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Identification of a Novel Heterodimeric Form of the Purine Biosynthetic Enzyme, Formylglycinamide Ribonucleotide Amidotransferase (FGARAT).

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Abstract

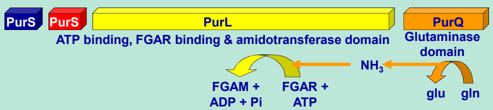
Formylglycinamide ribonucleotide amidotransferase (FGARAT) catalyzes the fourth step in the purine biosynthetic pathway. Two forms of this enzyme have been described in the literature, a monomeric form present in eukaryotes, beta and gamma proteobacteria, and a heterotetrameric form found in most other Bacteria and Archaea. The heterotetrameric enzyme is composed of PurS, PurL and PurQ subunits in a ratio of 2:1:1, while the monomeric enzyme has a domain organization corresponding to a gene fusion of *purS-purS-purL-purQ*. To investigate the evolution of this enzyme, FGARAT sequences were retrieved from the completed genomes of different families of organisms and compared.

Analysis of gene length and multiple sequence alignments from diverse organisms revealed a heterodimeric form of the enzyme in Chloroflexi, Planctomycetes and Delta Proteobacteria. These organisms contained a *purQ* gene but lacked a separate *purS*. Instead, they had a PurL of intermediate length that corresponded to a *purS-purS-purL* gene fusion. The intermediate-length PurL N-terminal domain showed sequence homology to the corresponding region of the monomeric PurL and to PurS sequences, despite the lack of sequence conservation between the latter two sequences. The multiple sequence alignment highlighted regions in which the intermediate-length PurL was more similar to the monomeric PurL, other regions that were more similar to the heterotetrameric PurL, residues conserved among all three forms and residues that were specific for each class of enzyme. These results suggest that FGARAT may have evolved via gene fusion from the heterotetramer to a heterodimer and ultimately to the monomer found in eukaryotes.

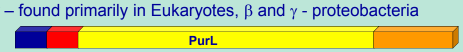
Introduction

Known forms of FGARAT

• Heterotetramer – found in most Archaea and most Bacteria



• Monomer – found primarily in Eukaryotes, β and γ - proteobacteria



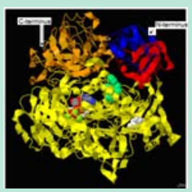


Figure 1. Domain/ Subunit organization of FGAR Amidotransferase Enzymes.
Color coding of subunits and domain scorrespond to domain colors shown in 3-D structure view of monomeric enzyme found in *Salmonella typhimurium (Anand et al., 2004)*.

Goal: Compare FGARAT sequences to investigate the evolution of this enzyme and determine the phylogenetic group in which the monomeric FGARAT first arose.

Methods

All completed genomes (as of 3/1/07) in the NCBI Microbial Genomes Database were searched for FGARAT sequences, either using existing annotation or by performing BLAST searches. After initial compilation of predicted ORF sizes, the FGARAT sequences from at least one representative of each taxonomic family were retrieved from the NCBI Database, and the sequences from multiple subunit FGARATs were assembled to mimic the domain organization of the monmer. Multiple sequence alignments (Clustal W) and phylogenetic trees were generated using the Megalign component of the Lasergene sequence analysis suite (DNASTAR).

Results

Table 1. 100 Representative Sequences used for Phylogenetic Analysis

Organism	Taxonomy	Sub-units	PurL	PurQ	PurS
Corynebacterium diphtheriae	Actinobacteria, Actinomycetales	1	1238	-	-
Bifidobacterium longum	Actinobacteria, Bifidobacteriales	1	1244	-	-
Methanocorpusculum labreanum	Archaea, Euryarchaeota, Methanomicrobiales	1	1231	-	-
Gramella forsetii KT0803	Bacteroidetes, Flavobacteriales	1	1225	-	-
Cytophaga hutchinsonii	Bacteroidetes, Sphingobacteriales	1	1231	-	-
Saccharomyces cerevisiae	Eukaryota, Fungi	1	1358	-	-
Homo sapiens	Eukaryota, Metazoa	1	1338	-	-
Dictyostelium discoideum	Eukaryota, Mycetozoa	1	1355	-	-
Arabidopsis thaliana	Eukaryota, Viridiplantae	1	1387	-	-
Clostridium acetobutylicum	Firmicutes, Clostridia	1	1255	-	-
Clostridium perfringens	Firmicutes, Clostridia	1	1266	-	-
Clostridium tetani	Firmicutes, Clostridia	1	1258	-	-
Streptococcus pyogenes	Firmicutes, Lactobacillales	1	1257	-	-
Streptococcus agalactiae	Firmicutes, Lactobacillales	1	1203	-	-
Fusobacterium nucleatum	Fusobacteria, Fusobacterales	1	1249	-	-
Rhodoferax ferrireducens	Proteobacteria, Beta, Burkholderiales	1	1409	-	-
Polaromonas naphthalenivorans	Proteobacteria, Beta, Burkholderiales	1	1340	-	-
Burkholderia pseudomallei	Proteobacteria, Beta, Burkholderiales	1	1356	-	-
Thiobacillus denitrificans	Proteobacteria, Beta, Hydrogenophilales	1	1291	-	-
Methylobacillus flagellatus	Proteobacteria, Beta, Methylophilales	1	1297	-	-
Neisseria meningitidis	Proteobacteria, Beta, Neisseriales	1	1320	-	-
Nitrosomonas europaea	Proteobacteria, Beta, Nitrosomonadales	1	1304	-	-
Azoarcus sp. EbN1	Proteobacteria, Beta, Rhodocyclales	1	1310	-	-
Desulfotalea psychrophila	Proteobacteria, Delta, Desulfobacterales	1	1267	-	-
Myxococcus xanthus	Proteobacteria, Delta, Myxococcales	1	1302	-	-
Aeromonas hydrophila	Proteobacteria, Gamma, Aeromonadales	1	1357	-	-
Colwellia psychrerythraea	Proteobacteria, Gamma, Alteromonadales	1	1323	-	-
Baumannia cicadellinicola	Proteobacteria, Gamma, Candidatus Baumannia	1	1297	-	-
Nitrosococcus oceani	Proteobacteria, Gamma, Chromatiales	1	1300	-	-
Salmonella typhimurium	Proteobacteria, Gamma, Enterobacteriales	1	1295	-	-
Escherichia coli	Proteobacteria, Gamma, Enterobacteriales	1	1295	-	-
Coxiella burnetii	Proteobacteria, Gamma, Legionellales	1	1296	-	-
Methylococcus capsulatus	Proteobacteria, Gamma, Methylococcales	1	1288	-	-
Hahella chejuensis	Proteobacteria, Gamma, Oceanospirillales	1	1298	-	-
Haemophilus influenzae	Proteobacteria, Gamma, Pasteurellales	1	1320	-	-
Pseudomonas syringae	Proteobacteria, Gamma, Pseudomonadales	1	1313	-	-
Francisella tularensis	Proteobacteria, Gamma, Thiotrichales	1	1290	-	-
Vibrio fischeri	Proteobacteria, Gamma, Vibrionales	1	1303	-	-
Xanthomonas campestris	Proteobacteria, Gamma, Xanthomonadales	1	1348	-	-
Magnetococcus sp. MC-1	Proteobacteria, Magnetococcus	1	1295	-	-
Methanococcus maripaludis	Archaea, Euryarchaeota, Methanococci	2	989	272	-
Methanospirillum hungatei	Archaea, Euryarchaeota, Methanomicrobiales	2	979	282	-
Dehalococcoides ethenogenes	Chloroflexi, Dehalococcoidetes	2	953	255	-
Rhodopirellula baltica	Planctomycetes, Planctomycetales	2	1009	292	-
Anaplasma marginale	Proteobacteria, Alpha, Rickettsiales	2	1016	260	-
Bdellovibrio bacteriovorus	Proteobacteria, Delta, Bdellovibrionales	2	1009	239	-
Desulfovibrio vulgaris	Proteobacteria, Delta, Desulfovibrionales	2	1009	269	-
Geobacter sulfurreducens	Proteobacteria, Delta, Desulfuromonadales	2	996	275	-
Syntrophobacter fumaroxidans	Proteobacteria, Delta, Syntrophobacterales	2	1009	269	-
Acidobacteria bacterium Ellin345	Acidobacteria, Acidobacteriales	4	768	231	80
Solibacter usitatus	Acidobacteria, Solibacteres, Solibacterales	4	742	232	81
Corynebacterium glutamicum	Actinobacteria, Actinomycetales	4	762	223	81
Corynebacterium jeikeium	Actinobacteria, Actinomycetales	4	839	223	84
Streptomyces coelicolor	Actinobacteria, Actinomycetales	4	752	226	90
Acidothermus cellulolyticus 11B	Actinobacteria, Actinomycetales	4	754	225	81
Rubrobacter xylanophilus	Actinobacteria, Rubrobacterales	4	727	220	74
Aquifex aeolicus	Aquificae, Aquificales	4	745	227	77
Pyrobaculum aerophilum	Archaea, Crenarchaeota, Thermoprotei	4	697	212	84
Archaeoglobus fulgidus	Archaea, Euryarchaeota, Archaeoglobi	4	765	271	80
Haloarcula marismortui	Archaea, Euryarchaeota, Halobacteria	4	720	228	84
Methanothermobacter thermautotrophicus	Archaea, Euryarchaeota, Methanobacteria	4	714	214	84
Methanocaldococcus jannaschii	Archaea, Euryarchaeota, Methanococci	4	733	230	83
Methanosarcina acetivorans	Archaea, Euryarchaeota, Methanomicrobia	4	715	232	88
Methanopyrus kandleri	Archaea, Euryarchaeota, Methanopyri	4	724	226	84
Methanococcoides burtonii	Archaea, Euryarchaeota, Methanosarcinales	4	715	231	82
Pyrococcus abyssi	Archaea, Euryarchaeota, Thermococci	4	705	223	84
Picrophilus torridus	Archaea, Euryarchaeota, Thermoplasmata	4	741	248	75
Thermoplasma acidophilum	Archaea, Euryarchaeota, Thermoplasmata	4	759	257	79
Haloquadratum walsbyi	Archaea, Halobacteria, Halobacteriales	4	725	228	91
Salinibacter ruber	Bacteroidetes, Sphingobacteriales	4	754	235	92
Chlorobium tepidum	Chlorobi, Chlorobiales	4	759	234	84
Synechococcus elongatus	Cyanobacteria	4	777	221	74
Gloeobacter violaceus	Cyanobacteria, Gloeobacteria	4	774	232	88
Nostoc sp. PCC 7120	Cyanobacteria, Nostocales	4	782	224	92
Trichodesmium erythraeum	Cyanobacteria, Oscillatoriales	4	775	231	87
Prochlorococcus marinus	Cyanobacteria, Prochlorales	4	803	217	90
Deinococcus radiodurans	Deinococcus-Thermus, Deinococcales	4	747	266	84
Thermus thermophilus	Deinococcus-Thermus, Thermales	4	725	227	84
Bacillus subtilis	Firmicutes, Bacillales	4	742	227	84
Carboxydothermus hydrogenoformans	Firmicutes, Clostridia	4	728	234	81
Thermoanaerobacter tengcongensis	Firmicutes, Clostridia	4	733	224	82
Moorella thermoacetica	Firmicutes, Clostridia, Thermoanaerobacteriales	4	733	236	84
Enterococcus faecalis	Firmicutes, Lactobacillales	4	739	224	83
Caulobacter crescentus	Proteobacteria, Alpha, Caulobacterales	4	739	220	79
Sinorhizobium meliloti	Proteobacteria, Alpha, Rhizobiales	4	743	223	80
Silicibacter pomeroyi	Proteobacteria, Alpha, Rhodobacterales	4	719	222	76
Gluconobacter oxydans	Proteobacteria, Alpha, Rhodospirillales	4	734	233	80
Candidatus Pelagibacter ubique	Proteobacteria, Alpha, Rickettsiales	4	730	227	80
Zymomonas mobilis	Proteobacteria, Alpha, Sphingomonadales	4	734	221	77
Rhizobium etli	Proteobacteria, Alpha, Rhizobiales	4	743	223	80
Anaeromyxobacter dehalogenans	Proteobacteria, Delta, Myxococcales	4	759	218	81
Campylobacter jejuni	Proteobacteria, Epsilon, Campylobacterales	4	728	215	81
Leptospira interrogans	Spirochaetes, Spirochaetales	4	745	219	82
Thermotoga maritima	Thermotogae, Thermotogales	4	603	213	82

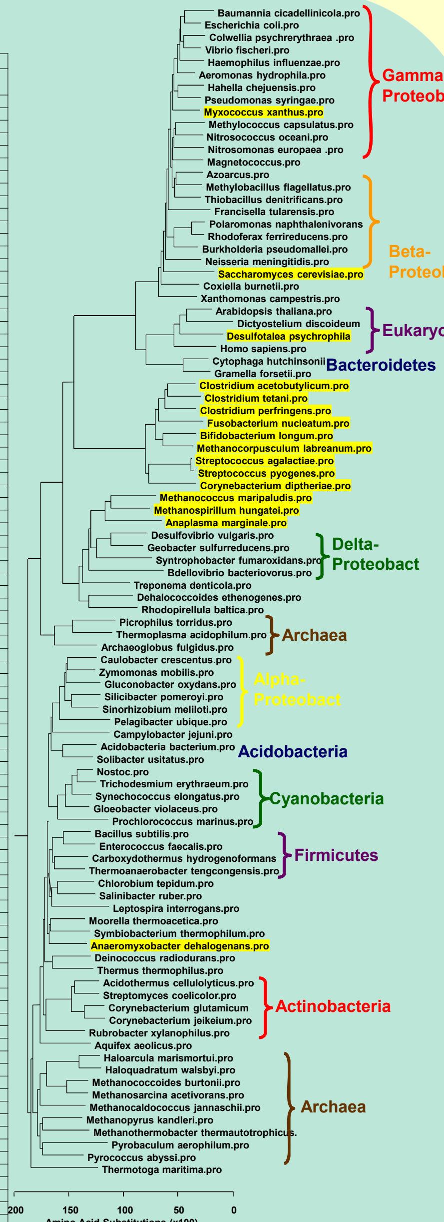


Figure 2. Phylogenetic Tree Based on Clustal W alignment of combined PurS-PurS-PurL-PurQ sequences.
Yellow highlights sequences that do not cluster with closely related organisms and may have been acquired by horizontal gene transfer.

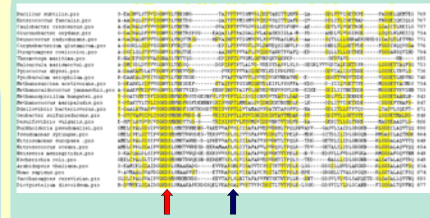
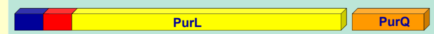


Figure 3. Segment of a multiple sequence alignment illustrating heterodimer sequences that are more similar to monomer (red arrow) or more similar to tetramer (blue arrow)

Conclusions

Heterodimeric FGARAT is present in Delta-Proteobacteria, Chlorofexi, Planctomycetes, & some Methanogens.



Future Work

•Examine apparent cases of horizontal gene transfer to identify source of unusual enzyme forms.

•Correlate protein structural characteristics with sequences conserved between heterodimer and monomer and between heterodimer and heterotetramer.

References

• Anand, R., Hoskins, A., Stubbe, J., et al. (2004). Domain Organization of *Salmonella typhimurium* Formylglycinamide Ribonucleotide Amidotransferase Revealed by X-ray Crystallography. Biochemistry 43:10328-10342.