cloned fragment revealed 3 open reading frames(ORFs); *nahAb*(324bp), *nahAc*(1350bp), and *nahAd*(585bp). Putative amino acid sequences of *nahAb*, *nahAc*, and *nahAd* gene from *P. fluorescens* SMEII show high homology to those from other *Pseudomonas* strains.

F314

Molecular Cloning of the *nahAa* Gene Encoding Feredoxin Reductase from *Pseudomonas fluorescens* SMEII

Su-Hee Jung*, Na-Ri Lee and Kyung-Hee Min
Dept.of Biology, Sookmyung Women's University, Seoul 140-742

We obtained 4.3kb PCR product from the genomic DNA of *Pseudomonas fluorescens* SMEII, which utilizes naphthalene. This DNA fragment, which carried the *nahAa*, *nahAb*, *nahAc*, and *nahAd* gene for upper naphthalene catabolism, was inserted into pT7Blue(R) vector. This recombinant DNA was subcloned by restriction enzyme *KpnI* to generate 1.8kb DNA fragment (pNA1), which was inserted into pUC19. Restriction endonuclease mapping of 1.8kb insert DNA of the pNA1 was carried out with *EcoRI*, *SphI*, *ApaI*, *AvaI*, and *PstI*. By means of bidirectional subcloning and dideoxynucleotide chain termination, we determined the nucleotide sequence of the *nahAa* gene. The results of sequence analysis, Southern hybridization, and SDS-PAGE showed that the recombinant plasmid pNA1 should be contain the *nahAa* gene.

F315

Control of Motility Development by Early Competence Genes in *Bacillus subtilis*

Bae-Kwang Kang*[1], Tae-Sook Kang[2], Jin-Cheol Yoo[3] and Oh-Hyoung Lee[1]
Dept. of Biology, Mokpo National University, Muangun 534-729[1]; Dept. of Medical Technology, Mokpo Science College, Mokpo 530-730[2]; Dept. of Pharmacy, Chosun University, Kwangju 501-759[3]

Many researchers have studied about the network system that controls spore formation and competence development but the field that controls motility development has received few attentions in connection with these two notable phenomena in *B. subtilis*. So we investigated the effect of mutations of competence-controlling genes on the motility development. Either deletion or over-expression of the *comS* whose gene product antagonizes MecA and thereby enhances competence development didn't affect the expression of *hag-lacZ*, a gene used to monitor the motility development, while the mutation of *codY* which is a known repressor of *srf* promoter that codes *comS* caused a slight increase in *hag-lacZ* expression. These controversing data implicated that the hag expression was not significantly influenced by the amount of ComS itself but rather by some events that were related to the *srf* promoter. Indeed the deletion of early competence genes, the *comQ-X-P-A*, which are known to promote *comS* expression, lowered the level of *hag* expression significantly. The *comA* mutation seemed to be responsible to this defect because its mutation alone lowered the *hag* expression to the same level that was observed when all 4 genes were deleted. On the contrary however, the disruption of *comP*, the sensor histidine kinase of two-component signal transduction systems and phosphorylates its response regulator ComA to ComA-P, resulted in the remarkable increasement of *hag* expression. Likewise, both *phrC* and *spo0K* mutations which are supposed to prevent ComA phosphorylation increased *hag* expression, while the *rapC* mutation which is believed to facilitate ComA phosphorylation lowered the

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.

F316

Construction of Hepatitis B Virus Vector for Liver Gene Therapy

Suho Shin, Dongho Woo, Dongheon Lee, Jinkyoung Rho and Guhung Jung*
School of biological Sciences, Seoul National University, Seoul 151-742

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.

F317

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

Younhee Kim*[1], Hee-yun Lee[2], Seungoe Lim[2], Dongheon Lee[2] and Guhung Jung[2]
Department of Oriental Medicine, Semyung University, Checheon 390-711*[1]; School of Biological Sciences, Seoul National University, Seoul 151-742[2]

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 x