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# Crystallographic analysis of proteins in the purine nucleotide biosynthetic pathway

プリンヌクレオチド生合成系タンパク質の結晶構造解析

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The *de novo* synthesis of purine nucleotides (AMP and GMP) proceeds by a 14-steps branched pathway via IMP. This pathway is fundamentally common to most organisms, but the organizations of the genes responsible for this pathway are various from one species to another. Most of the genes are organized in several operons in eubacteria. On the other hand, some genes of eukaryote are fused.

The feature observed in the reaction of this pathway is that all reactions are responsible for the C-N bond formation and several enzymes of this pathway share similar reaction mechanism. For example, three enzymes, amidophosphoribosyltransferase (PurF), FGAR amidotransferase (PurL) and GMP synthetase (GuaA), catalyze the transfer reaction of amide group of glutamine. Two enzymes, SAICAR synthetase (PurC) and adenylosuccinate synthetase (PurA), catalyze the amination reaction that depends on aspartic acid. And two enzymes, GAR transformylase (PurN) and AICAR transformylase (PurH), catalyze the transfer reaction of formyl group of $N^{10}$-formyltetrahydrofolate. Thus, it may be possible to know how this pathway has been organized and how the organization has been re-organized in each organism in the course of evolution by verifying whether the genes encoding these enzymes derive from the common ancestral gene. The purine nucleotide biosynthetic pathway is fundamentally common to most organisms and it must be a good model system to analyze organization and evolution of a metabolism pathway. Thus, we planned to determine the structures of all the proteins in this pathway of *Thermus thermophilus* HB8.

We started crystallization of 12 proteins of this biosynthesis pathway, and got single crystals for 8 proteins. Crystals of GuaA, AIR synthetase (PurM), Guanylate kinase (Gmk) and GAR synthetase (PurD) gave high-resolution data and we are proceeding the structure determination. The phase for GuaA and PurD were determined by multi wavelength anomalous diffraction (MAD) method and model building are in progress. 2.8 Å diffraction data was collected for PurM. Because crystals of Se-Met PurM could not be obtained, we are trying to determine the phase by molecular replacement or isomorphous replacement methods in parallel. In the case of Gmk, more than ten molecules are located in an asymmetric unit for the obtained crystal and MAD method with Se-Met Gmk might be required. Crystals of PurA, FGAR amidotransferase I (PurQ) and PurH gave only low resolution diffraction data and further search of crystallization conditions is in pregress.