the protein structure validated by *in vivo* assays could be a good alternative for the time consuming crystallographic method.

A2-017P

High expression of recombinant human Platelet Factor 4 in *Escherichia coli* and its bioactivities

N. L. Cheng, J. Xie, J. F. Zhang, Y. H. Zhang, B. F. Yu, X. J. Chen, X. M. Xu and B. Niu
Department of Biochemistry and Molecular Biology, Shanxi Medical University, Taiyuan, Shanxi, PR China.
E-mail: xiejunty@yahoo.com

In order to improve the expression of human Platelet Factor 4 (hPF4) in *Escherichia coli*, we have constructed a prokaryotic expression vector pBV220-hPF4 by DNA polymerase chain reaction (PCR) and DNA recombinant technology, 3′-UTR of PF4 cDNA was deleted and TAG was mutated to TAATAA. The yield of recombinant hPF4 is 160 mg/L in shaking flask culture. The expression level has been improved by 80-fold compared with that of PT7-7 hPF4 expression system. After we washed, dissolved and renatured the inclusion bodies, inhibition experiment of blood vessel proliferation in chicken chorioallantoic membrane was carried out to determine the bioactivities of hPF4. The experimental result demonstrated that hPF4 prepared by our methods had the inhibitory activity against angiogenesis.

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Identification of *Arabidopsis* mutants carrying T-DNA inserts in phosphoprotein phosphatase genes

É. Cseh[1], A. Demeter[1], L. Ökrész[2], I. Kovács[2], C. Koncz[3], L. Szabados[2], P. Gergely[1], V. Dombrádi[1] and I. Farkas[1]
[1]Department of Medical Chemistry, University of Debrecen, Debrecen, Hungary, [2]Institute of Plant Biology, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary, [3]Max Planck-Institut für Züchtungsforschung, Max Planck-Institut für Züchtungsforschung, Köln, Germany.
E-mail: mumina@freemail.hu

Although members of the phosphoprotein phosphatase (PPP) enzyme family are known to be involved in numerous essential cellular processes, their functions are poorly characterized in plants. We have exploited an *Arabidopsis* T-DNA insertion mutant collection to initiate functional analysis of PPP enzymes by reverse-genetics. Using a PCR screening technique described by Ríos et al. (Plant J. (2004) 32, 243-253), we have identified T-DNA insertions in genes encoding PP7, PP1 (TOPP-1), PP6At1, and a plant specific phosphatase (the product of At2g27210). Analysis of homozygous insertion mutants revealed that none of the PPP genes studied are essential for viability, as all PPP insertion mutant lines display wild type phenotype under normal growth conditions. Study of various stress responses to osmotic and hormonal stresses, cadmium(II) salt, sugars, and photooxidative stress induced by paraquat showed no significant difference between wild type and most PPP mutants. However, the PP6 mutant displayed increased sensitivity to abscisic acid inhibition of germination. In response to blue light irradiation, the PP7 mutant exhibited a loss of hypocotyl growth inhibition. PP6 and At2g27210 plant specific phosphatase mutant plants showed a similar deficiency in blue light-mediated inhibition of hypocotyl elongation. These results suggest that PP6, PP7 and At2g27210 may play important roles in the regulation of growth responses by blue light.

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Mutational and crystallographic studies of RNase HIII from *Bacillus stearothermophilus*: importance of N-terminal domain on substrate binding

H. Chon[1], K. Takano[1,3], H. Matsumura[2], Y. Koga[1] and S. Kanaya[1]
[1]Department of Material and Life Science, Osaka University, Suita, Osaka, Japan, [2]Department of Materials Chemistry, Osaka University, Suita, Osaka, Japan, [3]JST, PRESTO, Suita, Osaka, Japan. E-mail: hyongi@mls.eng.osaka-u.ac.jp

Many organisms possess multiple RNase H genes in their genomes. For example, the *Bacillus subtilis* genome possesses three RNase H-related genes. We have previously reported that two of them encode functional RNases H, RNases HII and HIII (Bsu RNases HII and HIII), while the other one, *ypdQ*, encodes an RNase HI homologue with no RNase H activity. Amino acid sequence of Bsu RNase HIII is similar to those of Bsu RNase HII and *Escherichia coli* RNase HII, but is different from that of *E. coli* RNase HI. However, the enzymatic properties of Bsu RNase HIII are similar to those of *E. coli* RNase HI rather than those of Bsu RNase HII and *E. coli* RNase HII. Recently, we cloned the gene encoding RNase HIII from *Bacillus stearothermophilus* (Bst RNase HIII) and characterized the enzymatic properties of the recombinant protein. Bst RNase HIII highly resembles to Bsu RNase HIII in enzymatic properties. Purpose of this study is to understand the molecular mechanism of the enzymatic function of RNases HIII. Limited proteolysis of Bst RNase HIII produced two peptide fragments cleaved at Leu83-Ala84, suggesting that Bst RNase HIII consists of the N- and C-terminal domains. The N-terminal domain alone and the C-terminal domain alone of Bst RNase HIII were overproduced in *E. coli*, purified and characterized. Determination of the kinetic parameters for RNase H activity and the binding analyses of the proteins to RNA/DNA hybrid using BIAcore indicated that the N-terminal domain of Bst RNase HIII is involved in substrate binding. To understand the structural basis for these results, Bst RNase HIII was crystallized and the X-ray diffraction data sets of the native crystal and the heavy-atom derivatives were collected. Structure determination by MIR method is in progress.

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The puzzling nature of cell wall anabolism: enzyme recruitment within and across pathways

J. J. Diaz-Mejia, E. Perez-Rueda and L. Segovia
Lab 8, Cellular Enginery and Biocatalysis., National Autonomous University of Mexico, Cuernavaca, Morelos, Mexico.
E-mail: jdime@ibt.unam.mx

Enzyme recruitment is one of the most important mechanisms to originate new reactions and metabolic pathways from the pre-existing ones. The two original models, the "patchwork" and "stepwise", agree on the necessity of gene duplication as a prerequisite for the recruitment, but have two main differences: (i) the distance (number of reactions) among the recruited enzymes and, (ii) the chemical similarity of the reactions. Some major constituents that distingue the bacterial cell wall from the eukaryotic and the archaeal counterparts are synthesized by the peptidoglycan (PG), folate (FL) and formyl-tetrahydrofolate (FTHF) anabolism, thus the study of these routes is important to understand the emergence of the three domains of life. In order to determine the amount and nature of the enzyme recruitment that have performed the assemble of these and other pathways, we have compared the chemical properties of their reactions, from a network