개발에 한 단계 더 나아가려 한다.

E327

Molecular Cloning of a Nucleoside Diphosphate Kinase (NDPK) Gene in *Aspergillus nidulans* and Biochemical Characteristics of *Aspergillus* NDPK Protein

Nak-Jung Kwon*, Hye-Jin Kang*[1], Nam-Shik Lee, Sun Young Kim[1], Eunhee Kim, Kong-Joo Lee[1] and Suhn-Kee Chae
Research Center for Biomedicinal Resources and Division of Life Science, Paichai University, Taejon 302-735; Center for Cell Signaling and College of Pharmacy, Ewha Womans University, Seoul 120-750[1]

NDPK catalyzes the transfer of the terminal phosphate group of a nucleoside triphosphate to a nucleoside diphosphate. Besides to NDPK activity, several other functions including suppression of metastasis in human, phytochrome responses in plants, and regulation of differentiation have been reported. Human NDPK was first isolated as a tumor metastasis suppressor, Nm23-H1. Six isotypes of Nm23 were known to date. Among them, Nm23-H1 and Nm23-H2, also known as the c-myc transcription factor、PuF, coexist as hetero-hexamer. Autophosphorylation and serine/threonine specific protein phosphotransferase activity were also reported for Nm23. However, relevant functions of Nm23 on the various cellular processes are hardly known. To investigate a possibility of using *Aspergillus nidulans* as a microbial model system for Nm23, a Nm23 homolog gene (*ankA*) has been isolated and various biochemical properties of recombinant as well as native NDPK purified using ATP-Sepharose affinity chromatography were examined. *Aspergillus* NDPK of 154 amino acids with 65% sequence similarity to human Nm23 was coded from an ORF of 462 bp, interrupted by four introns located on chromosome II. The 1.2 Kb transcript was detected in northern analysis. No evidence on the existence of other isotypes was obtained from non-stringent Southern and western analyses. *Aspergillus* NDPK was existed as a homo-tetramer (78 KD) judged from gel filtration chromatography. NDPK and autophosphorylation activities were demonstrated and both enzymatic activities were more thermostable than human NDPK-A. [Supported by a grant from KRF (#1998-001-F00771)].

E328

Growth of Several Mycobacterial Species on Carbon Monoxide and Methanol

Eun-Ha Hwang*, Sae-Woong Park, Jeong-Ae Kim, Hyuk Park, Dong-Oh Park and Young-Min Kim
Department of Biology, Yonsei University, Shinchon-dong 134, Seodaemun-gu, Seoul 120-749

Mycobacterium sp. strain JC1 DSM 3803 has been known to grow on carbon monoxide (CO) and methanol as the sole source of carbon and energy. We found in this experiment that several other mycobacteria tested such as *M. tuberculosis*, *M. neoaurum*, *M. parafortuitum*, *M. gastri*, *M. peregrinum*, *M. phlei*, *M. smegmatis* and *M. vaccae* were also able to grow on carbon monoxide as sole carbon and energy sources. The bacteria, but *M. tuberculosis*, also utilized methanol as the sole carbon and energy sources. CO-DH assay, CO-DH staining by activity, and Western blot analysis using antibody against *Mycobacterium* sp. strain JC1 CO-DH revealed that CO-DH is present in cell-free extracts prepared from all the mycobacteria grown in 7H9 medium supplemented with CO. Ribulose

bisphosphate carboxylase/oxygenase (RubisCO) activity was also detected in cell-free extracts prepared from all cells, except *M. tuberculosis*, grown on CO. The RubisCOs in cell-free extracts of *M. smegmatis* and *M. neoaurum*, however, did not cross react with antibody raised against *Mycobacterium* sp. strain JC1 RubisCO when it were subject to Western blot analysis. It was found that the mycobacterial species grown on methanol did not exhibit activities of methanol dehydrogenase, hydroxypyruvate reductase, and hexulose phosphate synthase, except that *M. gastri* showed hexulose phosphate synthase and NDMA-dependent methanol dehydrogenase activities.

E329

Characterization of Recombinant Gal β 1,4GlcNAc α 2,6-sialyltransferas e Expressed in Insect Cells

Hyung Gu Kim* and Jai Myung Yang
Department of Life Science, Sogang University, Seoul 121-742

Gal β 1,4GlcNAc α 2,6-sialyltransferase (ST6Gal I) catalyses the reation responsible for the attachment of sialic acid to N-linked glycoproteins. A truncated form of ST6Gal I, lacking 71 amino acids from N-terminus was produced in insect cells by using baculovirus expression system. For secretion out of the cell and easy purification, signal peptide of a mouse-derived IgM (20 amino acids) and part of protein A was inserted in front of ST6Gal I gene in frame. Immunoblot analysis done with rabbit antisera showed that the recombinant ST6Gal I was secreted into the culture media indicating that mammalian IgM signal peptide is effective for secretion of recombinant protein into the media in insect cells. The recombinant ST6Gal I was purified by immunoaffinity column and its biochemical characteristic was analyzed. The recombinant ST6Gal I retains a biological activity that catalyzes the transfer of sialic acid from CMP-NeuAc to the carbohydrate groups of asialofetuin. These results suggest that massive amount of biologically active form of sialyltransferase could be produced from baculovirus expression system.

E330

Characterization of Recombinant Gal β 1,3GlcNAc α 2,3-sialyltransferas e Expressed in Insect Cells

Sung Mo Yang*, Hyung Gu Kim and Jai Myung Yang
Department of Life Science, Sogang University, Seoul 121-742

Gal β 1,3GlcNAc α 2,3-sialyltransferase (ST3Gal III) catalyses the reation responsible for the attachment of sialic acid to N-linked glycoproteins. A truncated form of ST3Gal III, lacking 28 amino acids from N-terminus was produced in insect cells by using baculovirus expression system. For secretion out of the cell and easy purification, signal peptide of a mouse-derived IgM (20 amino acids) and part of protein A was inserted in front of ST3Gal III gene in frame. Immunoblot analysis done with rabbit antisera showed that the recombinant ST3Gal III was secreted into the culture media indicating that mammalian IgM signal peptide is effective for secretion of recombinant protein into the media in insect cells. The recombinant ST3Gal III was purified by immunoaffinity column and its biochemical characteristic was analyzed. The recombinant ST3Gal III retains a biological activity that catalyzes the transfer of sialic acid from CMP-NeuAc to the carbohydrate groups of asialofetuin. These results suggest that massive amount of biologically active form of sialyltransferase could be produced from baculovirus expression system.