

### ***Enterbacter aerogenes* 의 C-P Lyase (Phn) Operon Promoter의 기능규명**

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*Enterbacter aerogenes*는 C-P direct compound를 이용하는 기작으로 phosphonate pathway와 C-P lyase pathway를 가지고 있다. 그중 C-P lyase pathway에 관여하는 *phn* operon은 10개의 gene (*phn*FGHIJKLMNP)으로 구성되어 있고 regulatory gene 인 *phnF* 의 upstream쪽에서 *pho* box와 -10 region 그리고 RBS가 있음을 염기서열분석으로 확인하였으나 그 조절기작은 불분명하다. *phn* Operon promoter region의 기능을 확인하기 위하여 PCR을 통해서 cloning을 하였고 CAT assay를 시행한 결과, *Enterbacter aerogenes*에서도 *phn* operon의 transcriptional regulation은 PhoB protein에 의해 조절됨을 알 수 있었다.

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### **Mutagenic DNA Repair Pathways in *Aspergillus nidulans*: Effects of *uvsJ*, a *rad6* Homolog, on Survival, Cell Growth and Mutagenesis**

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RAD6 protein is indispensable to generate mutations in yeast. However, the function of RAD6 is mostly unknown except its ubiquitin conjugating (UBC) activity. In

*Aspergillus nidulans*, lack of mutagen-induced mutations has been observed in mutants of two different epistasis groups, *UvsI* and *UvsC*. To investigate whether the RAD6-dependent mutation pathway is also operated in *Aspergillus nidulans*, we have been cloned and characterized a *Rad6* homolog (*radB*) to find that *radB* is an allele of *uvsJ* previously assigned in *UvsF* group. In this study, null mutation of *uvsJ* was constructed by targeted gene replacement and the UBC enzymatic active site mutation, C88A was also generated to examine their effects on mutagenesis. Disruption of *uvsJ* caused growth retardation on an agar plate indicating its requirement on normal growth. Such a phenotype did not exhibited in *uvsJ1* mutant carrying a single point mutation at 58th amino acid histidine. We also found that *uvsJ1* was a temperature sensitive mutant showing the same level of mutagen-sensitivity to wild type at the permissive temperature 25°C but demonstrating high sensitivity at 37°C similar to *uvsJ* null mutants. In contrast to yeast *rad6* mutants, *uvsJ* null as well as *uvsJ1* mutants exhibited increased UV-induced mutation frequencies in a system detecting selenate resistant forward mutations which selects mainly the defects in the sulphate permease (*sB*) gene. Forced over-expression of *UVSJ*-[C88A] protein in wild type resulted in the change of colony morphology, indicating dominant-negative effects of the mutant protein on cell growth. [This work was supported by KOSEF (98-0501-005-1)]

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### **A Putative Timeless (TIM) Homolog of *Aspergillus nidulans* Partially Complements the MMS-sensitivity of *uvsH* Mutants**

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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 *Bam*HI 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