GECF: new knowledge base of gene expression in cyst formation

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Autosomal dominant polycystic kidney disease (ADPKD) is a common hereditary renal disease. It is characterized by abnormal growth of tubules, which gives rise to the progressive cyst formation, secretion of fluid and to renal failure. We developed a procedure for the standardization of microarray gene expression data of PKD2 transgenic mouse, Mx1 knock-out mouse and PKD2 TgKO MF/EF(Mouse Embryonic Fibroblast) Cell. We stored them in a single integrated database system, focusing on more effective data processing and interpretation. Another characteristic of the present database is that it has a systematic flexibility for statistical analysis and linking with other databases such as GenBank. Basically, we adopt an intelligent SQL querying system, as the foundation of our DB, in order to set up an interactive module which can automatically read the raw gene expression data in the standardized format. We maximize the usability of this DB, helping users study significant gene expression and identify biological function of the genes through integrated up-to-date gene information such as GO annotation and metabolic pathway. For collecting the latest information of selected gene from the database, we also set up the local BLAST search engine and nonredundant sequence database updated by NCBI server on a daily basis. We find that the present database is a useful query interface and data-mining tool, specifically for finding out the genes related to ADPKD. We apply this system to the identification and characterization of ADPKD related genes’ behavior.

Expression and purification of TdeA, a TolC like protein

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TdeA, belonging to the TolC family, is an outer membrane channel forming protein as an essential part of drug efflux and type-I secretion systems in gram negative bacteria. We have expressed and purified TdeA in the absence b-barrel domain that is inserted in the outer membrane. So far, it has not been reported high yield production of outer membrane proteins without the membrane insertion region. The recombinant protein described in this study reduces complicated purification step of integral membrane protein such as detergent extraction from the membrane fraction, and makes it much easier to study a general mechanism for bacterial efflux system in the absence of detergent that may block the protein-protein interaction.

Functional and structural roles of the Arg, Cys and Ser residues in Oryza sativa glutathione S-transferase

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Glutathione S-transferases (GSTs, EC 2.5.1.18) are a class of enzymes that catalyze detoxification of a wide variety of xenobiotics in many plants. Plant GSTs have been demonstrated that GST conjugation for a variety of herbicides is the major resistance and selectivity factor in plants, and provides a tool to control weeds in agronomic crops. In order to gain further insight on the relationship between structure and functions of a phi class glutathione S-transferase from rice, the one arginine, three cystein and four serine residues in OsGSTF3 (ORF ID : AF309384) were replaced with alanine by site-directed mutagenesis. The mutant enzymes were expressed in Escherichia coli and purified to electrophoretic homogeneity by affinity chromatography on immobilized GSH. The catalytic activities of the mutant enzymes were characterized with five different substrates. The three Cys mutant enzymes and S38A, S68A and S169A mutant enzymes did not affect the activity toward 1-chloro-2,4-dinitrobenzene. On the other hand, the substitution of Ser13 with alanine resulted in a decrease in the specific activity toward 1-chloro-2,4-dinitrobenzene to <15% of the wild-type value. The substitution of Arg18 with alanine also did not affect the catalytic mechanism. From these results on kinetic parameters and the dependence of kinetic parameters on pH, we propose that Ser13 plays an important role in catalysis.

Expression, purification and preliminary crystallographic characterization of human N-myc downstream-regulated gene 2 protein

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The N-myc downstream-regulated gene 2 (NDRG2) was initially identified as a candidate for tumor suppressor gene. The expression of NDRG2 in human glioblastoma tissues is significantly lower than that in the normal brain. Transfection of human glioblastoma U373 cells with NDRG2 markedly diminished proliferation of the glioblastoma cells. Recent investigations have shown that NDRG2 is up-regulated in Alzheimer disease brains and can induce the differentiation of dendritic cells. In addition, it has been reported that NDRG2 may facilitate neurite outgrowth of nerve growth factor-differentiated PC12 cells, implicating its involvement in differentiation. These findings suggest that NDRG2 might play important role in the regulation of cell growth and differentiation. Despite significant progress in cell biological investigations of NGR2, its function still remains unclear. Accordingly, we initiated structural studies of the NDRG2 protein. A truncated NDRG2 gene for overexpression in E. coli system was constructed based on secondary structure prediction. In this study, we present the expression, purification and initial X-ray crystallographic characterization of the truncated NDRG2 protein. This work was supported by a grant from the 21C Frontier Microbial Genomics and Applications Center.

Expression, purification and NMR structural studies of human melanocortin-4 receptor TM2 peptide

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The melanocortin receptor family consists of five subtypes (MC1R-MC5R) and belongs to the superfamily of G protein-coupled receptors (GPCRs) which activate the adenylate cyclase signal transduction pathway. Recently, it has been suggested that normal melanocortin receptor increases energy expenditure and decreases food intake, but genetic disruption of MC4R causes obesity. In fact, 4% of obesity patient have a defect from signal transduction by mutant MC4R/TM2. Therefore, MC4 receptors may be ideal pharmacological targets for treating disorders such as obesity and anorexia. Unfortunately, MC4 is membrane-bound protein that transverse the lipid bilayer of the cell membrane, so it is hard to express and characterize the membrane-bound three-dimensional structure using conventional solution NMR and X-ray crystallography. So far we have been optimized the large scale expression conditions and we are optimizing the purification system to get the pure target membrane protein. Here, we will show the results of expression, purification, and tentative NMR experiments.

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