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In vitro Carbohydrate Network of *Thermus caldophilus* GK24 and Its Application

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In order to construct *in vitro* carbohydrate network, a series of ORFs coding carbohydrate-related enzymes were identified from the genome sequences, using home-made DNA DB/analysis system. And they were cloned and overexpressed *in E. coli*, and then each enzyme reaction was characterized in terms of substrate specificity, products and reaction equilibrium. Then, a number of carbohydrate biosynthetic pathways were now being examined in terms of reaction flow and machinery assembly for carbohydrates. In this lecture, for example, *in vitro* machinery assembly of glycolysis and its operation, attempted in the presence of all the enzymes, substrates and cofactors will be discussed. And, in order to understand the assembly of the biosynthetic machinery in the cell, the 3D-structures of the enzymes corresponding to glycolysis were determined by computer-simulation and crystallo- graphy. Then, the 3D-structure dock ing experiments were proceeded for initial information on the enzyme-enzyme interactions in glycolysis machinery. Interestingly, the amino acids located within the recognition domains appeared to be conserved, compared with those of other microorganisms. On the other hand, glycolysis-related genes loci in *Thermus* genome were compared with those of other microbial genomes, in terms of similarity and gene order. It provided an unique window for searching glycolysis-related enzymes and regulatory proteins. A ccordingly, this microbial genome comparison showed that the gene orders among microbial genomes were directly related to the microbial genome evolution.

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Functional Genomics in *Escherichia coli* Construction of Basis of "-omics" Approaches

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Although *Escherichia coli* is undoubtedly one of the most well characterized organisms, the complete genome sequence revealed that more than 2000 ORFs still remain to be characterized. And also about 50 purified enzyme activities still remain to be assigned to their genes. Even though it is the present status of knowledge of *E.coli*, this organism is undoubtedly one of the ideal organisms for complete understandings of a cell because of its vast amount of biological knowledge and accumulation of methods in genetics, biochemistry and molecular biology. Our long-term vision is to understand *Escherichia coli* in sufficient detail to allow the construction of a dynamic molecular model of a simple, self-replicating cell. We have an unrivaled understanding of a multitude of enzyme reactions, metabolic pathways, macromolecular syntheses, and regulatory interactions in this quintessential model organism. Knowing how these processes interact to form a living cell will require a unified "Systems Biology" effort incorporating: (1) Bioinformatics: the cataloging, annotation, and curation of all *E. coli* information in a format that allows interoperative queries, (2) Function: the identification and characterization of all molecul a r constituents, (3) Physiometrics: measurements of the levels, fluxes, and interactions of key molecular constituents, and (4) Modeling: constructing a predictive description of the relationships between molecules, phenotypes and genotypes. Toward this, we performed to construct various kinds of resources and to develop bioinformatic tools for vast amount of data produced from transcriptome and proteome analysis. I would like to introduce our recent efforts for functional genomics of *Escherichia coli*.