Subsystem: Thiamin biosynthesis

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Thiamin pyrophosphate (vitamin B1) is an essential cofactor for several important enzymes of the carbohydrate metabolism. Many microorganisms, as well as plants and fungi synthesize thiamin, but it is not produced by vertebrates. Thiamin monophosphate is formed by coupling of two independently synthesized moieties, hydroxymethylthiamin-PP (HMP-PP) and hydroxyethylthiazole-P (HET-P).

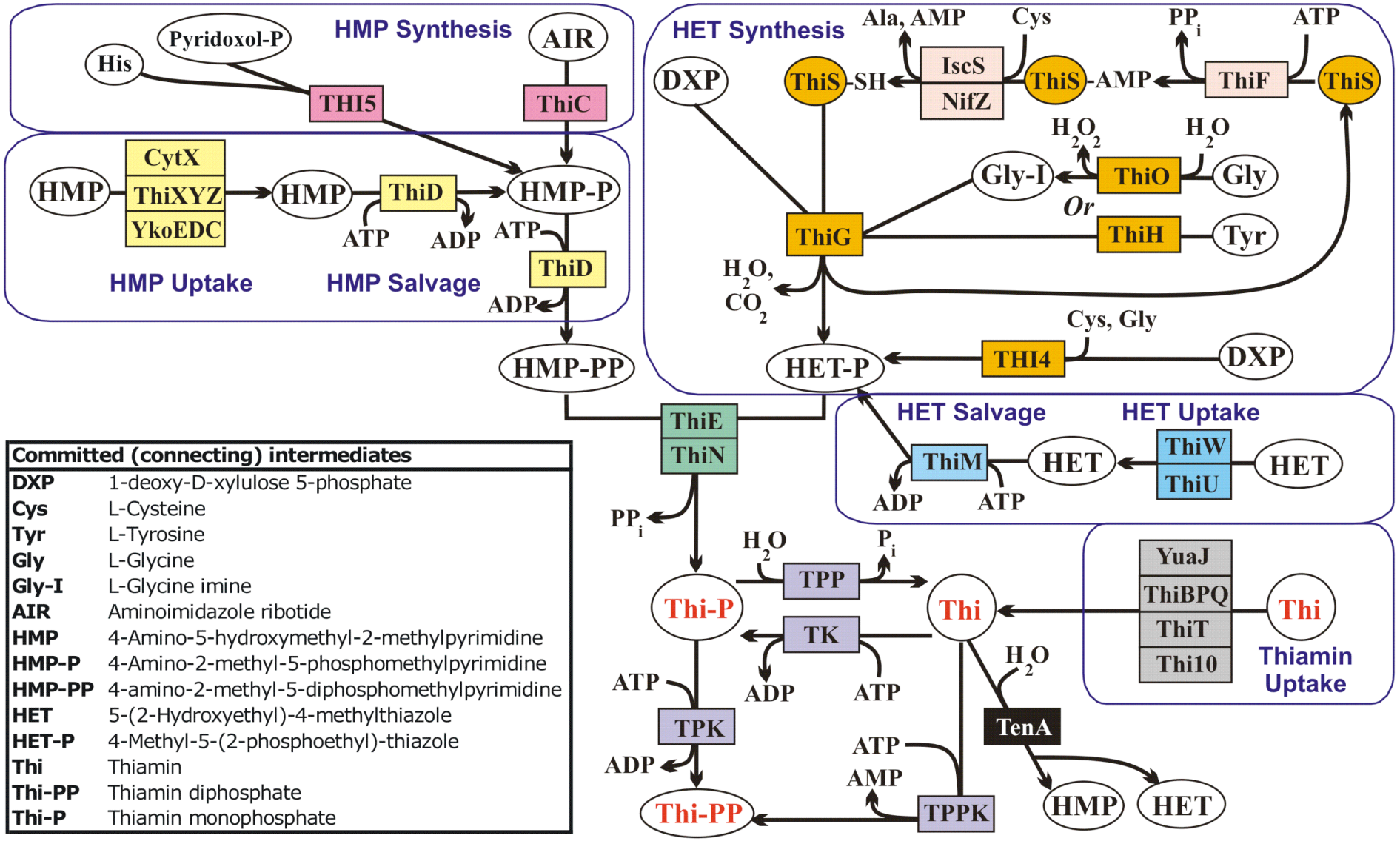
The HET moiety is biosynthesized in *Bacillus subtilis* and most other bacteria from DXP, Glycine, and cysteine in a complex oxidative condensation reaction [1]. This reaction requires five different proteins, ThiO, ThiG, ThiS, ThiF, and a cysteine desulfurase. Glycine oxidase ThiO catalyzes the oxidation of glycine to the corresponding glycine imine. Sulfur carrier protein adenylyl transferase ThiF catalyzes the adenylation of the carboxy-terminus of the sulfur carrier protein ThiS, and cysteine desulfurase catalyzes the transfer of sulfur from cysteine to the ThiS-acyl adenylate to give ThiS-thiocarboxylate. ThiG is the thiazole synthase and catalyzes formation of the thiazole from dehydroglycine, DXP, and ThiS-thiocarboxylate [2, 3, 4]. The thiazole moiety of thiamin in *E. coli* is derived from Tyrosine, cysteine, and DXP using another enzyme (ThiH) of yet unknown function instead of ThiO [5]. In contrast, the THI4 protein family has been shown to be involved in the thiazole synthesis in some eukaryotes [7]. The HET kinase ThiM is involved in the salvage of thiazole from the culture medium.

The conversion of 5-aminoimidazole ribonucleotide (AIR) into HMP is a fascinating reaction of the thiamin biosynthetic pathway in bacteria and is probably the most complex unresolved rearrangement in primary metabolism. The *thiC* gene complements all HMP requiring mutants in *E.coli*, and *B.subtilis* [6]. In yeast and plants, the pyrimidine moiety of thiamin is synthesized using a distinct gene (THI5 in yeasts), and the initial substrates appear to be histidine and pyridoxol-P [8, 9]. HMP-P is phosphorylated by the bifunctional HMP kinase/HMP-P kinase ThiD.

The ThiE protein catalyzes the formation of thiamin-P via coupling of HMP-PP and HET-P moieties. The ThiN protein recently identified in some ThiE-lacking archaea and in *T. maritima* [10] could complement *E.coli thiE* mutant strain and thus presents a case of non-orthologous gene displacement of the thiamin-P synthase ThiE [11]. The thiamin-phosphate phosphatase activity is present in yeast but corresponding gene has not yet been characterized. Bacteria synthesize TPP via single phosphorylation of TP using the ThiL kinase, whereas eukaryotes use distinct pathway to form an active coenzyme TPP: hydrolysis of TP to free thiamin is followed by pyrophosphorylation [8].

Search for thiamin-specific regulatory elements (*THI* riboswitches) and analysis of operon structures identified a large number of new candidate thiamin-regulated genes, mostly transporters, in various bacterial genomes [10]. In particular, the thiamin transporter function was assigned to *yuaJ* in the *Bacillus/Clostridium* group and *thiT* in Archaea. Also previously unknown HMP and HET transporter functions were tentatively assigned to several genes including *thiXYZ* and *ykoEDC* (ABC-type HMP transporters), *cytX* (HMP permease), *thiU* and *thiW* (HET permeases). Identification of the predicted uptake systems for thiamin precursors, HET and HMP, allowed to reconstruct the thiamin pathways in organisms that are unable to synthesize HET and HMP *de novo* and are forced to uptake them via specific transport system [10].

Fig. 1. Thiamin biosynthesis and uptake; HET and HMP salvage pathways



Committed (connecting) intermediates	
DXP	1-deoxy-D-xylulose 5-phosphate
Cys	L-Cysteine
Tyr	L-Tyrosine
Gly	L-Glycine
Gly-I	L-Glycine imine
AIR	Aminoimidazole ribotide
HMP	4-Amino-5-hydroxymethyl-2-methylpyrimidine
HMP-P	4-Amino-2-methyl-5-phosphomethylpyrimidine
HMP-PP	4-amino-2-methyl-5-diphosphomethylpyrimidine
HET	5-(2-Hydroxyethyl)-4-methylthiazole
HET-P	4-Methyl-5-(2-phosphoethyl)-thiazole
Thi	Thiamin
Thi-PP	Thiamin diphosphate
Thi-P	Thiamin monophosphate

ThiG	Thiazole biosynthesis protein ThiG
ThiH	Thiazole biosynthesis protein ThiH
ThiO	Glycine oxidase ThiO (EC 1.4.3.19)
ThiS	Sulfur carrier protein ThiS
ThiF	Sulfur carrier protein adenylyltransferase ThiF
THI4	Thiazole biosynthetic enzyme Thi4

ThiC	Thiamine biosynthesis protein ThiC
THI5	Hydroxymethylpyrimidine synthesis protein THI5
ThiM	Hydroxyethylthiazole kinase (EC 2.7.1.50)
ThiD	Phosphomethylpyrimidine kinase (EC 2.7.4.7)
ThiN	Thiamin-phosphate synthase ThiN (EC 2.5.1.3)
ThiE	Thiamin-phosphate pyrophosphorylase (EC 2.5.1.3)

TenA	Thiaminase II (EC 3.5.99.2)
TPK	Thiamine-monophosphate kinase (EC
TPPK	Thiamin pyrophosphokinase (EC 2.7.6.2)
TK	Thiamine kinase (EC 2.7.1.89)
TPP	Thiamin-phosphate phosphatase (EC 3.1.3.-)

Fig. 2. Thiamin biosynthesis. Subsystem spreadsheet.

		HMP synthesis	HET synthesis			Thi-P synthesis	HMP salvage		HET salvage		Thiamin Uptake
Organism	Variant Code	ThiC-1; THI5-35	ThiG	ThiH-3; ThiO-4	THI4	ThiE-11; ThiN-9	ThiD	HMP Uptake: CytX-21; ThiY-14,ThiX-15,ThiZ-16; YkoE-25,YkoD-26,YkoC-27	ThiM	HET Uptake: ThiW-22; ThiU-23	ThiB-18, ThiP-19, ThiQ-20; YuaJ-17, ThiT-24, THI10-36
Shewanella oneidensis MR-1	1	2216-1	2212	2211-3		2215-11	2215				
Escherichia coli K12	11	3907-1	3903	3902-3		3906-11	2078		2079		69-18, 68-19, 67-20
Mycobacterium tuberculosis CDC1551	2	438-1	431	429-4		428-11	437				
Streptomyces coelicolor A3	22	3885-1	2075	2073-4		2070-11	5516				5595-18, 5596-19, 5597-20
Thermotoga maritima MSB8	3	781-1			780	783-9	783	478-14, 479-15, 477-16			
Pyrobaculum aerophilum str. IM2 [A]	33	229-1			122	1538-9; 1770-9	1538, 1770				227-18, 226-19, 225-20; 123-24
Neurospora crassa [E]	4	9970-35			3898	184-11	6488		184		
Saccharomyces cerevisiae [E]	44	1690-35; 3168-35; 4461-35			2183	5452-11	4958, 5407, 5764		5452		3779-36, 5080-36, 5194-36
Methanococcus maripaludis S2 [A]	5	187-1			1352	1639-9; 1139-11	1639		1138		
Pyrococcus abyssi GE5 [A]	55	683-1			827	1140-9; 1413-9; 1141-11	1140, 1413	1143-21			836-18, 834-19, 835-20
Archaeoglobus fulgidus DSM 4304 [A]	6	2396-1			695	2059-11	2194		2060		
Streptococcus pneumoniae R6	7					630-11; 637-11	1449, 638	2000-14, 2001-15, 1999-16; 631-25, 632-26, 633-27	629, 636	635-22	
Haemophilus influenzae R2866	77					1610-11	1609	1550-14, 1549-15, 1548-16	1608	1611-23	472-18, 473-19, 474-20
Bradyrhizobium japonicum USDA 110	8	6659-1	6657	6655-4		6658-11	5905	2837-14, 2836-15, 2838-16			
Bacillus subtilis subsp. subtilis str. 168	88	878-1	1170	1168-4		1167-11; 3834-11	1172, 3807	1325-25, 1324-26, 1323-27	3835		3102-17
Silicibacter pomeroyi DSS-3	9		48	46-4		49-11	45	51-14, 54-15			
Brucella melitensis 16M	99		1734	1732-4		1735-11	1731	1736-14, 1738-15			284-18, 283-19, 282-20
Streptococcus pyogenes M5	10						161				1584-17
Treponema pallidum subsp. Pallidum	10						114				143-18, 142-19, 141-20

Functional variants:

#1: HMP synthesis (ThiC-1), HET synthesis (ThiG,ThiH-3), thiamin synthase (ThiE-11);
#2. HMP synthesis (ThiC-1), HET synthesis (ThiG,ThiO-4), thiamin synthase (ThiE-11);
#3: HMP synthesis (ThiC-1), HET synthesis (THI4), thiamin synthase (ThiN-9);
#4: HMP synthesis (THI5-35), HET synthesis (THI4), thiamin synthase (ThiN-9);
#5: HMP synthesis (ThiC-1), HET synthesis (THI4), thiamin synthases (ThiE-11 and ThiN-9);
#6: HMP synthesis (ThiC-1), HET synthesis (THI4), thiamin synthase (ThiE-9);
#7: HMP and HET salvage, thiamin synthase (ThiE-11);
#8: HMP synthesis (ThiC-1), HET synthesis (ThiG,ThiO-4), thiamin synthase (ThiE-11), HMP salvage;
#9: HMP salvage, HET synthesis (ThiG,ThiO-4), thiamin synthase (ThiE-11);

#10: only Thiamin Uptake;

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***Variants #11,22,33,44,55,77,88,99 are the same as #1,2,3,4,5,7,8,9, respectively, but also include additional genes for Thiamin Uptake.**

Analysis of regulatory elements. Predicted thiamin-regulated transporter genes [10]

1. YuaJ: predicted Thiamin Transporter (possibly H^+-dependent)

- Found only in bacteria from the *Bacillus/Clostridium* group
- All these species lack the known thiamin ABC transporter ThiBPQ;
- Occurs in genomes without thiamin biosynthetic pathway (*Streptococcus* spp.);
- Has 6 predicted transmembrane segments;
- Regulated by thiamin riboswitches;
- *Bacillus cereus* is able to uptake thiamin in a proton-dependent manner [12].

2. ThiXYZ and YkoEDC: predicted ATP-dependent HMP Transporters

- Found in some Proteobacteria and Firmicutes;
- Not found in genomes without thiamin biosynthetic pathway;
- Always co-occur with the ***thiD*** and ***thiE*** genes;
- Present in *Pasteurellae*, *Brucella* and Gram-positive cocci, lacking HMP synthase **ThiC**;
- Regulated by thiamin riboswitches;
- The substrate-binding component **ThiY** is homologous to the HMP synthase **THI5** from Yeast and has an N-terminal signal peptide for possible membrane binding.

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