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Research

Significance of two distinct types of tryptophan synthase beta chain in Bacteria, Archaea and higher plants

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Abstract

Background: Tryptophan synthase consists of two subunits, α and β . Two distinct subgroups of β chain exist. The major group (TrpEb_1) includes the well-studied β chain of *Salmonella typhimurium*. The minor group of β chain (TrpEb_2) is most frequently found in the Archaea. Most of the amino-acid residues important for catalysis are highly conserved between both TrpE subfamilies.

Results: Conserved amino-acid residues of TrpEb_1 that make allosteric contact with the TrpEa subunit (the α chain) are absent in TrpEb_2. Representatives of Archaea, Bacteria and higher plants all exist that possess both TrpEb_1 and TrpEb_2. In those prokaryotes where two *trpEb* genes coexist, one is usually *trpEb_1* and is adjacent to *trpEa*, whereas the second is *trpEb_2* and is usually unlinked with other tryptophan-pathway genes.

Conclusions: TrpEb_1 is nearly always partnered with TrpEa in the tryptophan synthase reaction. However, by default at least six lineages of the Archaea are likely to use TrpEb_2 as the functional β chain, as TrpEb_1 is absent. The six lineages show a distinctive divergence within the overall TrpEa phylogenetic tree, consistent with the lack of selection for amino-acid residues in TrpEa that are otherwise conserved for interfacing with TrpEb_1. We suggest that the stand-alone function of TrpEb_2 might be to catalyze the serine deaminase reaction, an established catalytic capability of tryptophan synthase β chains. A coincident finding of interest is that the Archaea seem to use the citramalate pathway, rather than threonine deaminase (IlvA), to initiate the pathway of isoleucine biosynthesis.

Background

Tryptophan biosynthesis is absent in mammals but is a general metabolic capability of prokaryotes, eukaryotic microorganisms and higher plants. It has been a classic system for elucidation of gene-enzyme relationships and regulation, thanks largely to the lifelong efforts of Charles Yanofsky [1]. Tryptophan is biochemically the most expensive of the amino acids to synthesize [2]. The clustered organization of the *trp* genes into an operon has been a model

system for many years and continues to receive attention. In the current genomics era, emerging surprises reveal there is still much to learn. These surprises include the revelations that operons are being organizationally reshuffled, invaded by insertion of apparently unrelated genes, disrupted by either partial or complete dispersal of genes to extra-operon locations, or complicated by the seemingly unnecessary presence of additional operon-gene copies located outside of the operon.

Tryptophan synthase, catalyzing the final step of tryptophan biosynthesis, is one of the most rigorously documented examples of an enzyme complex [3–6]. It consists of an α subunit, which cleaves indoleglycerol phosphate to indole and glyceraldehyde 3-phosphate, and a β subunit, which condenses indole and L-serine to yield L-tryptophan. The $\alpha\beta\alpha$ complex forms a tunnel into which enzymatically generated indole is released. The α monomers and β dimers contact one another via highly sophisticated mechanisms of allostery [7], and it is little wonder that the genes encoding these two subunits are almost always closely linked, frequently being translationally coupled.

Against this background, it seems curious that a significant number of organisms possess more than one gene encoding the β chain of tryptophan synthase. Usually, but not always, the ‘extra’ gene is unlinked to the gene encoding the α chain, and it also defines a distinct subgroup of the β chain. This has been recognized in the COGs database [8] as “alternative tryptophan synthase” (COG 1350). In this paper we present a detailed analysis of the distribution of the two subgroups of the β chain in prokaryotes within the context of the surprisingly dynamic past and ongoing alterations of organization for genes responsible for tryptophan biosynthesis.

Nomenclature

In recent publications involving bioinformatic analysis of aromatic amino-acid biosynthesis [9–14], we have implemented a nomenclature that attempts logical and consistent naming of genes and their gene products for different organisms. The problem is exemplified by the contemporary naming in two model organisms of 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) synthase, the initial enzyme step of aromatic amino-acid biosynthesis. In *Bacillus subtilis* the gene, appropriately enough, has been named *aroA*, but in *Escherichia coli* the equivalent function is represented by genes encoding three differentially regulated paralogs. These *E. coli* genes have been named *aroF*, *aroG* and *aroH*. In *B. subtilis* these latter three gene designations refer to 5-enolpyruvylshikimate-3-phosphate synthase (step 6 of chorismate synthesis), chorismate synthase (step 7) and chorismate mutase (initial step of phenylalanine and tyrosine biosynthesis). Even in *B. subtilis*, where the naming was intended to follow an orderly progression in terms of order of reaction steps, there is the complication that DAHP synthase is expressed as a fusion of two catalytic domains, one being a class of chorismate mutase called AroQ. This requires naming at the level of domain and the designation *aroQ•aroA* was implemented to denote such a fusion [9–12]. Thus, a single enzymatic function in one organism is accommodated through the cumulative expression of three paralog genes, but in another organism is only encoded by a portion of a single gene. A universal nomenclature is needed that labels at the level of domain, that labels in synchrony with order of reaction steps as much as possible, and that labels isofunctional paralogs at the same hierarchical level but with discriminating identifiers.

The status of nomenclature for the tryptophan pathway is not so chaotic as the genes in almost all prokaryotes have been named in line with *E. coli*, but even here distinct problems have arisen because gene fusions that exist in *E. coli* are often absent elsewhere. Much of the literature up to at least 1996 used five gene designations for the seven protein domains [15]. In *B. subtilis* these seven protein domains are encoded by separate genes [16]. Thus, *E. coli* *trpD* encodes the equivalent of *B. subtilis* TrpG and TrpD. The *E. coli* gene fusion has been re-designated with the convention of a bullet to represent a fusion: *trpG•trpD* [17]. Likewise, the *E. coli* *trpC* encodes the equivalent of *B. subtilis* TrpC and TrpF, and the *E. coli* gene fusion has been re-designated *trpC•trpF*. The nomenclature we advocate is more easily remembered because genes are named in the order of pathway reaction. Subunits for a given reaction are named at the same hierarchical level: anthranilate synthase (first reaction) consists of TrpAa and TrpAb, and tryptophan synthase (fifth reaction) consists of TrpEa and TrpEb. The implementation of a logical and consistent nomenclature, as illustrated in Figure 1, should be most helpful in the long term.

Results and discussion

Phylogenetic tree construction

Initial amino-acid alignments were generated using ClustalW software, version 1.4 [18]. Manual adjustments were made through visual inspection to bring conserved motifs and residues into register. This was implemented by use of the BioEdit multiple alignment tool [19]. Inferences about the evolutionary relationships within the TrpEa and TrpEb protein families were made using the PHYLIP package of programs [20]. The Protpars program was used to generate a maximum parsimony tree, and the neighbor-joining and Fitch programs were used to generate a distance-based tree. The distance matrix used in the latter programs was produced using the program Protdist with a Dayoff PAM matrix. The Seqboot and Consense programs were then used to assess the statistical strength of the tree using bootstrap resampling. Neighbor-joining (PHYLIP), Fitch and Margolash (Fitch in PHYLIP), and maximum parsimony methods [21] all produced trees consistent with one another. Despite low bootstrap values at many individual internal nodes, the clusters formed and arrangement of taxa within them were largely identical. Ninety TrpEb and 63 TrpEa sequences were analyzed.

Distinctly different types of TrpEb

TrpEb proteins divide into two distinctly different groups, as illustrated by the unrooted tree shown in Figure 2. The major group, denoted TrpEb_1, includes the well-studied enzymes from such organisms as *E. coli*, *Salmonella typhimurium*, and *B. subtilis*. The minor group, denoted TrpEb_2, is represented heavily, but not exclusively, by archaeal proteins. Among the current inventory of completed archaeal genomes, only *Methanococcus jannaschii* lacks TrpEb_2. Seven

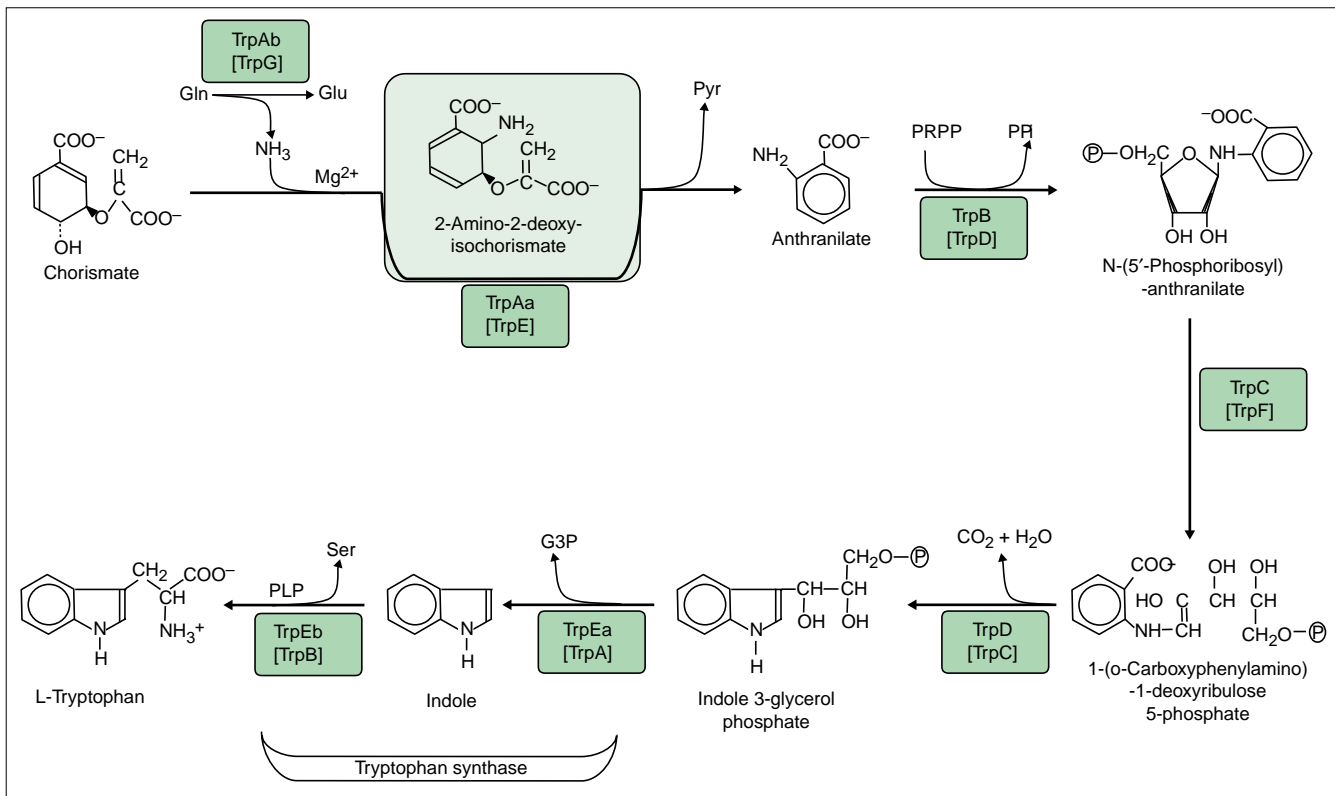


Figure 1

Biochemical pathway of tryptophan biosynthesis. Acronyms that are currently used for the seven monofunctional proteins of *Bacillus subtilis* are shown in brackets below the acronyms used in this paper. TrpAa, large aminase subunit of anthranilate synthase; TrpAb, small glutamine-binding subunit of anthranilate synthase; TrpB, anthranilate phosphoribosyl transferase; TrpC, phosphoribosyl-anthranilate isomerase; TrpD, indoleglycerol phosphate synthase; TrpEa, α subunit of tryptophan synthase; TrpEb, β subunit of tryptophan synthase.

archaeal genomes possess both TrpEb₁ and TrpEb₂ (*Methanosarcina barkeri* possesses two paralogs of TrpEb₁ in addition to one species of TrpEb₂). Six archaeal genomes possess TrpEb₂, but not TrpEb₁.

Bacterial TrpEb₂ proteins are thus far limited to *Aquifex*, *Thermotoga*, *Mycobacterium*, *Geobacter*, *Chlorobium* and *Rhodospseudomonas* genera. In addition, one of the multiple TrpEb proteins present in the higher plant, *Arabidopsis thaliana*, belongs to the TrpEb₂ subfamily. In view of the distinct divergence of TrpEb₂ from TrpEb₁, one might expect that either TrpEb₂ has lost the ability to interact allosterically with TrpEa, or perhaps that a divergent subgroup of TrpEa has coevolved with TrpEb₂. Multiple copies of TrpEb are often present in genomes. Examples include

cases where two TrpEb₁ species coexist, where two TrpEb₂ species coexist, or where TrpEb₁ and TrpEb₂ coexist in the same organism. A number of organisms (*M. barkeri*, *Rhodobacter capsulatus*, *Chlamydia psittaci*, and *Corynebacterium diphtheriae*) possess two copies of TrpEb₁. In each case the *trpEb₁* copy that is linked to *trpEa* is highly conserved, whereas the remaining copy has diverged to the extent that it may be a pseudogene (Table 2). The TrpEb₁ products of these probable pseudogenes have elongated branches (highlighted yellow) on the protein tree shown in Figure 2.

It is noteworthy that cyanobacterial and higher plant amino-acid sequences form a cohesive cluster for TrpEb₁, as shown in phylogram form in Figure 3 (left panel). An

Figure 2 (see figure on the next page)

Unrooted phylogenetic tree (radial view) of the TrpEb protein family consisting of subfamily TrpEb₁ (top) and TrpEb₂ (bottom). Phylogenetic reconstruction of the inferred amino-acid sequence was accomplished by the neighbor-joining method using the PHYLIP program. Organismal acronyms are defined in Table 1. For economy of space, a single branch is used to represent proteins that have diverged very recently, for example, TrpEb₁ proteins from *E. coli* and *S. typhimurium* (Eco/Sty). Archaeal proteins are highlighted in magenta. The detailed order of branching for TrpEb₁ proteins in cyanobacteria and higher plants is shown in Figure 3. Probable pseudogenes are shown in yellow.

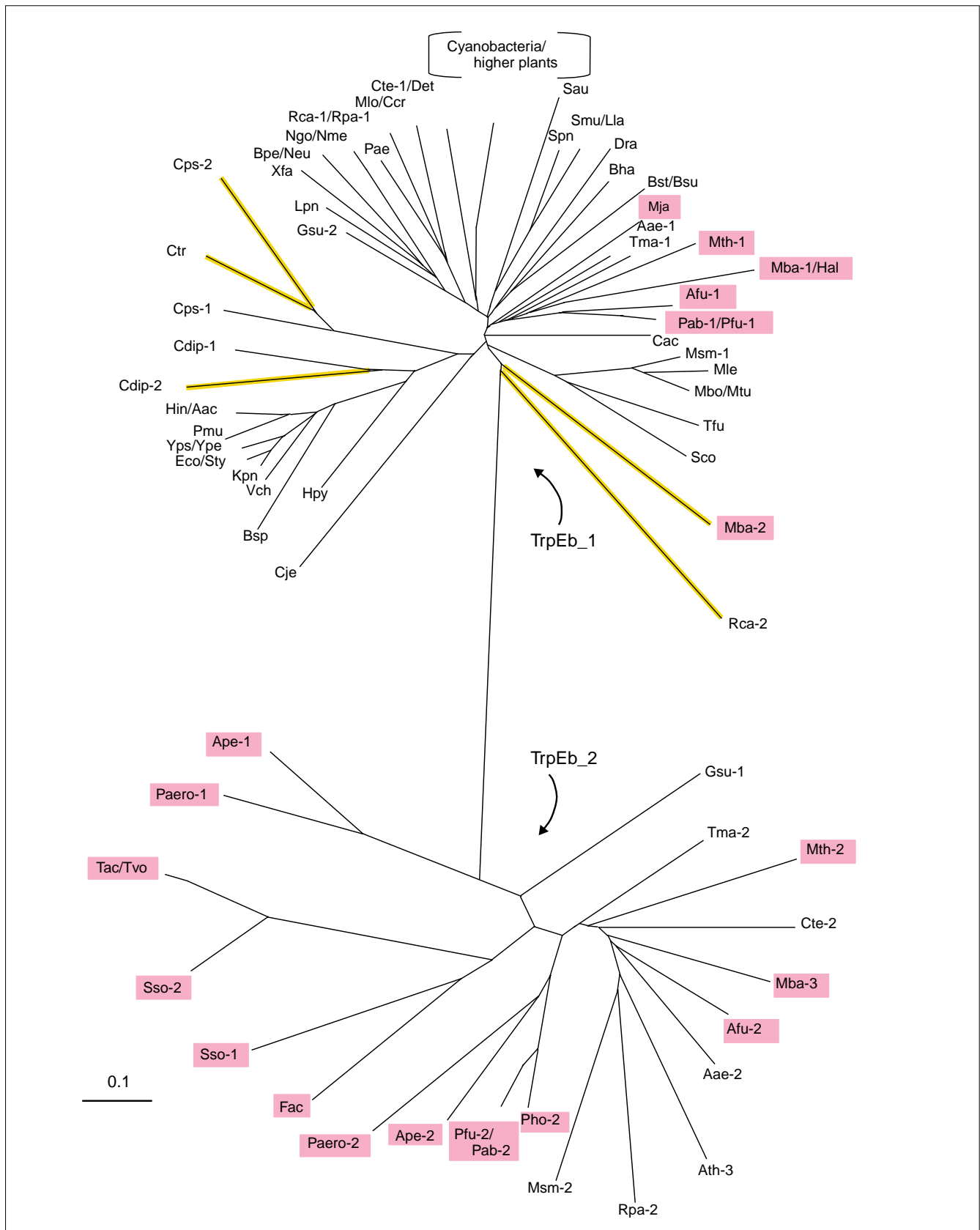


Figure 2 (see legend on the previous page)

Table 1

Key to sequence identifiers		NCBI GI number	
Species name	Acronym	trpEb-1	trpEb-2
<i>Actinobacillus actinomycetemcomitans</i>	Aac	N/A	
<i>Aeropyrum pernix</i>	Ape-1		7674393
<i>Aeropyrum pernix</i>	Ape-2		7674395
<i>Anabaena</i> sp.	Asp-1	N/A	
<i>Anabaena</i> sp.	Asp-2	N/A	
<i>Aquifex aeolicus</i>	Aae-1	6226273	
<i>Aquifex aeolicus</i>	Aae-2		7674374
<i>Arabidopsis thaliana</i>	Ath-1	136251	
<i>Arabidopsis thaliana</i>	Ath-2	1174779	
<i>Arabidopsis thaliana</i>	Ath-3		10176821
<i>Archaeoglobus fulgidus</i>	Afu-1	3334387	
<i>Archaeoglobus fulgidus</i>	Afu-2		7674372
<i>Bacillus halodurans</i>	Bha	10174280	
<i>Bacillus stearothermophilus</i>	Bst	226585	
<i>Bacillus subtilis</i>	Bsu	136270	
<i>Bordetella pertussis</i>	Bpe	N/A	
<i>Buchnera</i> sp. APS	Bsp	11182450	
<i>Campylobacter jejuni</i>	Cje	11269304	
<i>Caulobacter crescentus</i>	Ccr	136272	
<i>Chlamydia trachomatis</i>	Ctr	6226274	
<i>Chlamydia psittaci</i>	Cps-1	N/A	
<i>Chlamydia psittaci</i>	Cps-2	N/A	
<i>Chlorobium tepidum</i>	Cte-1	N/A	
<i>Chlorobium tepidum</i>	Cte-2		N/A
<i>Clostridium acetobutylicum</i>	Cac	N/A	
<i>Corynebacterium diphtheriae</i>	Cdip-1	N/A	
<i>Corynebacterium diphtheriae</i>	Cdip-2	N/A	
<i>Dehalococcoides ethenogenes</i>	Det	N/A	
<i>Deinococcus radiodurans</i>	Dra	7474051	
<i>Escherichia coli</i>	Eco	136273	
<i>Ferroplasma acidarmanus</i>	Fac		N/A
<i>Geobacter sulfurreducens</i>	Gsu-1	N/A	
<i>Geobacter sulfurreducens</i>	Gsu-2		N/A
<i>Haemophilus influenzae</i>	Hin	1174785	
<i>Halobacterium</i> sp.	Hal	14423973	
<i>Helicobacter pylori</i>	Hpy	7674399	
<i>Klebsiella pneumoniae</i>	Kpn	N/A	
<i>Lactococcus lactis</i>	Lla	267168	
<i>Legionella pneumophila</i>	Lpn	N/A	
<i>Mesorhizobium loti</i>	Mlo	13474230	
<i>Methanobacterium thermoautotrophicum</i>	Mth-1	3334383	
<i>Methanobacterium thermoautotrophicum</i>	Mth-2		7674371
<i>Methanococcus jannaschii</i>	Mja	2501412	
<i>Methanosarcina barkeri</i>	Mba-1	N/A	
<i>Methanosarcina barkeri</i>	Mba-2	N/A	
<i>Methanosarcina barkeri</i>	Mba-3		N/A
<i>Mycobacterium bovis</i>	Mbo	N/A	
<i>Mycobacterium leprae</i>	Mle	13093205	
<i>Mycobacterium smegmatis</i>	Msm-1	N/A	
<i>Mycobacterium smegmatis</i>	Msm-2		N/A
<i>Mycobacterium tuberculosis</i>	Mtu	3024761	
<i>Neisseria gonorrhoeae</i>	Ngo	N/A	
<i>Neisseria meningitidis</i>	Nme	11269306	
<i>Nitrosomonas europaea</i>	Neu	N/A	
<i>Nostoc punctiforme</i>	Npu-1	N/A	
<i>Nostoc punctiforme</i>	Npu-2	N/A	
<i>Pasteurella multocida</i>	Pmu	13432266	

Table 1 (continued)

Species name	Acronym	NCBI GI number	
		trpEb-1	trpEb-2
<i>Prochlorococcus marinus</i>	Pma	N/A	
<i>Pseudomonas aeruginosa</i>	Paeru	12230946	
<i>Pyrobaculum aerophilum</i>	Paero-1		N/A
<i>Pyrobaculum aerophilum</i>	Paero-2		N/A
<i>Pyrococcus abyssi</i>	Pab-1	14520675	
<i>Pyrococcus abyssi</i>	Pab-2		14423981
<i>Pyrococcus furiosus</i>	Pfu-1	N/A	
<i>Pyrococcus furiosus</i>	Pfu-2		N/A
<i>Pyrococcus horikoshii</i>	Pho		14591361
<i>Rhodobacter capsulatus</i>	Rca-1	N/A	
<i>Rhodobacter capsulatus</i>	Rca-2	N/A	
<i>Rhodospseudomonas palustris</i>	Rpa-1	N/A	
<i>Rhodospseudomonas palustris</i>	Rpa-2		N/A
<i>Salmonella typhimurium</i>	Sty	136281	
<i>Staphylococcus aureus</i>	Sau	13701169	
<i>Streptococcus mutans</i>	Smu	N/A	
<i>Streptococcus pneumoniae</i>	Spn	N/A	
<i>Streptomyces coelicolor</i>	Sco	6226276	
<i>Sulfolobus solfataricus</i>	Sso-1		14424473
<i>Sulfolobus solfataricus</i>	Sso-2		13814334
<i>Synechococcus</i> sp.	Syn	N/A	
<i>Synechocystis</i> sp.	Ssp	2501413	
<i>Thermomonospora fusca</i>	Tfu	N/A	
<i>Thermoplasma acidophilum</i>	Tac		13878841
<i>Thermoplasma volcanium</i>	Tvo		13541762
<i>Thermotoga maritima</i>	Tma-1	1717761	
<i>Thermotoga maritima</i>	Tma-2		7674388
<i>Vibrio cholerae</i>	Vch	11269279	
<i>Xylella fastidiosa</i>	Xfa	11269281	
<i>Yersinia pestis</i>	Ype	N/A	
<i>Yersinia pseudotuberculosis</i>	Yps	N/A	
<i>Zea mays</i>	Zma-1	1174780	
<i>Zea mays</i>	Zma-2	1174778	

analysis of TrpEa proteins from the same organisms yields a very similar phylogram output (Figure 3, right panel). This higher plant/cyanobacteria relationship is pleasingly consistent with the endosymbiotic hypothesis of organelle evolution. In each case *Prochlorococcus marinus* and *Synechococcus* species are the outlying sequence group, with the other cyanobacterial sequences (*Nostoc punctiforme* and *Anabaena* species) being closer to the higher plant sequences from *A. thaliana* and corn (*Zea mays*). The order of branching shown is supported by very high bootstrap values. Zma-3 is the TrpEa protein that has been proposed [22] to function independently of a TrpEb partner, producing indole for entry into a pathway other than tryptophan. In this case indole serves as a precursor for a defense metabolite that is active against insects, bacteria and fungi.

TrpEa in organisms lacking TrpEb_1

Six organisms (all Archaea) possess intact tryptophan pathways, but they lack TrpEb_1. TrpEb in *Thermoplasma volcanii*, *T. acidophilum*, and *Ferroplasma acidarmanus* is represented only by a single species of TrpEb_2. Although *Sulfolobus solfataricus*, *Aeropyrum pernix* and *Pyrobaculum*

Table 2**Exceptions to residue invariance**

Protein	Invariant residue*	Exceptions: organism (homologous residue)			
TrpEa	57P	Sso (A)			
	60D	Hal (E)			
	61G	Ctr (N)			
	64I	Mth (V)			
	183T	Ctr (R)			
	184G	Hin (S)			
	211G	Ctr (R)			
	212F/L	Ctr (R)			
	213G	Ctr (D)	Paero (A)	Ape (S)	
	234G	Ctr (K)			
	TrpEb_1	10G	Ctr (H)	Cps-2 (E)	Cps-1 (Y)
		21L	Cje (A)		
		41F	Asp-1 (Y)		
		55R	Mth-1 (K)		
		78E	Ccr (D)		
		79D	Rca-1 (E)	Paeru (E)	
		80L	Mth-1 (M)	Dra (Q)	
		82H	Mba-2 (Q)	Dra (F)	
		94Q	Rca-2 (E)		
		96L	Rpa-1 (M)	Spn (W)	Bsp (M)
97L		Cps-2 (I)			
102G		Bsp (K)			
114Q		Rpa-1 (M)	Paeru (M)		
124A		Zma-2 (R)	Cps-1 (G)		
133F/Y		Rca-2 (H)			
141R		Rca-2 (K)			
146V		Mba-1 (A)			
149M		Cps-2 (I)			
153G		Hal (D)			
156V		Cje (I)			
159V		Bha (A)			
162G		Cdip-2 (E)			
167K		Cdip-2 (S)	Sau (S)		
173A		Hal (T)	Rca-2 (C)		
177W		Cps-2 (F)	Rca-2 (Y)		
186Y	Cps-2 (F)	Rca-2 (F)			
208I	Xfa (V)				
213R/K	Bpe (L)				
224P	Cje (V)				
225D	Bha (T)	Rca-2 (A)			
267H	Rca-2 (N)				
269A	Sau (L)	Mba-1 (S)			
274G	Aac (A)				
299S	Rca-2 (T)				
310G	Mba-2 (S)				
345I	Rca-2 (V)				
383D	Yps (E)				
Protein	Invariant residue†	Exceptions: organism (homologous residue)			
TrpEb_2	P10	Paero-1 (gap)			
	Y14	Aae-2 (L)			
	E54	Sso-1 (Q)			
	L86	Msm-2 (F)			
	E87	Paero-2 (D)			
	E101	Paero-2 (Q)			
	S108	Paero-2 (N)			
	A114	Mba-3 (S)			
	W138	Sso-1 (R)			
	G176	Fac (N)			

Table 2 (continued)

Protein	Invariant residue†	Exceptions: organism (homologous residue)
	S184	Sso-1 (T)
	S203	Fac (T)
	I206	Msm-2 (M)
	A207	Fac (G)
	S209	Ape-1 (A)
	E210	Sso-2 (D)
	G226	Fac (A)
	N265	Paero-2 (S)
	P272	Mba-3 (E)
	G300	Cte-2 (A)
	F/Y325	Mba-3 (H)
	F371	Ape-1 (M)
	P379	Fac (A)
	S410	Ath-3 (C)

*Residues are numbered according to the *S. typhimurium* sequence.†Residues are numbered according to the *P. furiosus* sequence.

aerophilum all possess two species of TrpEb, both are the TrpEb_2 variety. Thus, in all six of these lineages TrpEa either might be unable to form a tight complex with TrpEb_2, or might have evolved different protein-protein contacts. In the latter case, distinct TrpEa subgroupings might be expected in parallel with the two TrpEb subgroupings. On the contrary, all TrpEa sequences fall into a single group (Figure 4). However, in contrast to sequences present in those Archaea that do possess TrpEb_1 (for example, *Archaeoglobus*, species of *Pyrococcus*, *Methanococcus*, *Methanobacterium*, *Methanosarcina* and *Halobacterium*), the six archaeal lineages that possess only TrpEb_2 have very distinctive elongated branches on the TrpEa tree (Figure 4). This suggests an elevated rate of evolutionary divergence, due either to selection for new productive contacts of TrpEa with TrpEb_2 or to lack of constraint to maintain TrpEa residues previously important for contacts with TrpEb_1.

The long branch of the TrpEa sequence of *Chlamydia trachomatis* reflects its likely status as a pseudogene. This is consistent with the observation that *C. trachomatis* TrpEb_1 (Figure 2) also seems to be a pseudogene. One does not expect positive selection for maintenance of function in *C. trachomatis* as it lacks an intact tryptophan pathway. Indeed, the alteration in *C. trachomatis* of many otherwise invariant amino-acid residues is evident from the information given in Table 2.

Overview comparison of TrpEb_1 and TrpEb_2

Figure 5 shows an alignment of the amino-acid sequence of TrpEb_1 from *S. typhimurium* with TrpEb_2 from *P. furiosus*. Each sequence is shown as a template for its own subfamily, as extracted from a refined multiple alignment. Conserved residues deduced from a full multiple alignment (available from the author on request) are indicated, as are the gap positions present in the full alignment. Functional roles in catalysis and allosteric regulation are indicated for

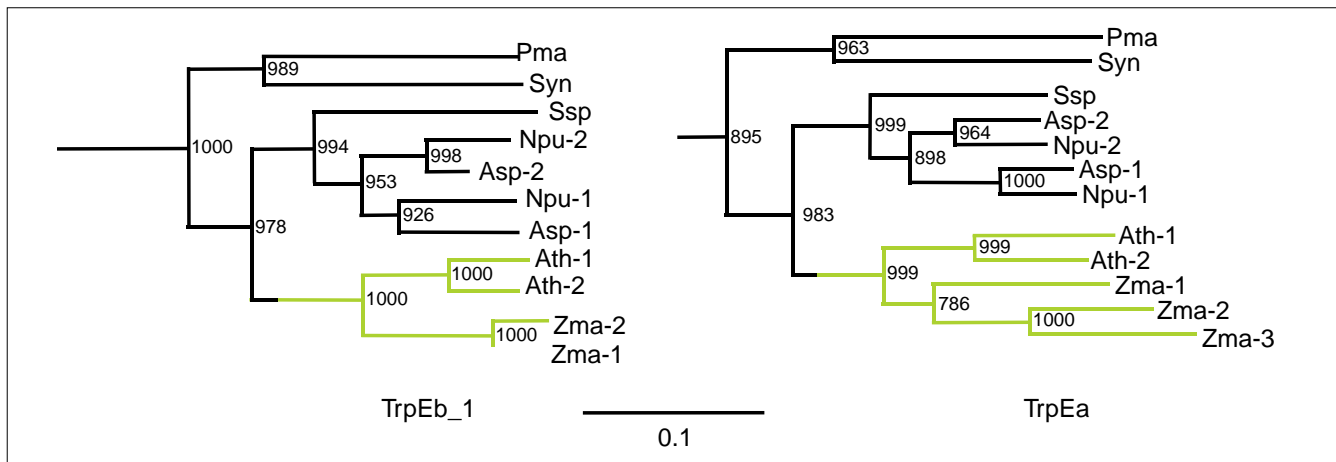


Figure 3

Unrooted phylogenetic tree (phylogram view) of cyanobacterial and higher plant TrpEb_1 (zoom-in expansion from Figure 2) and TrpEa (zoom-in expansion from Figure 4) protein sequences. The higher-plant lineage is shown in green. Bootstrap values (from 1,000 replications) supporting the order of branching shown are given at the nodes.

the *S. typhimurium* TrpEb_1 sequence in order to compare similarities and differences between TrpEb_1 and TrpEb_2 proteins. Residues that are ligands of pyridoxal phosphate or that interact with pyridoxal phosphate are scattered throughout the sequences, including the catalytic K87, and are highly conserved. Residue E109 has been shown to render indole more nucleophilic via proton abstraction from N1 [23]. The serine substrate-binding region is highly conserved, as is a monovalent cation (MVC) binding region [7] coordinating with G232, F/Y306, and G/A/S308. A number of indels (insertions/deletions) distinguish TrpEb_1 and TrpEb_2, and TrpEb_2 is about 50 residues longer overall than TrpEb_1. In addition to other residues conserved between both TrpEb_1 and TrpEb_2, each subgroup has its own repertoire of uniquely conserved residues. The COMM domain [24], a rigid but mobile domain as originally defined with *S. typhimurium* TrpEb_1 [25], differs from the corresponding region of the TrpEb_2 subfamily by the presence of an indel. Key TrpEb_1 regulatory residues (R141, K167) within this region as well as one residue near the MVC site (D305) are not conserved in the TrpEb_2 subgroup.

Loss of intersubunit contacts in TrpEa-TrpEb_2 systems

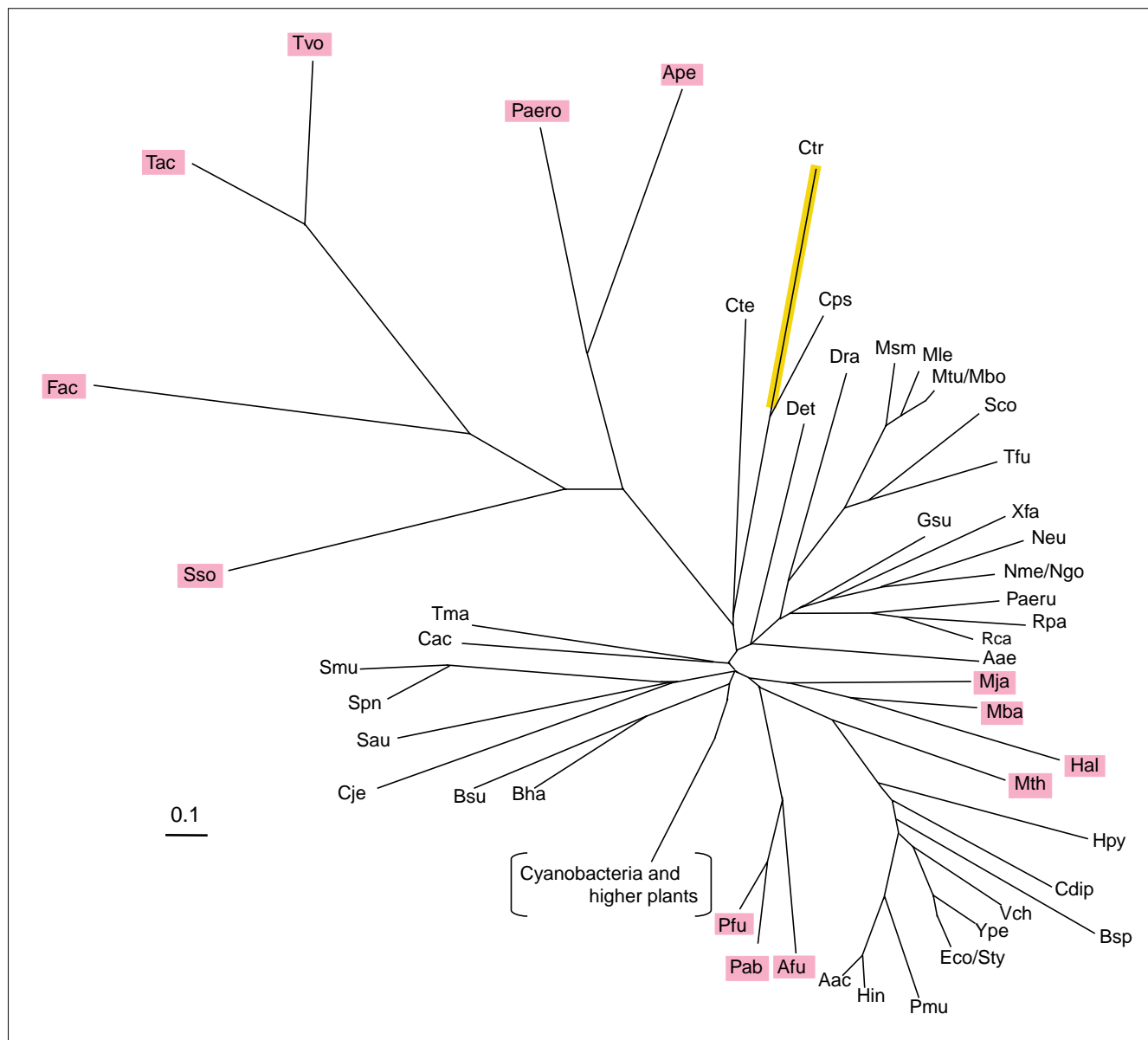
The tryptophan synthase of *S. typhimurium* is a rigorously documented example of substrate channeling in which indole generated as an intermediate is passed through an internalized tunnel ([7] and references therein). Ligand binding at the α -site and covalent transformations at the β -site accomplish mutually reinforcing overall allostery. The movable COMM domain is comprised of residues G102 to G189 in *S. typhimurium* TrpEb_2. COMM interacts with both the β -active site and with α -subunit loops 2 and 6 in response to allosteric signals. Within the COMM domain of TrpEb_1, S178 participates in intersubunit signaling with

G181 of TrpEa. Competing allosteric conformations are mediated by alternative salt bridges between K167 of TrpEb_1 and D305 of TrpEb_1 on the one hand, or between K167 of TrpEb_1 and D56 of TrpEa, on the other. When D305 of TrpEb_1 is not occupied with K167, it forms an alternative salt bridge with R141, as shown in Figure 5.

As intersubunit signaling between TrpEb_2 and TrpEa is either lacking or involves different contacts, one might expect the important catalytic residues, but not the allosteric residues, to be conserved in comparison of TrpEb_2 with TrpEb_1. This comparison is shown in Figure 5. Likewise, in those TrpEa proteins that lack a TrpEb_1 partner, instead being forced to function in concert with TrpEb_2, one might expect retention of catalytic residues but loss of allosteric residues. This comparison is shown in Figure 6. Of the intersubunit signaling pair TrpEb_1 S178/TrpEa G181, S178 is not conserved within its own subfamily and equivalent residues seem to exist [26]. It is, however, striking that G181 is invariant in all TrpEa proteins, except for those belonging to the six archaeal lineages lacking TrpEb_1. It is even more striking that K167, which participates in the alternative β - β or β - α salt bridges is invariant in TrpEb_1, but absent in TrpEb_2. Likewise, the salt-bridge partner D305 is invariant in TrpEb_1, but absent in TrpEb_2. The salt-bridge partner D56 in TrpEa is invariant except for the six archaeal lineages that lack TrpEb_1. Figure 5 shows that TrpEb_2 sequences carry an insertion of 16 residues in the general region corresponding to the COMM domain of TrpEb_1 proteins.

Tryptophan gene organization in TrpEb_2-containing genomes

The organization of tryptophan-pathway genes in the ten archaeal genomes and six bacterial genomes that are thus far known to possess TrpEb_2 are displayed on a 16S rRNA tree

**Figure 4**

Unrooted phylogenetic tree (radial view) of the TrpEa protein family. Organismal acronyms are defined in Table 1. Phylogenetic reconstruction of the inferred amino-acid sequence was accomplished by the neighbor-joining method using the PHYLIP program. The detailed order of branching for cyanobacterial and higher-plant sequences is shown in Figure 3. Archaeal proteins are visualized in magenta, and the yellow line indicates the TrpEa protein from *C. trachomatis*, which is probably encoded by a pseudogene.

in Figure 7. *Pyrococcus horikoshii* has lost the entire pathway, and only *trpEb_2* is present. In *Aquifex* and *Chlorobium* all *trp* genes are dispersed, and *trpEa* is not linked to either *trpEb_1* or *trpEb_2*. In Bacteria and Archaea the gene order *trpEb_1* → *trpEa* is one of the most highly conserved of genomic gene arrangements. Often translational coupling exists, as seen in Figure 7 for *P. furiosus*, *P. abyssi*, *M. thermoautotrophicum*, *T. volcanium*, *Archaeoglobus fulgidus* and *T. maritima*. In each of the latter genomes *trpEb_2* is outside the *trp* operon. Both

species of *Thermoplasma* possess an otherwise intact *trp* operon that lacks *trpEb_1* (also not present elsewhere in the genome). Hence, by default it appears that the unlinked *trpEb_2* must function for tryptophan biosynthesis in these organisms. In *A. pernix* and *S. solfataricus* all of the *trp* genes are adjacent, but *trpEb_2* flanks *trpEa*, and *trpEb_1* is absent from these genomes. In each case, a second unlinked copy of *trpEb_2* is present. In *Geobacter sulfurreducens*, both *trpEa* and *trpEb_1* (presumably partnered based on the results shown in Figure 4) are unlinked to one another, and

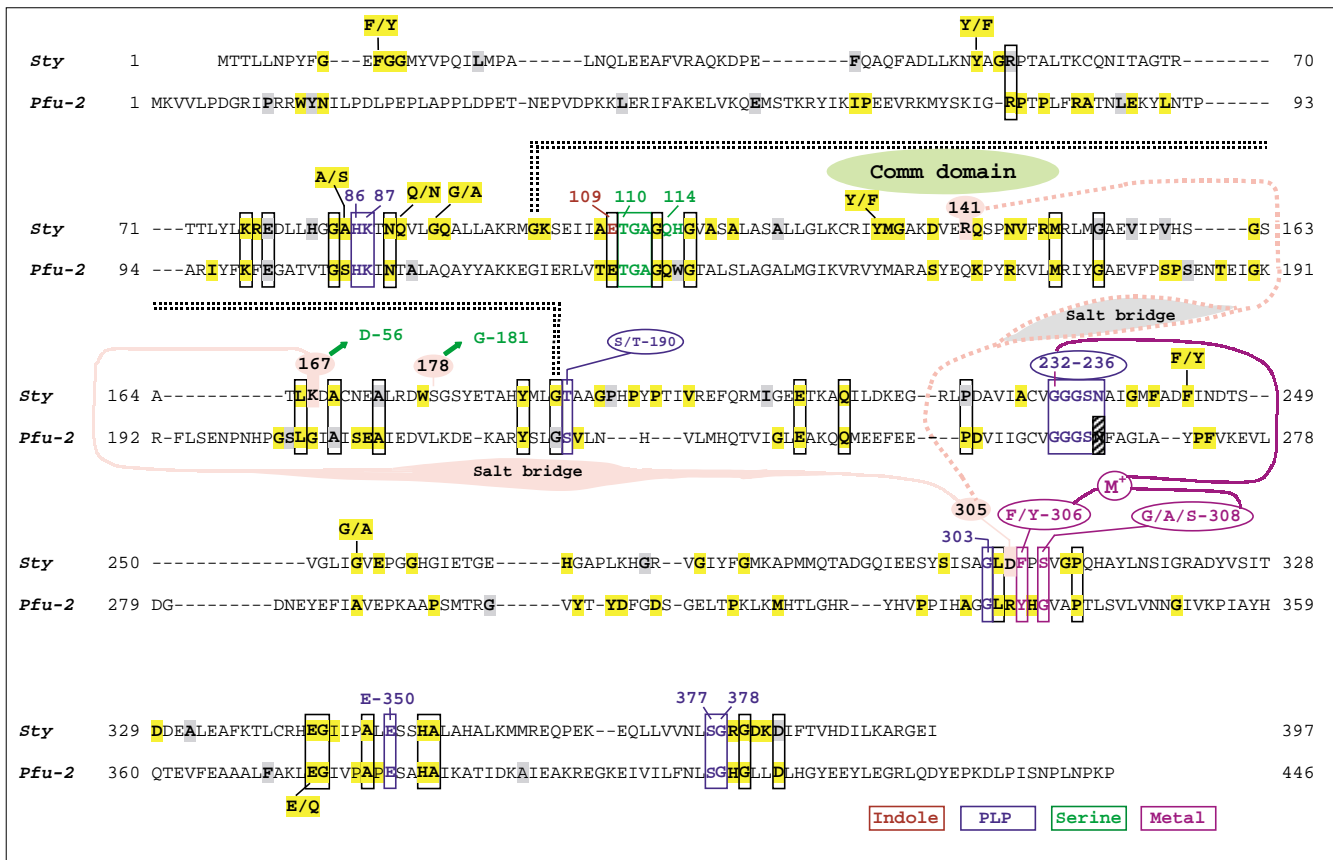


Figure 5
 Alignment of TrpEb_1 from *S. typhimurium* (Sty) and of TrpEb_2 from *P. furiosus* (Pfu-2). The sequences are shown as they appear in a comprehensive alignment, including gaps. Invariant residues in each subfamily are highlighted and near-invariant residues (delineated in Table 2) are shaded. Invariant or near-invariant residues common to both subfamilies are boxed. Residues shown in Sty to be relevant to metal coordination or to the binding of indole, serine or PLP are color-coded as indicated at the bottom.

both are outside an otherwise intact *trp* operon. In this case it seems curious indeed that the operon contains *trpEb_2*.

A snapshot of the incredibly dynamic alteration of tryptophan gene organization in prokaryotes is apparent from Figure 7. Of the organisms shown in Figure 7, the most consistent linkage is that of *trpAa* and *trpAb*. In *A. fulgidus* *trpD* and *trpB* are fused, whereas in *Rhodospseudomonas palustris* *trpAa* and *trpAb* are fused. Operons are sometimes incomplete as with *P. aerophilum* and *G. sulfurreducens*, or fragmented, as with *R. palustris*. In *A. permix* an inverted arrangement yields a divergent transcription of *trpEa* → *trpEb_2* → *trpC* → *trpD* and *trpB* → *trpAa* → *trpAb*. This is also one of the very few cases where the order is *trpEa* → *trpEb* instead of the usual *trpEb* → *trpEa*.

The *Ferroplasma* genome illustrates a case where a non-tryptophan pathway gene, *aroA* encoding DAHP synthase, is a member of the operon (translationally coupled). The implied transcriptional control of DAHP synthase by L-tryptophan potentially could produce growth inhibition by L-tryptophan

because of limitation of precursors needed for L-phenylalanine and L-tyrosine biosynthesis. This is because no other genes encoding DAHP synthase appear to be present in the genome. It would be interesting to know whether the *Ferroplasma* DAHP synthase is sensitive to allosteric control or not. The phenomenon of growth inhibition triggered by exogenous amino acids is exemplified by the effect of exogenous L-phenylalanine upon DAHP synthase in *Neisseria gonorrhoeae* [27] and in other organisms ([27] and references therein).

What is the function of TrpEb_2? As previously discussed, it seems clear that TrpEb_2 has sometimes been pressed into service with TrpEa to function as tryptophan synthase. What might be its function in those situations where *trpEb_2* is isolated away from a typical tryptophan operon, which possesses closely linked or translationally coupled genes specifying *trpEa* and *trpEb_1*?

TrpEb_1 from *S. typhimurium* is the prototype member of a superfamily of pyridoxal phosphate (PLP)-dependent



Figure 6 Alignment of TrpEa from *S. typhimurium* with TrpEa proteins restricted to co-function with a TrpEb_2 type of β subunit. Catalytic residues Sty E49 and D60, as well as other conserved residues shown to be important for indoleglycerol phosphate binding are shown in blue. Other invariant (highlighted) or near-invariant (shaded) residues are marked in yellow. Conserved residues that important for intersubunit signaling with TrpEb_1 are shown in green.

enzymes that are of remote relationship and that catalyze β -replacement and β -elimination reactions [28-30]. These include *O*-acetylserine sulfhydrylase, threonine deaminase, threonine synthase, cystathionine β -synthase, 1-aminocyclopropane-1-carboxylate deaminase, L-serine dehydratase, and D-serine dehydratase. Isolated TrpEb_1 does catalyze the reaction of L-serine dehydratase (deaminase) *in vitro*, but does not support significant levels of the other activities. It does not seem likely that TrpEb_2 catalyzes the latter reactions either, as Psi-Blast of TrpEb_2 sequences did not return hits for them any more avidly than was the case for TrpEb_1 queries.

Perhaps the most likely 'alternative' function of TrpEb_2 proteins is a catalytic activity already established as an *in vitro* activity of isolated TrpEb_1, of which there are two. The L-serine + indole \rightarrow H₂O + L-tryptophan reaction might function alone for tryptophan biosynthesis in cells that acquire indole from the environment. Little information about the availability and utilization of indole in nature

seems to exist. Model organisms such as *E. coli* and *B. subtilis* transport indole poorly and it tends to be toxic, but these organisms lack TrpEb_2.

As stated above, the second established *in vitro* activity of isolated TrpEb_1 dimers is L-serine dehydratase (deaminase): L-serine \rightarrow pyruvate + ammonia. It is indeed suggestive that the presence of TrpEb_2 correlates almost perfectly with the absence of the primary L-serine deaminase (COG1760) used in nature (Table 3). This SdaA class of serine deaminase is an iron-sulfur protein, not a pyridoxal 5'-phosphate protein [31]. It is absent from the Archaea. Although widely distributed in the Bacteria, it is not present in *Thermotoga*, *Aquifex*, *Chlorobium*, *Geobacter* and *Rhodopseudomonas*. *Mycobacterium smegmatis* is the only TrpEb_2-containing organism to possess an SdaA homolog.

There are other candidates for carrying out the serine deaminase reaction. A serine deaminase that is PLP-dependent exists in eukaryotes, but seems to be absent or rare in

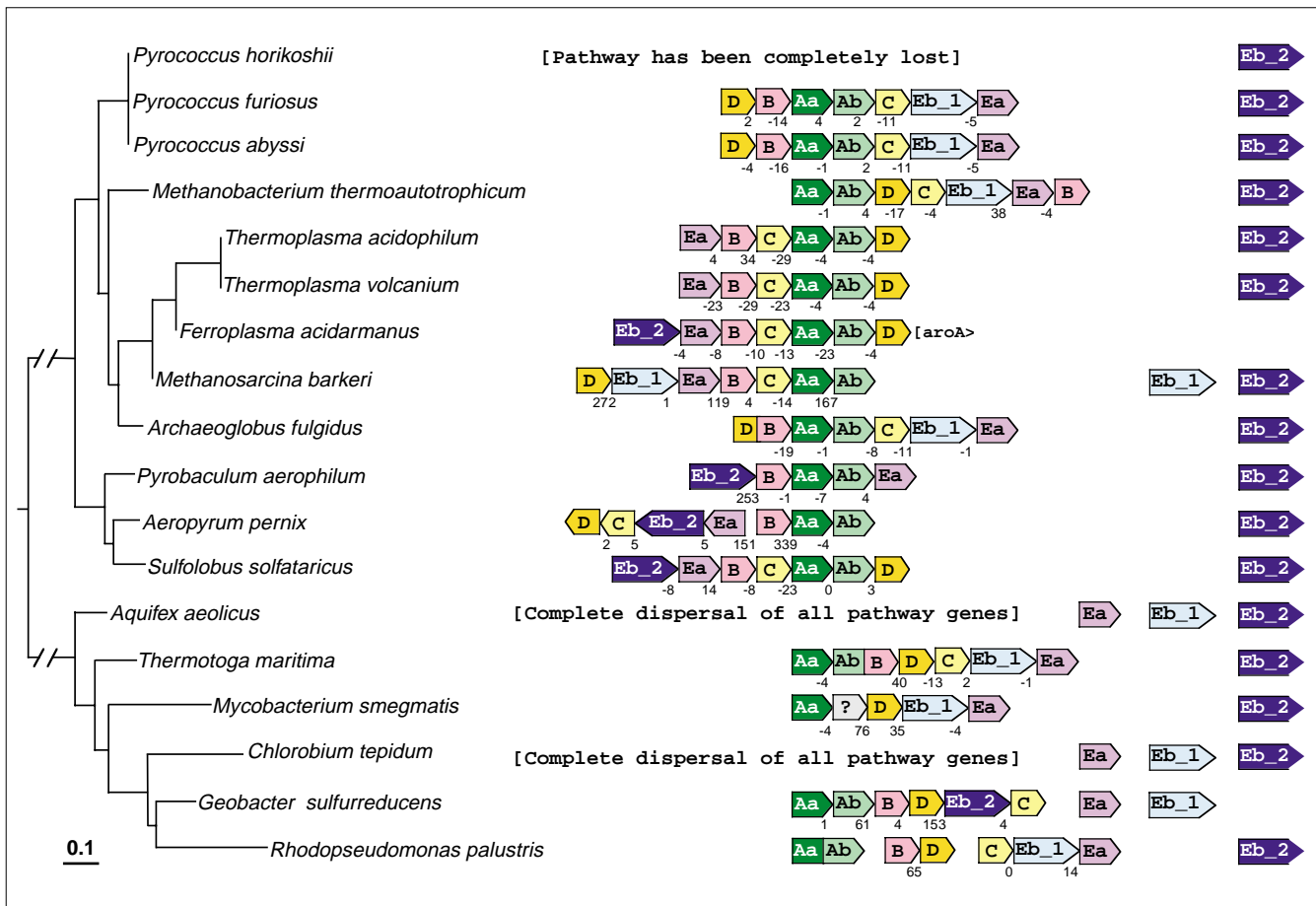


Figure 7 Organization of tryptophan-pathway genes in those archaea and bacteria that possess *trpEb_2*. The 16S rRNA tree of the organisms that possess *trpEb_2* is shown at the left. The tree was obtained from the SUBTREE program of the Ribosomal Database [41,42]. Each of the eight genes is color-coded differently. For economy of space *trpD* is D, *trpC* is C, etc. *aroA* in *F. acidarmanus* belongs to the AroA_β grouping [12]. Flanking genes are shown as boxes touching one another with intergenic spacing indicated. Minus values indicate translational coupling. The open gray box is a conserved hypothetical protein. Pointed box ends indicate the direction of transcription. Space between boxes indicates a lack of genetic linkage.

prokaryotes. Threonine dehydratase has a lesser capability to carry out the serine dehydratase reaction *in vitro*. 'Biosynthetic' (IlvA) and 'catabolic' (TdcA) threonine deaminase are homologs, except that IlvA has a unique carboxy-terminal extension that provides an allosteric module. Most archaea lack *ilvA*, and only a limited number possess catabolic threonine dehydratase.

If archaea lack *ilvA*, what is the source of α -ketobutyrate for isoleucine biosynthesis? It appears likely that α -ketobutyrate is generated instead by the 'pyruvate' pathway in which citramalate is generated as the initial intermediate. A few tracer studies in the older literature have indicated derivation of α -ketobutyrate from pyruvate rather than from threonine [32,33], and (R)-citramalate synthase activity has been shown recently in *M. jannaschii* (MJ1392) [34]. This pathway was initially shown to exist in *Leptospira* [35], and it seems likely that more examples will surface. Indeed, it is

interesting that an enteric bacterium has a latent potential to replace the threonine deaminase step with a pyruvate-derived pathway under appropriate selective conditions [36]. The citramalate pathway for conversion of pyruvate to 2-ketobutyrate involves a carbon-chain elongation mechanism that uses an initial step of condensation with acetyl-CoA, followed by rearrangement, oxidation and elimination of a carbon to produce a keto acid differing from the original substrate by the presence of an additional carbon. This mechanism is familiar in nature, analogous steps being used in the TCA cycle, the ketoacid pathway of lysine biosynthesis, and in leucine biosynthesis. These analogous steps in different pathways were originally used in formulating the recruitment hypothesis for evolutionary acquisition of new function [37], and it is interesting that the citramalate synthase gene shown in *M. jannaschii* had previously been annotated as α -isopropylmalate synthase (which catalyzes the initial step of leucine biosynthesis).

Table 3**Possible sources of serine deaminase in organisms containing TrpEb_2**

Organisms	Serine deaminases		Threonine deaminases	
	Fe-S*	PLP*	TdcA*	IlvA*
<i>Pyrococcus horikoshii</i>	-	-	-	-
<i>Pyrococcus furiosus</i>	-	-	-	-
<i>Pyrococcus abyssi</i>	-	-	-	-
<i>Methanobacterium thermoautotrophicum</i>	-	-	-	-
<i>Thermoplasma acidophilum</i>	-	-	+	-
<i>Thermoplasma volcanium</i>	-	-	+	-
<i>Ferroplasma acidarmanus</i>	-	-	+	-
<i>Methanosarcina barkeri</i>	-	-	-	-
<i>Archaeoglobus fulgidus</i>	-	-	-	-
<i>Pyrobaculum aerophilum</i>	-	-	+	-
<i>Aeropyrum pernix</i>	-	-	+	-
<i>Sulfolobus solfataricus</i>	-	-	+	-
<i>Aquifex aeolicus</i>	-	-	-	-
<i>Thermotoga maritima</i>	-	-	+	-
<i>Mycobacterium smegmatis</i>	+	-	+	-
<i>Chlorobium tepidum</i>	-	-	-	-
<i>Geobacter sulfurreducens</i>	-	-	+	-
<i>Rhodospseudomonas palustris</i>	-	-	+	-

*Sequences that were used to query the databases at NCBI [38], at JGI [39] for Fac and Mba, and at ERGO [40] for Paer and Rpa were gi 2501150 (iron-sulfur (Fe-S) serine deaminases), gi 134387 (PLP-dependent serine deaminases), gi 135723 (catabolic threonine deaminase), and gi 135720 (biosynthetic threonine deaminase).

Overall, the cumulative evidence indicates that established sources of L-serine dehydratase are low or absent in organisms that possess TrpEb_2, and therefore points to a plausible role for TrpEb_2 as L-serine dehydratase.

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