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We have been isolated several genomic DNA clones which complemented MMS-sensitivity of *uvsH* mutants in *Aspergillus nidulans*. Physical mapping of those clones revealed two-kinds of genomic DNAs. One contained the *uvsH* gene encoding a yeast RAD18 homolog [Mol. Gen. Genet. (1995) 248; 174-181]. The other genomic clone which complemented MMS-sensitivity in part but failed to complement UV-sensitivity of *uvsH* mutant was further analyzed. In this genomic clone, the *uvsH*-partial complementing activity was localized within the 7 Kb *BamH*I DNA fragment and mapped on chromosome IV. Determination of the nucleotide sequence of the clone showed a putative ORF of 3357 bp encoding 1119 amino acids with similarity to Timeless (TIM) protein of mouse and human. About 4 Kb size of transcript was detected in northern analysis. Furthermore, the transcripts were abundantly induced in MMS-treated cells. A putative ORF failed to complement MMS-sensitivities of other mutagen sensitive mutants, *uvsC*, *uvsD* and *uvsJ*, indicating a specific complementation activity on the defects of *uvsH* mutants. Null mutation of the putative TIM homolog was generated by the targeted gene replacement and confirmed by Southern analysis and PCR. The null mutants exhibited slight sensitivity to MMS.

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Isolation and Characterization of a *MSH2* Homolog, Involving in Mismatch DNA Repair in *Aspergillus nidulans*

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Mismatched bases in *E. coli* are repaired by MutHLS system. MutS proteins are required for recognition of mismatched bases. Several MutS homologs have been identified in yeast and human cells. At least three of these eukaryotic MutS homologs are involved in the recognition/binding of mispaired nucleotides. MSH2 plays a key role in mispair recognition whereas MSH3 and MSH6 appear to modify the specificity of this recognition. Alterations of the human mismatch repair genes have been linked to hereditary non-polyposis colon cancer (HNPCC) as well as to sporadic cancers that exhibit microsatellite instability. In this study, we isolated an *E. coli MutS* and *Msh2* homolog in *Aspergillus nidulans* using the PCR based sib-selection method with degenerated primers from the chromosome specific genomic DNA library. Within a positive clone, a 6 Kb *Sal*I DNA fragment was subcloned and subject to DNA sequencing. This subclone contained an ORF of 2,886 bp, interrupted by one intron of 56 bp confirmed by sequencing of RT-PCR products, encoding a polypeptide of 962 amino acids. The expected polypeptide showed 73%, 45%, and 43% amino acid sequence similarity to MSH-2 of *N. crassa*, MSH2 of yeast, and hMSH2 of human, respectively. The gene was named *MshT* and localized on chromosome III. The transcript size was about 4 Kb in northern analysis and the amount of transcript was induced with MMS treatment. [Supported by KOSEF]

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Cloning and Characterization of Several Putative Genes Possibly Involved in the Utilization of Carbon Monoxide in *Mycobacterium* Sp. Strain JC1 DSM 3803

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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