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Several putative genes, *orf3, orf4, orf5, orfA, orfX,* and *orfY* in the downstream of *cutBCA*, the three structural genes for carbon monoxide dehydrogenase (CO-DH) responsible for oxidation of CO in *Mycobacterium* sp. strain JC1, were cloned and characterized. The deduced amino acid sequences of *orf3, orf4, and orf5* showed high homology with those of genes assumed to be involved in the utilization of CO in *Oligotropha carboxidovorans, Pseudomonas thermocarboxydovorans,* and *Bradyrhizobium japonicum* and the deduced products of hypothetical genes possibly related to the utilization of CO in *Mycobacterium tuberculosis*. The *orf4* gene was specifically transcribed under CO-autotrophic growth condition. The *orfA, orfX, and orfY* were located 1,620 bp downstream of *orf5*. Deduced amino acid sequence of *orfA* showed high sequence homology with acyl-CoA dehydrogenases of*Streptomyces coelicolor* A3(2) and *Deinococcus radiodurans,* and probable acyl-CoA dehydrogenase of *M. tuberculosis*. The *orfX* and the N-terminal region of *orfY* showed high homology in amino acid sequence with the probable 3-oxoacyl-(acyl-carrier protein) reductase and the acyl-CoA dehydrogenase of *M. tuberculosis*, respectively.

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Binding of a Putative Regulatory Protein (CutR) to *cutB-cutR* Intergenic Region in *Mycobacterium* Sp. Strain JC1

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Carbon monoxide dehydrogenase (CO-DH) is an enzyme responsible for the oxidation of CO to carbon dioxide in carboxydobacteria. Recently, several genes including CO-DH structural genes *cutBCA*, a putative regulatory gene *cutR*, and several accessory genes which may be involved in the utilization of CO were cloned from *Mycobacterium* sp. strain JC1. In this work, we tried to demonstrate that CutR binds to the upstream region of *cutBCA*. It was found that transcription of *cutBCA* started at nucleotide T located 78 bp upstream of translational start codon of *cutB*. The transcription of putative regulatory gene *cutR* seperated by 314 bp from the divergently oriented *cutBCA* began at nucleotide T which is located in the translational start codon of *cutR*. CutR was purified after overexpression in *Esherichia coli*. The molecular size of the purified CutR was estimated to be 36kd based on the denaturing polyacrylamide gel electrophoresis, which coincides with the mass (34,029) calculated from deduced amino acid sequence of CutR. Gel mobility shift assay indicated that the CutR bound specifically to *cutB-cutR* intergenic region.

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The Transcription Regulation of a Yeast Ribosomal Small Subunit Protein, *RPS3*

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Yeast RPS3 protein is a component of the ribosomal subunit. It is an essential gene in *Saccharomyces cerevisiae*. The yeast RPS3 protein has 66% homology with human rpS3 protein. The human rpS3 has a DNA repair endonuclease activity and is a component of the ribosomal small subunit. We cloned 1.6kb sequence of yeast *RPS3* upstream promoter

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