. The residues identified in these mutants appear to play a role in the interaction with other protein(s) for Gcn4p activation. As a result, we found that SSB2 (Stress-Seventy subfamily B2) was the suppressor gene by overexpressing the yeast genomic library in Gcn4p mutant strains. Further characterization of this suppressor gene is to be discussed.

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Selection of a Mutant Defective in Growth at Low pH Media

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Salmonella enterica serovar Typhimurium is a facultative intracellular pathogen, able both to invade and to s vive within eukaryotic cells and to grow in various extracellular environments. As the pH of the Salmonella-containing vacuole inside host cells has been shown to acidify to between pH 4.0-5.0, and as several proteins have been known to be induced to survive in the acidic environments within the hos', low pH might be a physiological stimulus for expression of genes needed in macrophage. In this study, we selected a mutant defective in growth at pH 5.0 by mutagenesis using transposon (Tn10dTc). We cloned the region that Tn10dTc was inserted and found an unidentified ORF, which exists between cspH and envE. By Northern hybridization, it was shown that its expression was induced at pH 5.0-5.5 but not at pH 7.0. In addition, it was expressed only in log phase, not in stationary phase. Consequently, ATR (Acid Tolerance Response) was tested about the

Tn10dTc insertion mutant. Surprisingly, it showed an increased level of ATR in comparision with wild type UK1 in log phase ATR test while no such effect was detected in stationary phase ATR test. To complement this phenomenon of the mutant, several plasmids were constructed and introduced into the mutant. As the result, it was proposed that the region was critical in log phase ATR. Finally, its transcriptional start site was examined by primer extension assay and sequencing analysis. Taken all together, it is suggested that the unidentified ORF is acid-inducible and may be important in survival of Salmonella enterica serovar Typhimurium inside macrophage.

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The Expression, Regulation and Promoter Analysis of cspH, One of the Cold Shock Genes in *Salmonella typhimurium*

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cspH is one of the genes encoding cold shock proteins (CSPs) in *Salmonella typhimurium*. Previously, we showed that its promoter was active not only upon cold shock condition (approximately 15℃) but also at 37℃ and proved that its 5′-untranslated region (UTR) was unusually short unlike the long 5' -UTRs in the other cold-shock inducible genes. In this study, we showed that mRNA of cspH was more stable than that of other csp genes at 37℃ using analysis of mRNA stability. It was shown that the 14 base downstream box (DB) locating 12 base downstream of the initiation codon of cspH mRNA and complementary to a region near the decoding region of 16S rRNA was essential for the mRNA translation during the growth acclimation phase immediately after cold shock. The cspH-lacZ fusion plasmid revealed that a minimal promoter sequence consisting of 55 bp was sufficient to generate its growth phase-dependent expression and cold-shock induction pattern. Furthermore, we found that a putative Fis binding site was present upstream cspH promoter. Using the fis mutant strain contaning wild type cspH-lacZ translational fusion and the wild type strain containing Fis site deleted cspH-lacZ translational fusion plasmid, we revealed that Fis regulated the expression of cspH at 37℃.

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The Regulation of *rfaYZ* against Oxidative Stress in *Escherichia coli*

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Promoters inducible by paraquat, a superoxide-generating agent, were isolated from *Escherichia coli*, using a promoter-probing plasmid pRS415 with promoterless *lacZ* gene. Twenty two promoters induced by paraquat, were selected and further characterized. One of SoxRS-dependent promoters, *rfaY*p, was characterized. The *rfaY* gene is found in the middle of LPS core biosynthetic gene cluster in *E. coli*. *rfaY*p-*lacZ* fusion was induced 10 fold by paraquat and other superoxide generators (menadione, plumbagin, and lawsone) in single copy state, while no induction was observed by H_2O_2, et nol, and heat shock. Induction of *rfaY* disappeared by introducing a *soxRS* mutation into the fusion strain, indicating that *rfaY* is a member of the *soxRS* regulon. The transcriptional start site was determined by primer extension analysis. The -10 and -35 boxes of *rfaY*p were predicted. The Northern