Subsystem: Lysine Biosynthesis DAP Pathway

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I. Introduction

A biosynthesis of L-lysine in various organisms is associated with an amazing diversity of pathways and enzymes involved in metabolism of this amino acid. Two independent strategies of lysine biosynthesis, so-called **alpha-aminoadipate (or AAA) and diaminopimelate (or DAP) pathways,** were initially characterized in fungi and bacteria, respectively. An elegant genomic survey of these pathways reveals an intriguing evolutionary history of these pathways and their relationship with other metabolic systems, such as TCA, Leucine and Arginine biosynthesis [1]. Additional variants of AAA pathway traditionally perceived as a strictly eukaryotic route, were recently uncovered in archaea and deep-branched bacteria of Thermophilus/Deinococcus group [2-4] (a respective SEED subsystem is currently under development).

The subsystem *Lysine Biosynthesis DAP Pathway* is focusing on the analysis of typically bacterial scenarios of lysine production from aspartate (see a diagram). In addition to metabolic and biotechnological importance of its end-product, lysine, this subsystem generates an important intermediate, diaminopimelate (DAP), a building block of peptidoglycan in the cell wall of many (but not all) bacteria. Although DAP pathways and all involved enzymes were studied in great detail in classical models (eg *E.coli*, and *C.glutamicum*), genes encoding a three-step conversion of tetrahydrodipicolinate to LL-2,6-biaminoheptanedioate remain unknown in many other important bacteria (see a diagram). Application of genome context analysis techniques, including chromosomal clustering and shared regulatory elements, allowed to elucidate DAP pathways in a number of diverse bacteria [5]. In the current SEED subsystem we attempted to capture and project the results of this analysis over a larger variety of bacterial species.

II. Subsystem notes, functional variants, open problems and conjectures

(The following slides provide abbreviations of functional roles and diagrams)

A complexity in DAP subsystems comes from the existence of at least three major variations:

Var.1: succinylation-dependent branch (as in *E.coli* and many Gram negative bacteria)

Var.2: acetylation-dependent branch (as in *B.subtilis* and many Gram positive bacteria)

Var.3: dehydrogenase shunt (as in *Bacteroides thetaiotaomicron*)

Var.4: combination of Var.1 and 3 (as in *C.glutamicum* and several related species)

(Var99: no choice could be made between # 2 and 3; missing genes)

An additional complexity arises from the fact that all three enzymes forming the succinylation and acetylation branches belong to large families of paralogs and enzymes with broad specificity. A case of aminotransferase (SDAPAT or ADAPAT) appears particularly challenging. Some data in the literature, as well as the analysis of other subsystem related to metabolism of Arginine, Glutamine and Ornithine, suggests that at least some of the proteins in the argD/astC family may display a very broad specificity. Possible substrates may include both acetylated and succinylated derivatives as well as free ornithine and DAP. That means that the same enzyme may participate in a large variety of pathways (subsystems)

Other problems and observations:

- Missing DAPE in some Gram-positive organisms (eg Leuconostoc mesenteroides; Oenococcus oeni; Lactococcus lactis, etc). Possible candidates: homologs of DAPE1 (predicted based on chromosomal clustering in S.aureus by D.Rodionov and O.Vasieva).
- Streptococcus pyogenes, agalactiae and equi do not have a functional pathway. They should salvage exogenous Lys and use it for cell wall synthesis.
- Fusobacterium nucleatum does not have a pathway but contain DAPE (Lys salvage and reverse reaction?).
- Missing DAPDS in: Campylobacter jejuni, Helicobacter hepaticus

Helicobacter pylori, Wolinella succinogenes DSM 1740 [B]

- Missing SDAPAT in Actinobacillus actinomycetemcomitans and Haemophilus influenzae
- In Thermotoga maritima the whole pathway (variant #2) is in one chromosomal cluster, except missing ADAPAT. Inferred: argD homolog (TM1785)?

Functional Roles, Abbreviations, Subsets and Alternative Forms of Enzymes

Column	Abbrev	Functional Role
1	AK	Aspartokinase (EC 2.7.2.4)
2	ASD	Aspartate-semialdehyde dehydrogenase (EC 1.2.1.11)
3	DHPS	Dihydrodipicolinate synthase (EC 4.2.1.52)
4	DHPR	Dihydrodipicolinate reductase (EC 1.3.1.26)
5	THPST	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase (EC 2.3.1.117)
6	SDAPAT	N-succinyl-L,L-diaminopimelate aminotransferase (EC 2.6.1.17)
7	DAPDS	N-succinyl-L,L-diaminopimelate desuccinylase (EC 3.5.1.18)
8	THPAT	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-acetyltransferase (EC 2.3.1.89)
9	ADAPAT	N-acetyl-L,L-diaminopimelate aminotransferase (EC 2.6.1.-)
10	DAPDA	N-acetyl-L,L-diaminopimelate deacetylase (EC 3.5.1.47)
11	DAPDH	Meso-diaminopimelate D-dehydrogenase (EC 1.4.1.16)
12	DAPE	Diaminopimelate epimerase (EC 5.1.1.7)
13	DAPE1	Diaminopimelate epimerase alternative form predicted for S.aureus (EC 5.1.1.7)
14	DAPDC	Diaminopimelate decarboxylase (EC 4.1.1.20)
15	DAPC	N-succinyl-L,L-diaminopimelate aminotransferase alternative (EC 2.6.1.17)
16	ADAPATp	N-acetyl-L,L-diaminopimelate aminotransferase, possible (EC 2.6.1.-)

Alternative forms

Subsets of roles

Subset	
*ADAPAT	9,16
*DAPE	12,13
*SDAPAT	6,15
Acetylation_pathway	1,2,3,4,8,9,10,12,14
DAP_dehydrogenase_pathway	1,2,3,4,11,14
Succinylation_pathway	1,2,3,4,5,6,7,12,14

Subsystem spreadsheet

Subsystem: Lysine Biosynthesis DAP Path

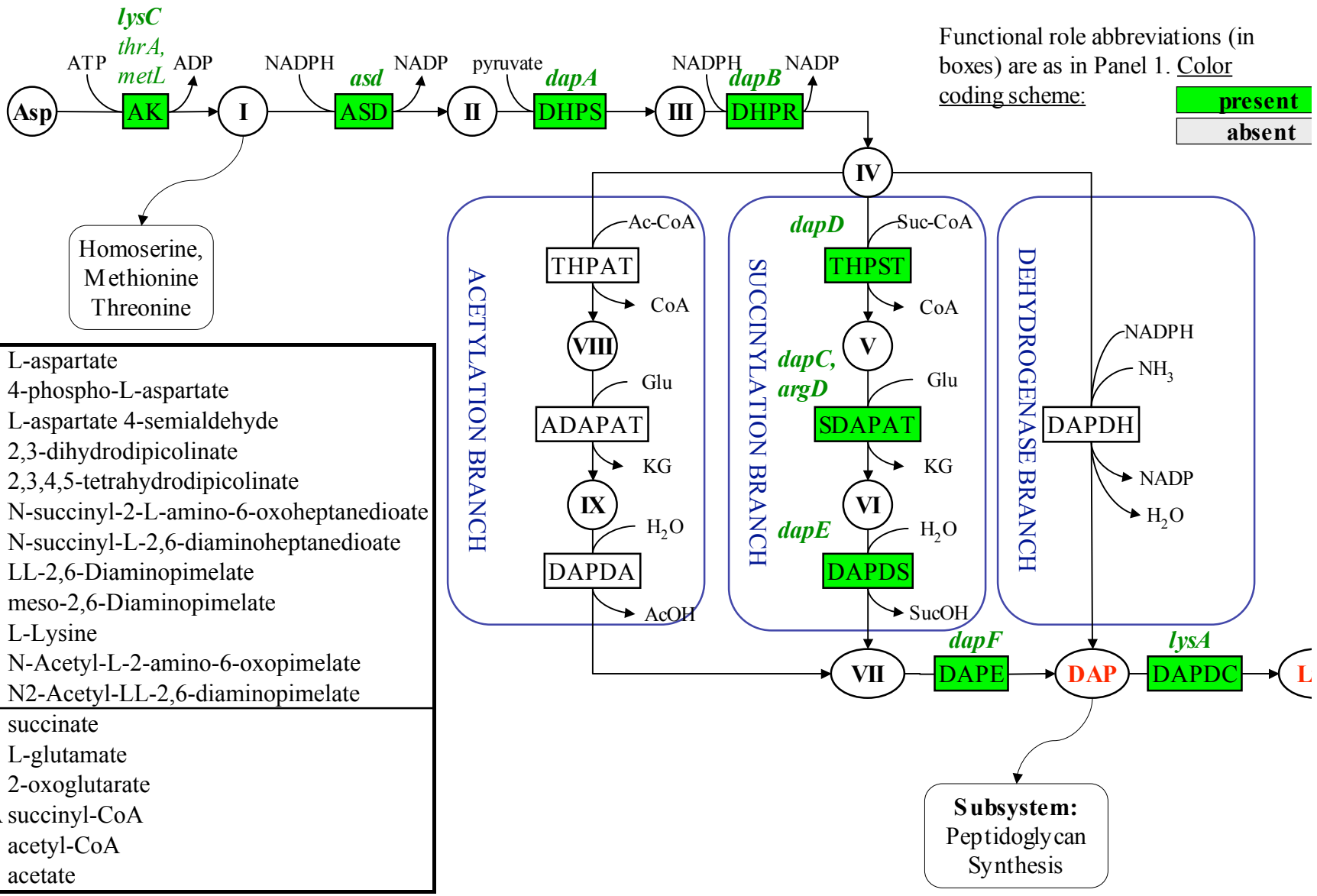
a fragment of the SEED display with selected examples

Organism	Variant Code	AK	ASD	DHPS	DHPR	THPST	*SDAPAT	DAPDS	THPAT	*ADAPAT	DAPDA	DAPDH	*DAPE	DAPDC
Streptococcus agalactiae 2603V/R [B]	-1	337	1024							19-9				
Fusobacterium nucleatum subsp. vincentii ATCC 49256 [B]	-1												1347-12	
Escherichia coli K12 [B]	1	2, 3859, 3933	2293, 3367	2447, 3169	31	166	1731-6, 3294-6	2440					3735-12	2791
Vibrio cholerae O1 biovar eltor str. N16961 [B]	1	2335, 2653, 3549, 386, 539	2011, 2080	1752, 2129	2362	2301	2587-6	2124					125-12, 126-12	124
Bacillus subtilis subsp. subtilis str. 168 [B]	2	1679, 2850, 380	1678	1680, 247	2253		4040-6		1420	1402-9	1421		3223-12	2343
Thermotoga maritima MSB8 [B]	2	1502, 541	1507	1505	1504		1766-6		1503	?	1500		1506-12	1501
Staphylococcus aureus subsp. aureus N315 [B]	2	1203	1269	1270, 311	1271		183-6, 840-6		1272	930-9	1273		2344-12, 1274-13	121, 1275
Bacteroides thetaiotaomicron VPI-5482 [B]	3	2403	3634	895	3319							1979	548-12	1374
Corynebacterium efficiens YS-314 [B]	4	220	221	1864	1866	1163, 1165	1529-6, 1161-15	1166				2498	1837-12	1277
Proteus mirabilis HI4320 [B]	4	1393, 2477, 3668	2512, 446	1359, 2328, 3426, 3460	2762	3041	2593-6	1351				513	771-12, 772-12	133, 2235
Nostoc sp. PCC 7120 [B]	99	3951	3987	3986	2849	?	1390-6	?	?	?	5241		2355-12, 5148-12	3304
Prochlorococcus marinus subsp. pastoris MED4 [B]	99	527	521	522	1479	?	?	?	or ?	?	?		1410-12	1164

Matching colors highlight genes that occur close to each other on the chromosome. Genes (proteins) assigned with respective functional roles are shown by unique FIG IDs. Alternative forms are indicated by additional numbers, dash-separated.”Missing genes” are indicated by “?”. Some of the examples are further illustrated by projection on a subsystem diagram.

Example: *E.coli (variant 1)*

Subsystem: Lysine Biosynthesis DAP Pathw



Functional role abbreviations (in boxes) are as in Panel 1. Color coding scheme:

present

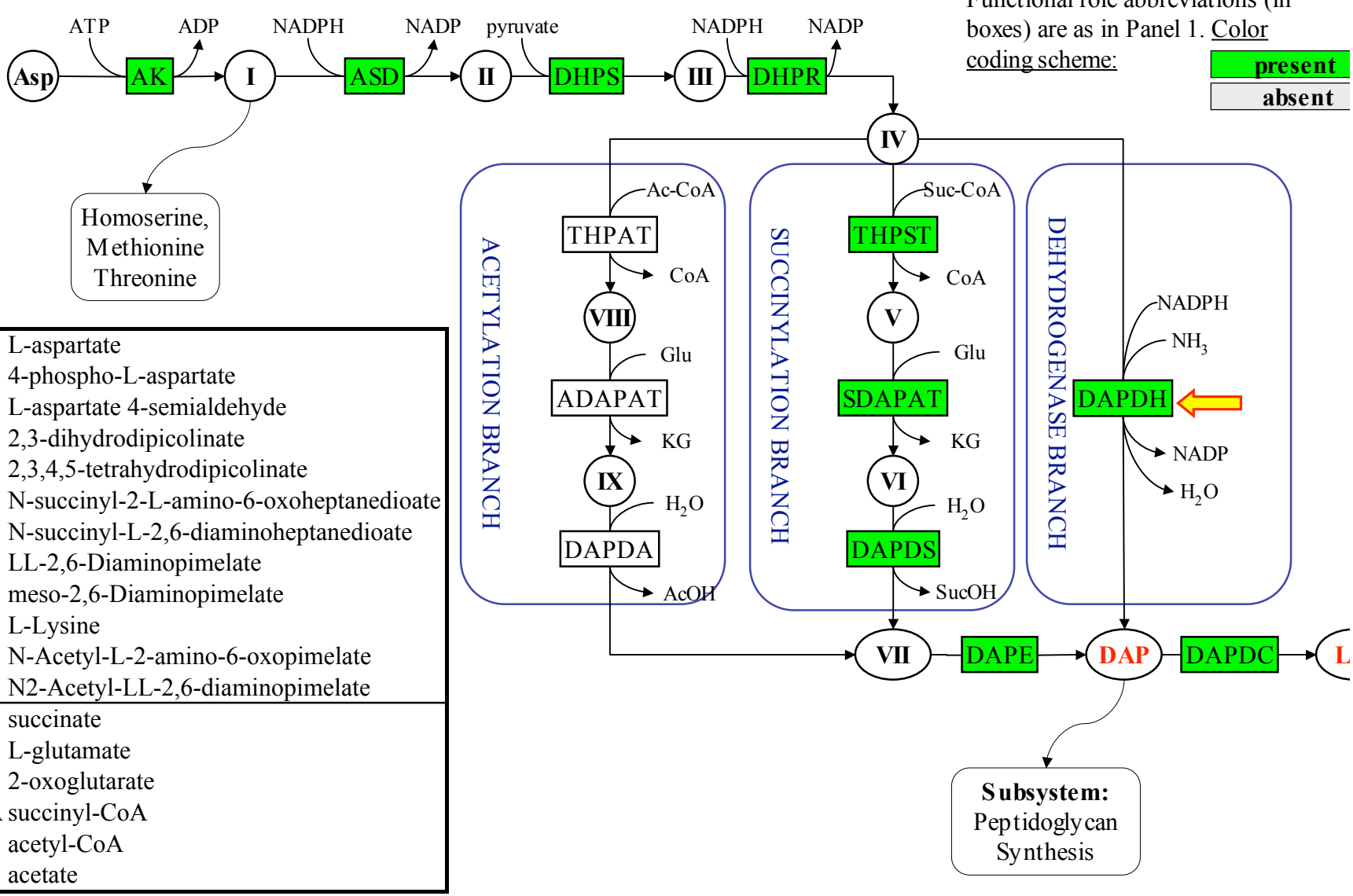
absent

Asp	L-aspartate
I	4-phospho-L-aspartate
II	L-aspartate 4-semialdehyde
III	2,3-dihydrodipicolinate
IV	2,3,4,5-tetrahydrodipicolinate
V	N-succinyl-2-L-amino-6-oxoheptanedioate
VI	N-succinyl-L-2,6-diaminoheptanedioate
VII	LL-2,6-Diaminopimelate
DAP	meso-2,6-Diaminopimelate
Lys	L-Lysine
VIII	N-Acetyl-L-2-amino-6-oxopimelate
IX	N2-Acetyl-LL-2,6-diaminopimelate
SucOH	succinate
Glu	L-glutamate
KG	2-oxoglutarate
Suc-CoA	succinyl-CoA
Ac-CoA	acetyl-CoA
AcOH	acetate

Organism	Variant Code	AK	ASD	DHPS	DHPR	THPST	*SDAPAT	DAPDS	THPAT	*ADAPAT	DAPDA	DAPDH	*DAPE	DAPDC
Escherichia coli K12 [B]	1	2, 3859, 3933	2293, 3367	2447, 3169	31	166	1731-6, 3294-6	2440					3735-12	2791

Example: *Corinebacteria (variant 4)*

Subsystem: Lysine Biosynthesis DAP Pathw

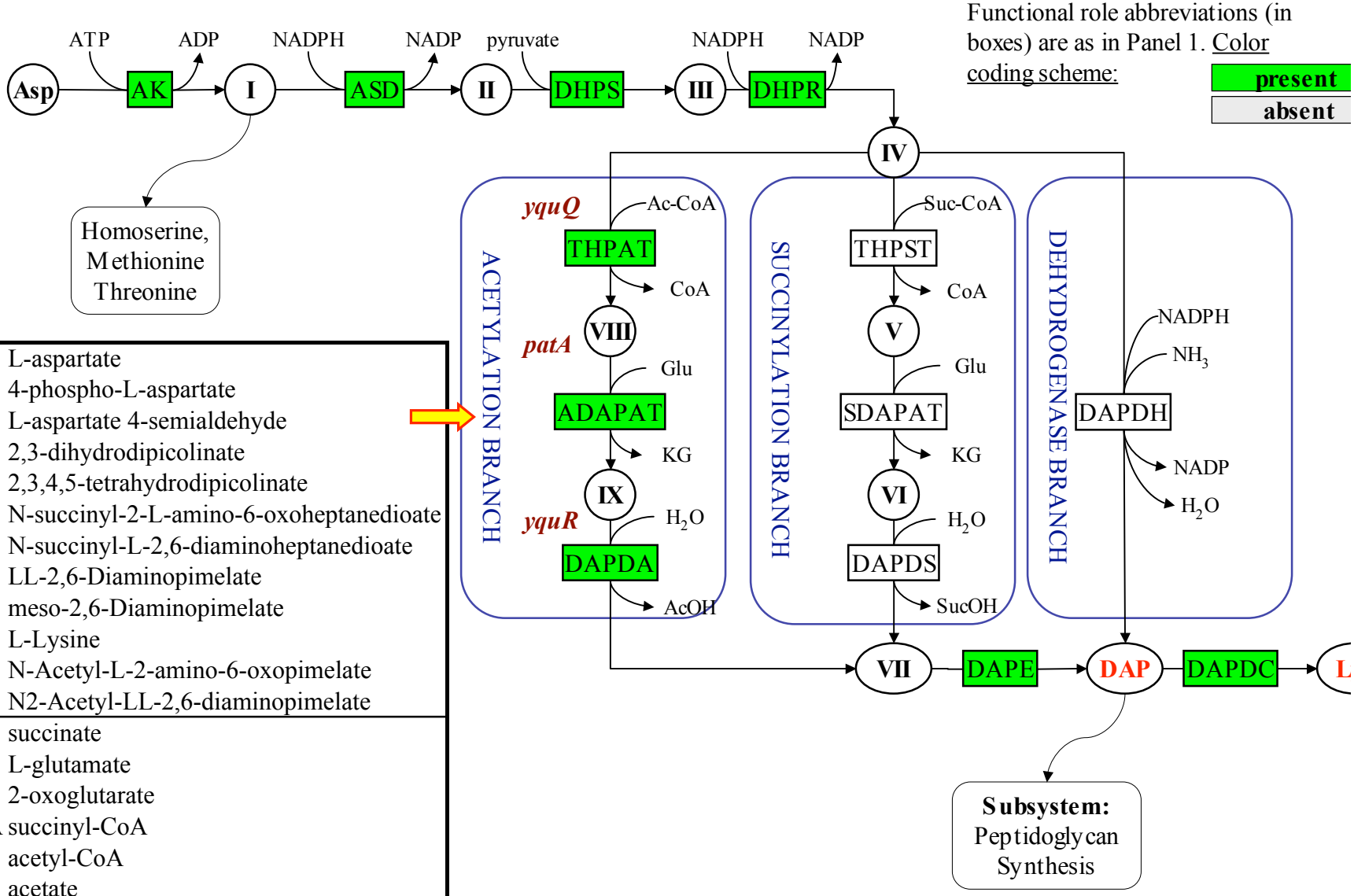


Asp	L-aspartate
I	4-phospho-L-aspartate
II	L-aspartate 4-semialdehyde
III	2,3-dihydrodipicolinate
IV	2,3,4,5-tetrahydrodipicolinate
V	N-succinyl-2-L-amino-6-oxoheptanedioate
VI	N-succinyl-L-2,6-diaminoheptanedioate
VII	LL-2,6-Diaminopimelate
DAP	meso-2,6-Diaminopimelate
Lys	L-Lysine
VIII	N-Acetyl-L-2-amino-6-oxopimelate
IX	N2-Acetyl-LL-2,6-diaminopimelate
SucOH	succinate
Glu	L-glutamate
KG	2-oxoglutarate
Suc-CoA	succinyl-CoA
Ac-CoA	acetyl-CoA
AcOH	acetate

Organism	Variant Code	AK	ASD	DHPS	DHPR	THPST	*SDAPAT	DAPDS	THPAT	*ADAPAT	DAPDA	DAPDH	*DAPE	DAPDC
Corynebacterium efficiens YS-314 [B]	4	220	221	1864	1866	1163, 1165	1529-6, 1161-15	1166				2498	1837-12	1277

Example: *B.subtilis (variant 2)*

Subsystem: Lysine Biosynthesis DAP Pathw



Asp	L-aspartate
I	4-phospho-L-aspartate
II	L-aspartate 4-semialdehyde
III	2,3-dihydrodipicolinate
IV	2,3,4,5-tetrahydrodipicolinate
V	N-succinyl-2-L-amino-6-oxoheptanedioate
VI	N-succinyl-L-2,6-diaminoheptanedioate
VII	LL-2,6-Diaminopimelate
DAP	meso-2,6-Diaminopimelate
Lys	L-Lysine
VIII	N-Acetyl-L-2-amino-6-oxopimelate
IX	N2-Acetyl-LL-2,6-diaminopimelate
SucOH	succinate
Glu	L-glutamate
KG	2-oxoglutarate
Suc-CoA	succinyl-CoA
Ac-CoA	acetyl-CoA
AcOH	acetate

Organism	Variant Code	AK	ASD	DHPS	DHPR	THPST	*SDAPAT	DAPDS	THPAT	*ADAPAT	DAPDA	DAPDH	*DAPE	DAPDC
Bacillus subtilis subsp. subtilis str. 168 [B]	2	1679, 2850, 380	1678	1680, 247	2253		4040-6		1420	1402-9	1421		3223-12	2343

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