# Subsystem: Methionine metabolism

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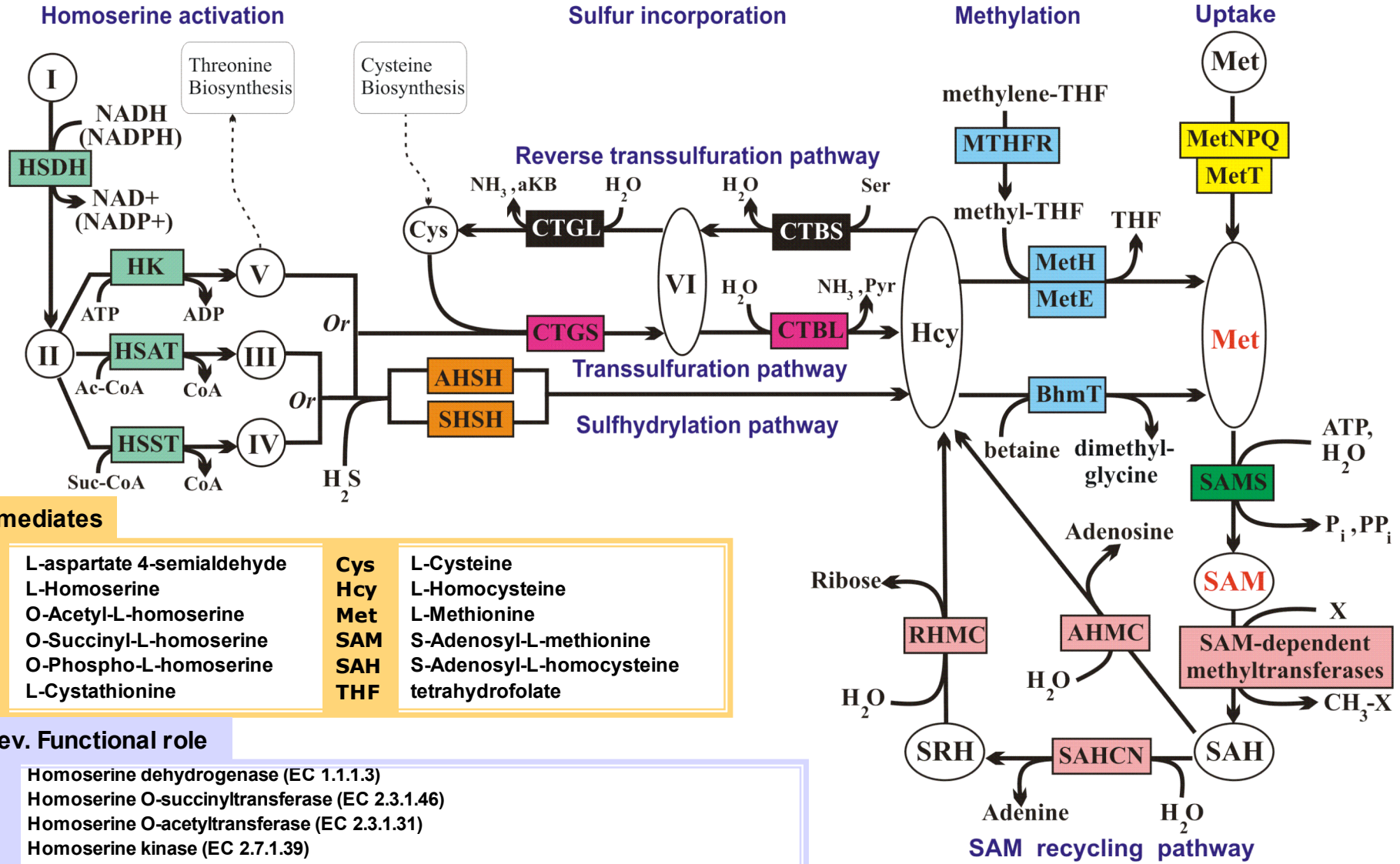
Methionine and cysteine are the two sulfur-containing amino acids. In addition to its general function as a component of proteins, methionine is specifically required for translation initiation and is crucial for a variety of methyltransferase reactions as a precursor of S-adenosyl-Methionine (SAM). Main stages of methionine biosynthetic pathway (Figure 1) are as follows. Homoserine is derived from aspartate semialdehyde by the homoserine dehydrogenase (HSDH). Acylation of homoserine is catalyzed either by homoserine succinyltransferase (HSST) or by homoserine acetyltransferase (HSAT), unrelated to HSST. Yet another variation in homoserine esterification exists in plants, where O-phospho-L-homoserine is produced in a reaction catalyzed by homoserine kinase (HK). Cysteine serves as a precursor for methionine biosynthesis via the Transsulfuration pathway mediated by two enzymes, cystathionine gamma-synthase (CTGS) and cystathionine beta-lyase (CTBL). An alternative pathway for methionine biosynthesis, the sulfhydrylation pathway, utilizes inorganic sulfur instead of cysteine and is catalized by O-acetylhomoserine or O-succinylhomoserine sulfhydrylases (AHSH or SHSH). Both pathways can utilize O-succinyl-L-homoserine or O-acetyl-L-homoserine in different bacterial species. The methylation of homocysteine by methyl-THF in bacteria can be catalyzed by two types of methionine synthases. Reaction catalyzed by coenzyme B12-dependent protein MetH is more than 100-fold faster than the reaction catalyzed by B12-independent isoenzyme MetE. In many bacteria the methyl group of methionine is donated by methyl-THF, which is formed by reduction of methylene-THF in a reaction catalyzed by MTHFR. S-adenosylmethionine (SAM) is synthesized from methionine and ATP by SAM synthetase. Utilization of SAM as a methyl donor results in formation of S-adenosylhomocysteine (SAH), which is then cleaved to homocysteine and adenosine by SAH hydrolase, or in two consequent stages, by S-adenosylhomocysteine nucleosidase (SAHCN) and ribosylhomocysteinase (RHMC). Remarkably, multiple functional variants of methionine biosynthesis in it's flow from homoserine to homocysteine exist in different species:

1. *as in Escherichia coli.* Full Transsulfuration pathway (has CTGS, CTBL) via O-succinyl-L-homoserine (has HSST).
2. *as in Staphylococcus aureus.* Full Transsulfuration pathway via O-acetyl-L-homoserine (has HSAT).
3. *as in Chlorobium tepidum*. Full Sulfhydrylation pathway (has AHSH) via O-acetyl-L-homoserine.
4. *as in Streptococcus spp.*. Full Sulfhydrylation pathway via O-succinyl-L-homoserine.
5. *as in Listeria spp.* Both Sulfhydrylation and Transsulfuration pathways via O-acetyl-L-homoserine
6. *as in Clostridium acetobutylicum*. Both Sulfhydrylation and Transsulfuration pathways via O-succinyl-L-homoserine.
7. *as in plants*. Full Transsulfuration pathway via O-phospho-L-homoserine (has HK).
8. *as in Bacillus cereus*. Both Sulfhydrylation and Transsulfuration pathways via O-succinyl-L-homoserine or O-acetyl-L-homoserine.

Also there are at least four functional variants of the methylation of homocysteine to methionine:

- *as in Bacillus subtilis*. Only B12-independent methionine synthase (MetE)
- *as in Thermotoga maritima.* Only B12-dependent methionine synthase (MetH)
- *as in E. coli.* Both B12-dependent and B12-independent methionine synthases
- *as in Oceanobacillus iheyensis*. Betaine--homocysteine S-methyltransferase (use betaine instead of methyl-tetrahydrofolate).

# Fig. 1. Methionine biosynthesis, uptake, SAM recycling and reverse Met to Cys pathways




## Intermediates

| | | | |
|---|---|---|---|
| I | L-aspartate 4-semialdehyde | Cys | L-Cysteine |
| II | L-Homoserine | Hcy | L-Homocysteine |
| III | O-Acetyl-L-homoserine | Met | L-Methionine |
| IV | O-Succinyl-L-homoserine | SAM | S-Adenosyl-L-methionine |
| V | O-Phospho-L-homoserine | SAH | S-Adenosyl-L-homocysteine |
| VI | L-Cystathionine | THF | tetrahydrofolate |

## Abbrev. Functional role

| | |
|---|---|
| HSDH | Homoserine dehydrogenase (EC 1.1.1.3) |
| HSST | Homoserine O-succinyltransferase (EC 2.3.1.46) |
| HSAT | Homoserine O-acetyltransferase (EC 2.3.1.31) |
| HK | Homoserine kinase (EC 2.7.1.39) |
| AHSH | O-acetylhomoserine sulfhydrylase (EC 4.2.99.10) |
| SHSH | O-succinylhomoserine sulfhydrylase (EC 4.2.99.9) |
| CTGS | Cystathionine gamma-synthase (EC 2.5.1.48) |
| CTBL | Cystathionine beta-lyase (EC 4.4.1.8) |
| MetH | 5-methyltetrahydrofolate--homocysteine methyltransferase (EC 2.1.1.13) |
| MetE | 5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase (EC 2.1.1.14) |
| MTHFR | 5,10-methylenetetrahydrofolate reductase (EC 1.7.99.5) |
| BhmT | Betaine--homocysteine S-methyltransferase (EC 2.1.1.5) |
| SAMS | S-adenosylmethionine synthetase (EC 2.5.1.6) |
| AHMC | Adenosylhomocysteinase (EC 3.3.1.1) |
| SAHCN | S-adenosylhomocysteine nucleosidase (EC 3.2.2.9) |
| RHMC | Ribosylhomocysteinase (EC 3.2.1.148) |
| CTGL | Cystathionine gamma-lyase (EC 4.4.1.1) |
| CTBS | Cystathionine beta-synthase (EC 4.2.1.22) |

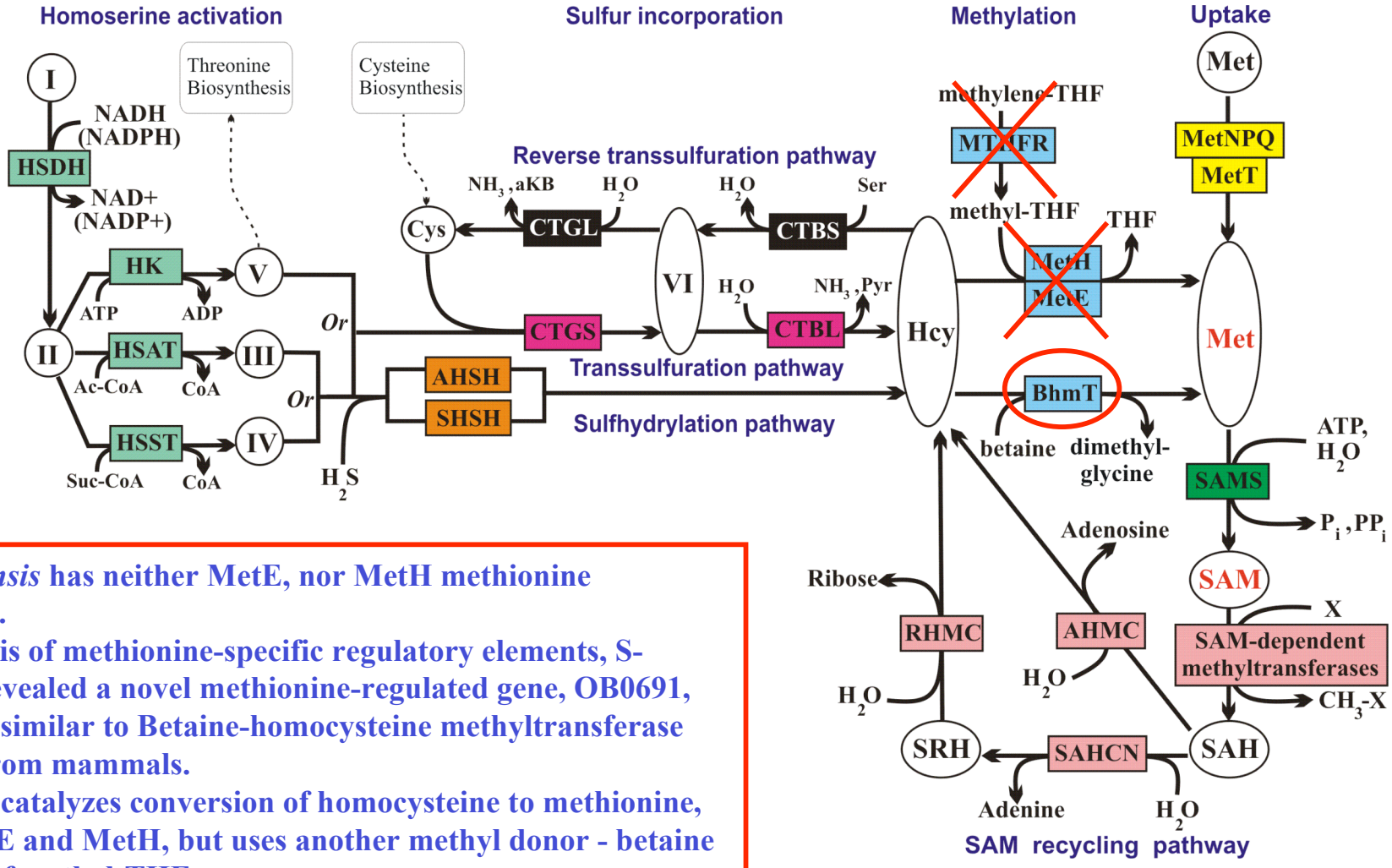
# Fig. 2. Methionine biosynthesis and uptake. Subsystem spreadsheet.

| | | Homoserine activation | | | | Sulfhydrylation | Transsulfuration | | Methylation | | | | Methionine uptake | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Organism | Variant Code | HSDH | HSST | HSAT | HK | AHSH/ SHSH | CTGS | CTBL | MetH | MetE | MTHFR | BhmT | MetNPQ | MetT |
| *Escherichia coli K12* | 1 | 2, 3859 | 3922 | | 3 | | 3858 | 2956 | 3928 | 3756 | 3860 | | 200, 199, 198 | |
| *Vibrio cholerae O1 biovar eltor str. N16961* | 1 | 2335, 2653 | 1592 | | 2334 | | 2652 | 1648 | 385 | 1681 | 2654 | | 895, 894, 893 | |
| *Bacillus subtilis subsp. subtilis str. 168* | 1 | 3232 | 2197 | | 3230 | | 1188 | 1189 | | 1320 | 1101 | | 3280, 3279, 3278 | |
| *Staphylococcus aureus subsp. aureus Mu50* | 2 | 1328 | | 12 | 1330 | | 359 | 358 | | 356 | 357 | | 462, 463, 464; 837, 838, 839 | 2326 |
| *Haemophilus influenzae Rd KW20* | 2 | 88 | | 1208 | 87 | | 85 | 116 | | 1615 | 1378 | | 589, 590 | |
| *Deinococcus radiodurans R1* | 3 | 1461 | | 1055 | 2570, 576 | 1056, 2368 | | | 1149 | | ? | | 1539, 1540, 1541 | |
| *Pseudomonas aeruginosa PAO1* | 3 | 3735 | | 391 | 5492 | 3107, 5022 | | | 2202 | | 1612 | | | 97 |
| *Chlorobium tepidum TLS* | 3 | 1999 | | 599 | 2003 | 598 | | | 1829 | | 1349 | | | |
| *Streptococcus pneumoniae R6* | 4 | 1218 | 1433 | | 1217 | 1094 | 1376 | | | 514 | 515 | | 150, 147 | |
| *Bacteroides fragilis NCTC9343* | 4 | 697 | 706 | | | 1983, 3161, 3403 | | | 1191, 1270 | 2696 | 760 | | | 2185 |
| *Thermotoga maritima MSB8* | 4 | 541 | 873 | | 539 | 874 | 1259 | | 265 | 1274 | 267 | | | |
| *Listeria monocytogenes EGD-e* | 5 | 2537, 541 | | 589 | 2535 | 590 | 1672 | 1671 | | 1673 | 1670 | | 2409, 2408, 2407; 282, 281, 283 | |
| *Geobacter sulfurreducens PCA* | 5 | 1682 | | 2446 | | 1174, 2409 | 936 | 937 | 2901 | | 2954, 855 | | | |
| *Pasteurella multocida Pm70* | 5 | 113 | | 866 | 114 | 738 | 995 | 794 | | 420 | 235 | | 1729, 1730, 1731 | |
| *Clostridium acetobutylicum ATCC 824* | 6 | 1163 | 1979 | | 1396 | 276, 2916 | 559 | 560 | 745 | | 461 | | 1149, 1150, 1151 | |
| *Shewanella oneidensis MR-1* | 6 | 3113, 3680 | 1541 | | 3112 | 1016, 1655 | 3681 | 1991 | 960 | 762 | 3679 | | | 1010 |
| *Synechococcus sp. WH 8102* | 6 | 706 | 845 | | 1476 | 846 | 669 | 670 | 1233 | | 2258 | | | |
| *Bacillus cereus ATCC 10987* | 8 | 2038, 2604, 5497 | 5498 | 4838 | 2040 | 5499 | 4301 | 4302 | 4297 | 4297 | 4298 | | 195, 196, 197; 341, 342, 343; 5090, 5089, 5087 | 1550 |
| *Arabidopsis thaliana* | 7 | 19798, 19799, 24322, 3465 | | | 8718 | | 12026, 3688 | 17034, 17035, 17036 | | 12333, 12334, 23963 | 11589, 17355, 17356, 17357 | | | |
| *Oceanobacillus iheyensis HTE831* | 9 | 472 | 444 | | 470 | 2639, 3050 | 2952 | | | | | 699 | 2098, 2097, 2096; 2381, 2380, 2379 | |
| *Streptococcus pyogenes SSI-1* | 10 | | | | | | | | | | | | 1624, 1626 | |

Functional variants: #1: Transsulfuration pathway via O-succinyl-L-homoserine; #2: Transsulfuration pathway via O-acetyl-L-homoserine; #3: Sulfhydrylation pathway via O-acetyl-L-homoserine; #4: Sulfhydrylation pathway via O-succinyl-L-homoserine; #5: Both pathways via O-acetyl-L-homoserine. #6: Both pathways via O-succinyl-L-homoserine; #8: Both pathways via O-succinyl-L-homoserine or O-acetyl-L-homoserine; #7: Transsulfuration pathway via O-phospho-L-homoserine in plants; #9: BhmT instead of the methionine synthases MetE/MetH; #10: Absence of the *de novo* methionine biosynthetic pathway and the presence of the methionine uptake genes only.

# Open problems, conjectures, comments

## Case 1. Missing methionine synthase gene in *Oceanobacillus iheyensis* – filling the gap




***O. iheyensis*** **has neither MetE, nor MetH methionine synthase.**

**Analysis of methionine-specific regulatory elements, S-boxes, revealed a novel methionine-regulated gene, OB0691, which is similar to Betaine-homocysteine methyltransferase BhmT from mammals.**

**BhmT catalyzes conversion of homocysteine to methionine, like MetE and MetH, but uses another methyl donor - betaine instead of methyl-THF.**

***O. iheyensis*** **is predicted to use a eukaryotic-type methionine synthase BhmT and thus does not require the methylene-THF reductase MetF, which is absent in this bacterium.**

## Case 2. Analysis of the methionine-specific regulatory elements. Prediction of novel methionine-specific transporters.

**MetT: predicted Methionine Transporter from the Na+:H+ Antiporter Superfamily** - Found in some Gram-positive and Gram-negative bacteria

- Has **11** predicted transmembrane segments;
- Regulated by the methionine riboswitch **S-box** in Gram-positive bacteria (e.g. *Clostridium* spp.);
- Regulated by the methionine repressor **MetJ** in some Gram-negative bacteria (e.g. *Vibrio* spp.);
- Occurres in some genomes (*C.perfringens*) that lack both the methionine biosynthetic pathway and known ABC-type methionine transporter MetNPQ, and in this case it is regulated by **S-box**;

# Case 3. Missing methionine biosynthesis genes in Cyanobacteria – open questions

Multiple variations of methionine biosynthesis in it's flow from homoserine to homocysteine exist in different cyanobacteria. *Proclorococci* and *Synechococcus* WH 8102, containing orthologs of HSST and AHSH (clustered on a chromosome), as well as CTGS and CTBL (clustered as well) apparently posses both pathways, each utilizing O-succinyl-L-homoserine (HSST is asserted, while HSAT is not). Based on the presence of HSAT and AHSH homologs in their genomes, *A. variabilis* and *N. punctiforme* utilize only sulfhydrylation pathway with succinyl-CoA as precursor for homoserine esterification. The sulfhydrylation pathway in *S. elongatus* apparently utilizes O-acetyl-L-homoserine (clustered HSST and AHSH orthologs can be asserted in its genome). Surprisingly, none of these pathway variations can be asserted in genomes of *Synechocystis* 6803, *Thermosynechococcus elongatus,* and several other cyanobacterial species. Since orthologs of homoserine dehydrogenase, as well all enzymes catalyzing conversions from homocysteine to methionine are present, these organisms are expected to posses a functional route of L-homocysteine biosynthesis from homoserine. One possibility is that these cyanobacteria harbor a completely novel pathway of homocysteine biosynthesis, yet to be discovered. Alternatively, they may follow the plant route through O-phosphoryl-L-homoserine (orthologs of homoserine kinase are evident in all cyanobacterial genomes). However, homologs of cystathionine gamma-synthase (CTGS) and cystathionine beta-lyase (CTBL), catalyzing these conversions in plants are "missing" from these cyanobacterial genomes and are likely to be encoded by non-orthologous genes. The search for the gene candidates for the "missing" enzymes is in progress and is based on the following assumptions:

**1.** strong gene candidates are expected to be present mainly in the cyanobacterial genomes missing the known pathways of homocysteine biosynthesis, and absent in the organisms where other pathways are evident (**occurrence profile**);

**2.** methionine-specific regulatory sites are likely to be present upstream of the candidate genes (**co-regulation**);

**3.** candidate genes are likely to be adjacent on a chromosome - based on a strong pattern of orthologs clustering in the known pathways of homocysteine biosynthesis (**clustering on a chromosome**).

## Subsystem spreadsheet

| Organism | HSDH | HSST | HSAT | AHSH | CTGS | CTBL | MetH | MetE | MTHFR | SAMS | AHMC |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Crocosphaera watsonii WH 8501* | 3291 | | | | | | | 5009 | 4239 | 4094 | 1914 |
| *Gloeobacter violaceus PCC 7421* | 4295 | | | | | | 477 | | 789 | 2577 | 3183 |
| *Synechocystis sp. PCC 6803* | 2356 | | | | | | 2469<br>2473 | | 1144 | 308 | 1500 |
| *Thermosynechococcus elongatus BP-1* | 277 | | | | | | 1027 | 1090 | 1770 | 977 | 2389 |
| *Synechococcus elongatus PCC 7942* | 1397 | 2172 | | 2173 | | | 702 | | 639 | 1756 | 1953 |
| *Anabaena variabilis ATCC 29413* | 2331 | | 3872 | 3873 | | | 4254<br>6365 | | 6434 | 2194<br>2334 | |
| *Nostoc punctiforme* | 2895 | | 5301 | 5302 | | | 4055 | | 1885 | 2892 | 2754 |
| *Nostoc sp. PCC 7120* | 4427 | | | | | | 619 | | 1093 | 3551<br>4431 | 1724 |
| *Trichodesmium erythraeum IMS101* | 4266 | | | | | | 106<br>1229 | 2279 | 4433 | 4394 | 1091 |
| *Synechococcus sp. WH 8102* | 706 | 845 | | 846 | 669 | 669 | 1233 | | 2258 | 1980 | 116 |
| *Prochlorococcus marinus str. MIT 9313* | 1141 | 875 | | 874 | 225 | 225 | 728 | | 2005 | 1664 | 138 |
| *Prochlorococcus marinus subsp. marinus str. CCMP1375* | 1148 | 799 | | 798 | 404 | 404 | 957 | | 176 | 349 | 1783 |
| *Prochlorococcus marinus subsp. pastoris str. CCMP1986* | 1047 | 640 | | 639 | 405 | 405 | 874 | | 153 | 309 | 1621 |
| *Prochlorococcus marinus MED4 [B]* | 1204 | 1714 | | 1715 | 1 | 1 | 1421 | | 295 | 134 | 548 |