hag expression. All these results suggest that unphosphorylated ComA are required to optimum *hag* expression. This implys that *comA* acts as a molecular switch that controls both motility and competence during the cellular differentiation process. A possible model that emphasizes the role of *comA* in the network control mechanism was proposed.

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Construction of Hepatitis B Virus Vector for Liver Gene Therapy

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Hepatitis B Viruses (HBV) specifically target the liver, where they ficiently infect quiescent hepatocytes. Thus, human hepatitis B virus has potential to be converted into vectors for liver-directed gene transfer. We constructed the HBV vectors for transferring foreign gene to liver by trans complementation of recombinant HBV replaced HBV polymerase with green fluorescence protein (GFP) and HBV polymersae in human HepG2 cell. In this report, we investigated the in vivo characteristics of this vector system with respect to its potential for gene transfer to liver cell. The constructed HBV vectors that formed core particles were identified by western blotting. Encapsidated recombinant HBV pregenomic RNA at core particle level and virus particle level was also confirmed by southern blotting and endogenous polymerase assay. These HBV vectors will be beneficial for liver gene therapy and will be useful tools to study the unknown virus entry-process of human HBV into liver cell.

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Fidelity of DNA Synthesis by Hepatitis B Viral Polymerase Expressed in Insect Cells

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The fidelity of DNA synthesis plays a major role of generating spontaneous mutation. HBV polymerase shows similarities to HIV-RT in that they have sequence homology in polymerase and have reverse transcription step during replication. Like HIV, HBV has high mutation rates in generating mutant viruses upon which selection can act to produce drug resistant HBV variants. Thus, it is critical to evaluate the contribution of the HBV polymerase to mutations. FLAG-tagged wild type (FLAG/pol) and mutant (FLAG/D551A) HBV polymerases have been expressed in insect cells and purified by using immunoaffinity column chromatography. The purified FLAG/pol and FLAG/D551A proteins migrated 90 kD on SDS-PAGE and the position was confirmed by using immunoblot analysis. The purified FLAG/pol showed polymerization activity, but the mutant FLAG/D551A did not, suggesting that the activity was derived from FLAG/pol. No 3′→5′ exonuclease proofreading activity was detected in FLAG/pol, like other retroviral reverse transcriptases. Efficiencies of misinsertion of FLAG/pol and HIV-1 RT were compared. FLAG/pol incorporated purine:pyrimidine (A:C and G:T) or pyrimidine:purine (T:G and C:A) mispairs with similar efficiencies (1.6×10^{-5} - 8.3×10^{-4}) as HIV-1 RT (3.1×10^{-4} - 7.5×10^{-4}), whereas it did not incorporate pyrimidine:pyrimidine (T:T, T:C, C:C and C:T) or purine:purine (A:A, A:G, G:A and G:G) mispairs (2.9×10^{-7} - 7.0×10^{-7} and $1.3 \times$

10^{-8} - 6.6 x 10^{-8}, respectively) efficiently, compared with HIV-1 RT (8.4 x 10^{-5} - 8.0 x 10^{-4} and 4.3 x 10^{-5} - 7.5 x 10^{-4}, respectively). The higher efficiency of misinsertion by HBV polymerase at purine:pyrimidine and pyrimidine:purine mispairs was achieved by the lower Km for the dNTP being misinserted. The data suggest that HBV polymerase is error-prone depending on the template, and HBV genetic variability may be related to the ability of HBV polymerase to form purine:pyrimidine or pyrimidine:purine mismatches during DNA replication

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Role of PhoU, a Negative Regulator of Pho-regulon, in Polyamine-dependent Transcriptional Expression of *paiAB* Operon of *E. coli*: *phoU*$^+$ is Required for Transcriptional Expression of *paiAB*

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In an attempt to elucidate the role of PA in the PA-dependent transcriptional regulation of *paiAB* locating 29.3 min. of *E. coli* chromosome, we have isolated a mutant (tentatively named *parE*) defective in putrescine-dependent expression of *paiA::lacZ*. The *parE* was mapped both genetically and physically at 84.1-84.2 min (3,915 kb - 3,921 kb) in *E. coli* chromosome. The 5.92 kb *Hin*dIII/*Pst*I fragment of the genomic DNA bearing whole *pstSCAB-phoU* operon complemented the *parE*. The expression of the PhoU protein from P*lac* complemented the *parE*. The *parE* mutant showed constitutive expression of *phoA* encoding bacterial alkaline phosphatase. Based upon the results, it was concluded that the negative regulator gene of the phosphate regulon, *phoU*, is identical to *parE*, and is required for the PA-dependent transcriptional expression of *paiA*. Sequence analysis of the *paiA* promoter upstream region revealed presence of two well-conserved PhoB-box centered at -76 bp and -57 bp, respectively. These results demonstrate that PA plays an important role in the phosphate-mediated global transcriptional regulation of gene expression.

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Regulation of Polyamine-dependent Transcription of *paiAB* Operon of *E. coli*: ArcA is Required for Transcriptional Expression of *paiAB*

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In an effort to uncover physiological role of polyamine (PA), our group has recently identified a novel operon in *Escherichi coli*, *paiAB* mapped at 29.3 mim., whose expression is totally dependent on PA with an extent of induction as high as 105-fold. The PA-dependent expressions of *paiAB* under aerobic conditions are about 105-fold higher than under anaerobic conditions. A mutation in the regulator gene (arcA) of the two component Arc-system, controlling the transcriptional expression of a group of genes involved in aerobic respiratory metabolism, was found to enhance the PA-dependent *paiAB* expression about 50% compared to an isogenic *arcA*$^+$. Sequence analysis of *paiAB* promoter upstream region revealed the presence of one perfect ArcA$^-$ binding site overlapping -35 bp region. Electrophoretic mobility shift analysis, using purified ArcA protein and *paiAB* promoter DNA, showed direct binding of ArcA.