Subsystem: NAD and NADP Biosynthesis

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I. Introduction

Nicotinamide dinucleotide (NAD) and its phosphorylated analog (NADP) are indispensable co-factors of numerous oxydoreductases in all forms of cellular life. In addition to that, NAD is a co-substrate for a number of other enzymes such as bacterial DNA ligase, various ADP-ribosylating factors, and a few others. NAD-dependent protein deacetylases of CobB/Sir2 family recently attracted much attention due to their perceived role in a number of regulatory processes in eukaryotic cells [1,2]. Biogenesis and maintenance of NAD pool in various species is one of the most important paradigms of metabolic biochemistry.

Although most of the classic biochemical studies related to NAD biosynthesis were performed in 60-80s [3], many key genes remained unknown until recently. Sequencing and comparative analysis of genomes enabled identification of previously missing genes (for overview see [4,5,6]) and allowed us to project the accumulated knowledge from a handful of model organisms to many others. We have attempted to capture this growing understanding within a framework of the SEED subsystem.

II. Subsystem notes

A table of functional roles (mostly enzymes) directly involved in various aspects of NAD biosynthesis is given in Panel 1. A subsystem diagram reflects the key reactions and pathways leading to the formation of NAD and NADP is presented in Panel 2. Note, that this subsystem in its entirety (including alternative, non-orthologous, forms of enzymes, as defined in Panel 1) is not implemented in any single organism. We have analyzed occurrence of individual functional roles in ~ 300 complete or almost complete genomes included in the current version of the subsystem (available in this release of The SEED). Among analyzed species, 21 archaea, 13 eukaryotes and the rest are bacteria. This analysis allows to infer the presence or absence of semi-independent modules implemented by sub-sets of roles (see Panel 1 and 2), eg “de novo biosynthesis from aspartate” or “salvage of vitamin B3 via NAM/NAPRT”, etc. We have attempted to capture various combination of such modules and other features (eg alternative forms) using a concept of functional variants assigned with numeric “bar-codes” (see Panel 3 and a series of examples after it). The analysis of distribution of functional variants across a variety of species contributes to understanding of the evolution, physiology and enzymology of NAD biosynthesis. In addition to that, it allows to map open problems (eg missing genes) and to suggest a strategy for their elucidation. A short summary of such problems is provided in Section III.

III. Open problems and comments

I. Major cases of missing genes (in genomes with variant codes not containing "9")

1. Missing GAT: **(X1XX)** In total of 18 variants: In at least 122 genomes

Candidates: Nit-paralogs. Alternatively: utilization of non-Gln donor

2. Missing ASPOX: **(3XXX)** In total of 4 variants: In at least 8 genomes

Candidates: SUC/FDH paralogs.

3. Missing NAM: **(XX2X)** In total of 6 variants: In at least 18 genomes

Candidates: isochorismatase family paralogs. Alternatively: salvage of Nicotinic acid form of B3.

4. Missing PnuC: (some XXX1 and XXX2) In total of 4 variants: In at least 9 genomes

Candidates: other membrane proteins, clustered. Alternatively: only intracellular recycling of NmR

Note: in addition to that a gene of NmR transporter has not been identified in eukaryotes (variant XXX3)

5. Missing NMNAT: (XXX2) In total of 2 variants: In at least 7 genomes

Candidates: NaMNAT with broader specificity. Alternatively (more likely): a still unknown NMN deamidase (globally missing gene) converts NMN to NaMN. It was described in *E.coli*, and it is inferred in *Pseudomonas aeruginosa* **[7]** and other genomes with variant code 1112))

6. Missing KFA; (some 4XXX) In total of 3 variants: In 7 genomes

Candidates: distant homologs of KFA or other putative hydrolases (clustered).

II. Miscellaneous missing genes (variant codes containing "9", except obvious "technical problems")

1. Missing NADK: In variants 1918 (*Thermus thermophilus*), 8948 (*Deinococcus radiodurans*), and several others
In at least 10 bacterial genomes

2. Missing QSYN: In variants 9118 (*Desulfitobacterium hafniense*), 9218 (*Bordetella* ssp), and several others
In at least 8 bacterial genomes

III. Out-of-context ("excessive") genes

1. QAPRT homologs in the absence of the de novo pathway: In some 8XXX variants, for example:
variant 8118 (all Streptococcus pneumoniae and pyogenes) In at least 16 bacterial genomes

Rationale: paralog with a different activity. Alternatively: salvage of quinolinate? (in pathogens)

2. PnuC-like transporter homologs in the absence of RNK (in some XXX8) In at least 40 bacterial genomes

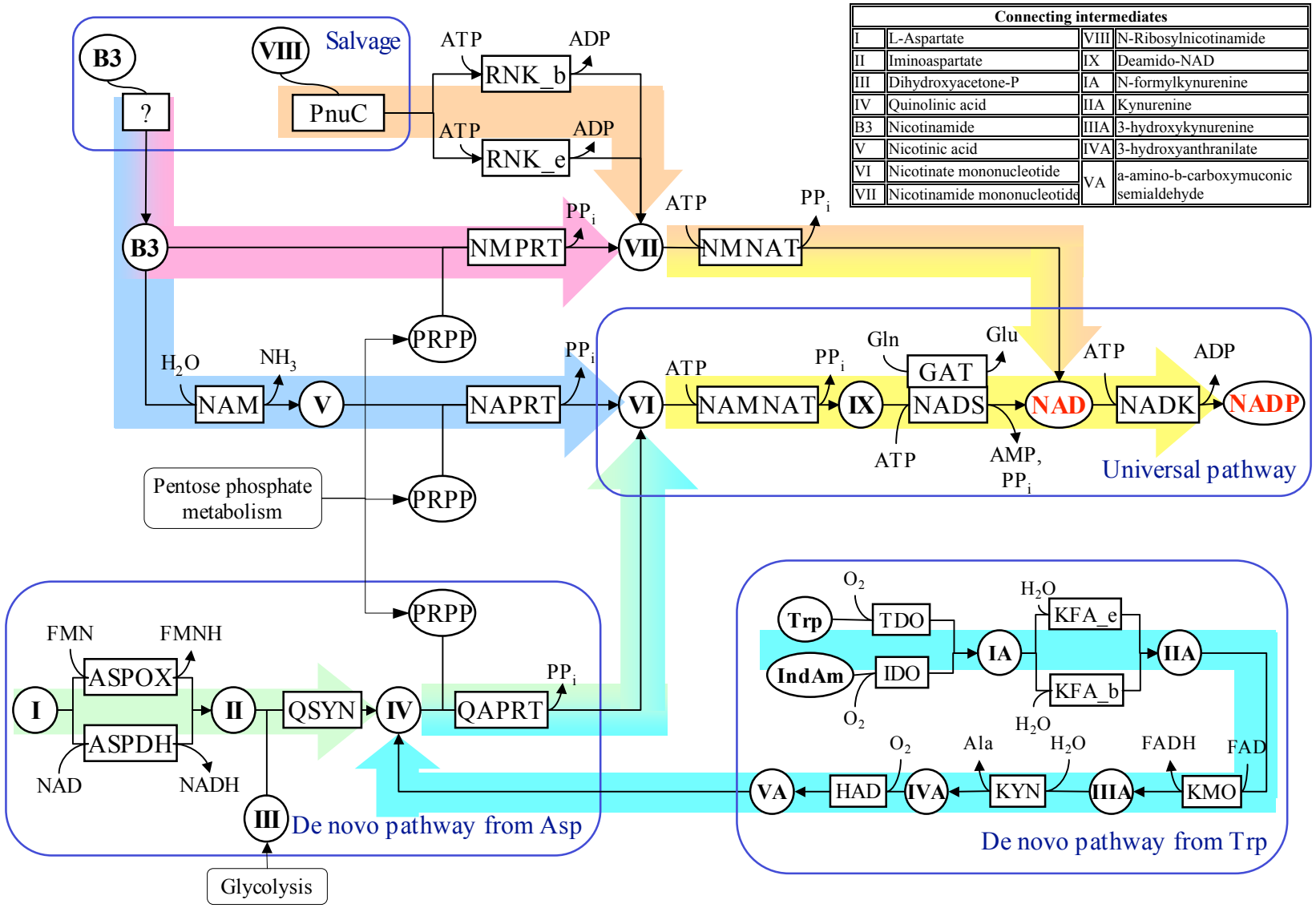
Rationale: transport of other compounds (eg thiamin, as proposed in [**8**]). Alternatively: missing RNK.

1. Functional Roles, Abbreviations, Subsets and Alternative Forms of Enzymes

Alternative forms			Subsystem:NAD/NADP Biosynthesis	Subsets of roles
*TDO	1	TDO	Tryptophan 2,3-dioxygenase (EC 1.13.11.11)	*De novo biosynthesis from Trp*
	2	IDO	Indoleamine 2,3-dioxygenase (EC 1.13.11.42)	
*KFA	3	KFA_e	Kynurenine formamidase (EC 3.5.1.9)	
	4	KFA_b	Kynurenine formamidase, bacterial (EC 3.5.1.9)	
	5	KMO	Kynurenine 3-monooxygenase (EC 1.14.13.9)	
	6	KYN	Kynureninase (EC 3.7.1.3)	
	7	HAD	3-hydroxyanthranilate 3,4-dioxygenase (EC 1.13.11.6)	
*ASPOX	8	ASPOX	L-aspartate oxidase (EC 1.4.3.16)	*De novo biosynthesis from Asp*
	9	ASPDH	Aspartate dehydrogenase [same functional role as] (EC 1.4.3.16)	
	10	QSYN	Quinolinate synthetase (EC 4.1.99.-)	
	11	QAPRT	Quinolinate phosphoribosyltransferase (EC 2.4.2.19)	*In both de novo pathways*
	12	NAMNAT	Nicotinate-nucleotide adenylyltransferase (EC 2.7.7.18)	*Universal pathway(s) via NaMN or/and NMN*
	13	NMNAT	Nicotinamide-nucleotide adenylyltransferase (EC 2.7.7.1)	
	14	NADS	NAD synthetase (EC 6.3.1.5)	
	15	GAT	Glutamine amidotransferase chain of NAD synthetase	
	16	NADK	NAD kinase (EC 2.7.1.23)	
	17	NAM	Nicotinamidase (EC 3.5.1.19)	*2-step salvage of Nicotinamide (via NaMN)*
	18	NAPRT	Nicotinate phosphoribosyltransferase (EC 2.4.2.11)	
	19	NMPRT	Nicotinamide phosphoribosyltransferase (EC 2.4.2.12)	*1-step salvage of Nicotinamide (via NMN)*
	20	PNUC	Ribosyl nicotinamide transporter, pnuC-like	*Salvage of Ribosyl-nicotinamide (via NMN)*
*RNK	21	RNK_b	Ribosylnicotinamide kinase (EC 2.7.1.22)	
	22	RNK_e	Ribosylnicotinamide kinase, ekaryotic (EC 2.7.1.22)	

2. Subsystem diagram

Functional role abbreviations are as in Panel 1. Key intermediates are shown in circles with Roman numerals explained in the inset. Reactions are shown by arrows (directionality shows the flow of biosynthesis without reflecting reversibility). Thick arrows highlight subsystem modules (pathways) using the same colors as for the respective sub-sets of roles (Panel 1)



3. Subsystem variants

Patterns of functional roles used to define major subsystem variants.

Four-positional variant codes XXX (left column) reflect the presence of certain features or modules, such as:
Pos.1: presence/absence and a type of de novo biosynthesis;
Pos.2: known or missing GAT;
Pos.3: presence/absence and a type of B3 salvage pathways
Pos.4: presence/absence and a type of nicotinamide ribose salvage pathways.
Some of the examples (marked by bold font and an asterisk) are illustrated and explained in the following slides

A presence of a gene assigned with a required functional role is shown by "+". Optional features (such as a transporter PnuC, which may not be always reliably assigned) are marked by "±".

Variant codes	Patterns of relevant functional roles distinguishing variants																	Total genomes	EXAMPLES
	De novo pathways								Universal pathway					Salvage/recycling					
	*TDO	*KFA	KMO	KYN	HAD	*ASPOX	QSYN	QAPRT	NAMNAT	NMNAT	NADS	GAT	NADK	NAM	NAPRT	NMPRT	PNUC	*RNK	
Variants with a universal pathway (PNAT-NADS-NADK) with a known ([X2XX]) or missing ([X1XX]) GAT, and:																			
+ de novo biosynthesis from aspartate via ASPOX-QSYN-QAPRT [1{1,2}XX], and																			
++ salvage of: B3 via NAM-NAPRT, and/or VIII via (PNUC)-RNK-(NMNAT) pathways																			
1111						+[8]	+	+	+	+	+	?	+	+	+		+	+[21]	15	***Escherichia coli K12****
1211						+[8]	+	+	+	+	+	+	+	+	+		±	+[21]	14	*Nostoc punctiforme*
1112						+[8]	+	+	+		+	?	+	+	+		±	+[21]	6	*Pseudomonas aeruginosa*
1282						+[8]	+	+	+		+	+	+				+	+[21]	1	*Novosphingobium aromaticivorans*
++ salvage of B3 via (NAM)-NAPRT or/and NMPRT pathways																			
1118						+[8]	+	+	+		+	?	+	+	+				32	*Bacillus subtilis*
1218						+[8]	+	+	+		+	+	+	+	+				35	***Aquifex aeolicus****
1128						+[8]	+	+	+		+	?	+		+				2	*Fusobacterium nucleatum*
1228						+[8]	+	+	+		+	+	+		+				6	*Coxiella burnetii RSA 493*
1138						+[8]	+	+		+	+	?	+			+			1	*Francisella tularensis*
1238						+[8]	+	+	+	+	+	+	+			+			1	*Synechocystis sp. PCC 6803*
1248						+[8]	+	+	+	+	+	+	+	+	+	+			9	*Acinetobacter sp. ADP1*
-- no evidence of salvage pathways																			
1188						+[8]	+	+	+		+	?	+						2	*Wolinella succinogenes*
1288						+[8]	+	+	+		+	+	+						21	***Prochlorococcus marinus****
or + de novo biosynthesis from aspartate via ASPDH-QSYN-QAPRT [2{1,2}XX]																			
2118						+[9]	+	+		+	+	?	+	+	+				2	***Pyrobaculum aerophilum****
2218						+[9]	+	+	+		+	+	+	+	+				1	*Thermotoga maritima*
2128						+[9]	+	+		+	+	?	+		+				3	*Methanococcoides burtonii*
2188						+[9]	+	+		+	+	?	+						4	*Methanocaldococcus jannaschii*
or + de novo biosynthesis from aspartate with missing ASPOX/ASPDH [3 {1,2} X X]																			
3111						?	+	+	+	+	+	?	+	+	+		+	+[21]	2	*Shigella flexneri 2a*
3118						?	+	+	+	+	+	?	+	+	+				2	*Corynebacterium glutamicum*
3188						?	+	+	+	+	+	?	+						3	***Helicobacter pylori **
3288						?	+	+	+	+	+	+	+						1	*Ehrlichia canis str. Jake*
or + de novo biosynthesis from tryptophan [4{1,2}XX]																			
4213	+[2]	?	+	+	+		+	+	+	+	+	+	+	+	+			+[22]	3	*Saccharomyces cerevisiae*
4243	+	+[3]	+	+	+		+	+	+	+	+	+	+	+	+	+		+[22]	2	***Homo sapiens****
4241	+[1]	?	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+[21]	1	*Stenotrophomonas maltophilia*
4248	+[1]	?	+	+	+		+	+	+	+	+	+	+	+	+	+			2	*Xanthomonas axonopodis*
or + only salvage pathways [8{1.2}XX]																			
8111									+	+	+	?	+	+	+		+	+[21]	1	*Lactococcus lactis*
8211									+	+	+	+	+	+	+		+	+[21]	1	*Proteus mirabilis*
8213									+	+	+	+	+	+	+			+[22]	1	*Schizosaccharomyces pombe*
8118									+		+	?	+	+	+				40	***Staphylococcus epidermidis **
8218									+		+	+	+	+	+				11	*Bartonella quintana*
8121									+	+	+	?	+		+		+	+[21]	1	*Leuconostoc mesenteroides*
8128									+		+	?	+		+				3	*Mycoplasma penetrans HF-2*
8228									+		+	+	+		+				3	*Plasmodium falciparum*
8138									+		+	?	+			+			2	*Mycoplasma genitalium*
8141									+	+	+	+	+	+	+	+	+	+[21]	1	*Oenococcus oeni PSU-1*
Variants without a universal pathway [88XX] byppassing NADS-independent:																			
- salvage of B3 via NMPRT and/or VIII via (PNUC)-RNK-NMNAT																			
8831										+			+			+	±	+[21]	5	*Mannheimia haemolytica*
8881										+			+				±	+[21]	4	***Haemophilus influenzae****
- inferred salvage of NAD																			
8884													+						10	*Wolbachia pipientis*

NAD and NADP Biosynthesis

Missing genes inferred by the functional context analysis are shown by "?". Only three major "missing gene" cases are reflected in this table. Other cases are less frequent. At least some of them are due to "technical problems" (eg incomplete genomes, imperfect ORF detection, etc). The respective variants codes contain "9" in one of the four positions (not shown in this table).

Several functional roles (marked with "*") aggregate two alternative enzyme families (as defined in Panel 1). The occurrence of a specific form is shown by a role number (as in Panel 1).

Modified from the full subsystem spreadsheet in the SEED, which also provides more detailed notes.

4. Subsystem basic spreadsheet

a fragment of the SEED display with selected examples

		De novo biosynthesis from tryptophan					*De novo biosynthesis from aspartate*			*Universal pathways via NaMN or/and NMN*					*Salvage/recycling pathways*				
Organism	**Variant Code**	***TDO**	***KFA**	**KMO**	**KYN**	**HAD**	***ASPOX**	**QSYN**	**QAPRT**	**NAMNAT**	**NMNAT**	**NADS**	**GAT**	**NADK**	**NAM**	**NAPRT**	**NMPRT**	**PNUC**	***RNK**
Escherichia coli K12 [B]	11110						2545-8	736	109	640	4298	1723	?	2581	1751, 1851	916		737	4298-21
Aquifex aeolicus VF5 [B]	12180						549-8	1313	605	27		669	669	632	695	733			
Acinetobacter sp. ADP1 [B]	12480						2359-8	594	55		2376	2619	2619	2039	3255	3236	883	948	
Prochlorococcus marinus MED4 [B]	12880						350-8	1670	259	727		726	726	292, 957					
Pyrobaculum aerophilum str. IM2 [A]	21180						2190-9	2191	2190		995, 1576	841	?	2192, 410	2109	2101			
Helicobacter pylori J99 [B]	31880						?	1270	1269	1252		309	?	1429					
Stenotrophomonas maltophilia K279a [B]	42410	3084-1	?	134	133	132			2412	428	1580, 769	1014	1014	139	1584	4301	1582	1646, 768	769-21
Homo sapiens (Human) [E]	42430	24524-1, 29968-2	14251-3	3138	18474	17562			11798	232, 2579, 2580	232, 2579, 2580	6020	6020	17183, 24887, 84, 86		30577	29005, 29006, 3598		15176-22, 15177-22, 31182-22
Staphylococcus epidermidis ATCC 12228 [B]	81180									1280		1596	?	696	1601	1597			
Plasmodium falciparum 3D7 [E]	82280									4879	4879	4491	4491	4359		3634			
Organism	**Variant Code**	***TDO**	***KFA**	**KMO**	**KYN**	**HAD**	***ASPOX**	**QSYN**	**QAPRT**	**NAMNAT**	**NMNAT**	**NADS**	**GAT**	**NADK**	**NAM**	**NAPRT**	**NMPRT**	**PNUC**	***RNK**
Haemophilus influenzae Rd KW20 [B]	88810								1404		731			70					731-21
Wolbachia pipientis wMel [B]	88840													775					

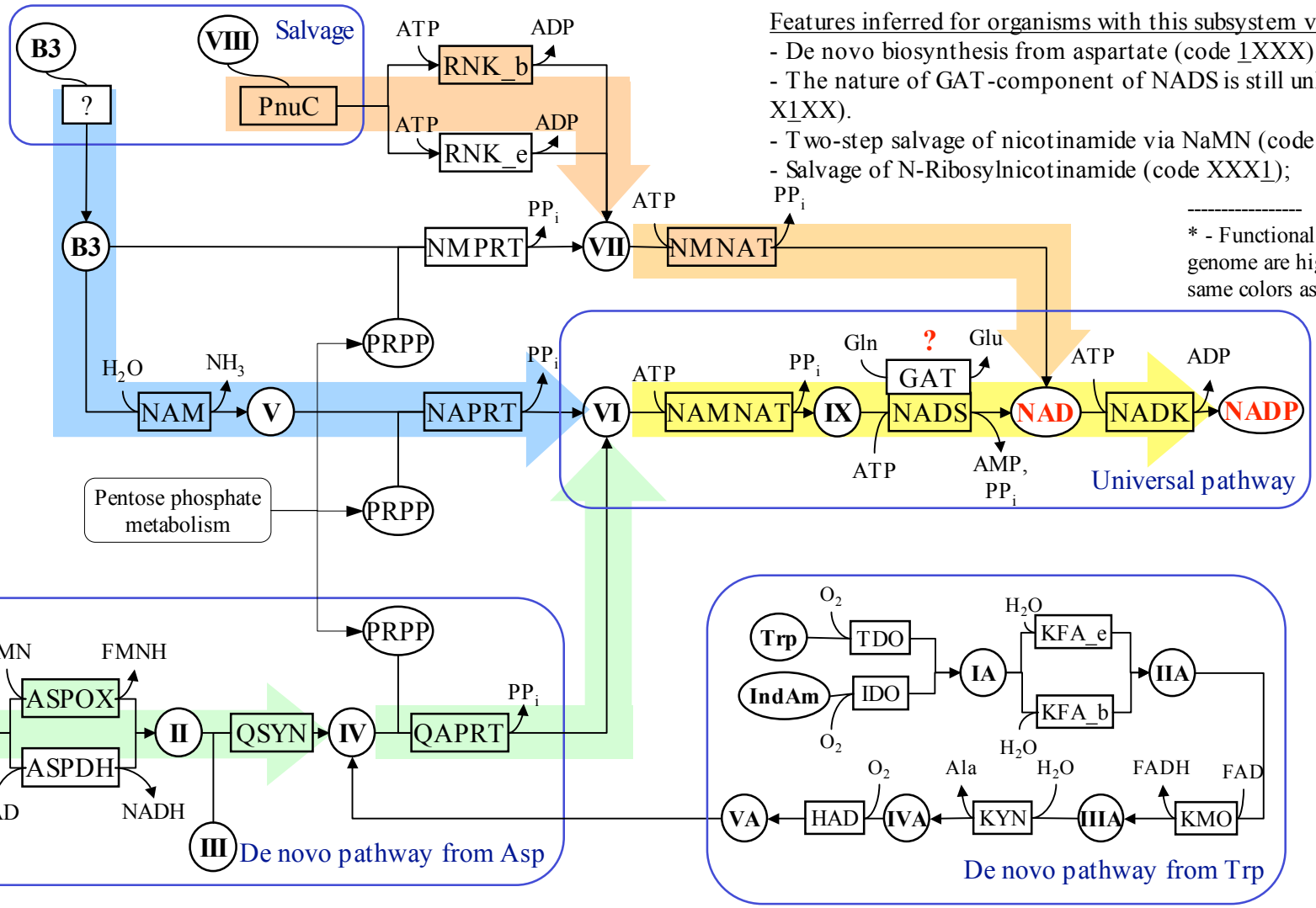
In this table, highlighting reflects a proximity of genes on the chromosome. In each row, matching colors mark genes that occur in the same chromosomal cluster. Some of these examples are further illustrated by projection on a subsystem diagram. Genes (proteins) assigned with respective functional roles are shown by unique FIG IDs. Alternative forms are indicated by underlined numbers. Inferred missing gene cases are marked with "?".

Examples of subsystem variants

Variant 1111: *E.coli K-12*

(and 14 other genomes)

Organism	Variant Code	*TDO	*KFA	KMO	KYN	HAD	*ASPOX	QSYN	QAPRT	NAMNAT	NMNAT	NADS	GAT	NADK	NAM	NAPRT	NMPRT	PNUC	*RNK
Escherichia coli K12 [B]	11110						2545-8	736	109	640	4298	1723		2581	1751, 1851	916		737	4298-21



Features inferred for organisms with this subsystem variant*:

- De novo biosynthesis from aspartate (code 1XXX);
- The nature of GAT-component of NADS is still unknown (code X1XX).
- Two-step salvage of nicotinamide via NaMN (code XX1X);
- Salvage of N-Ribosylnicotinamide (code XXX1);

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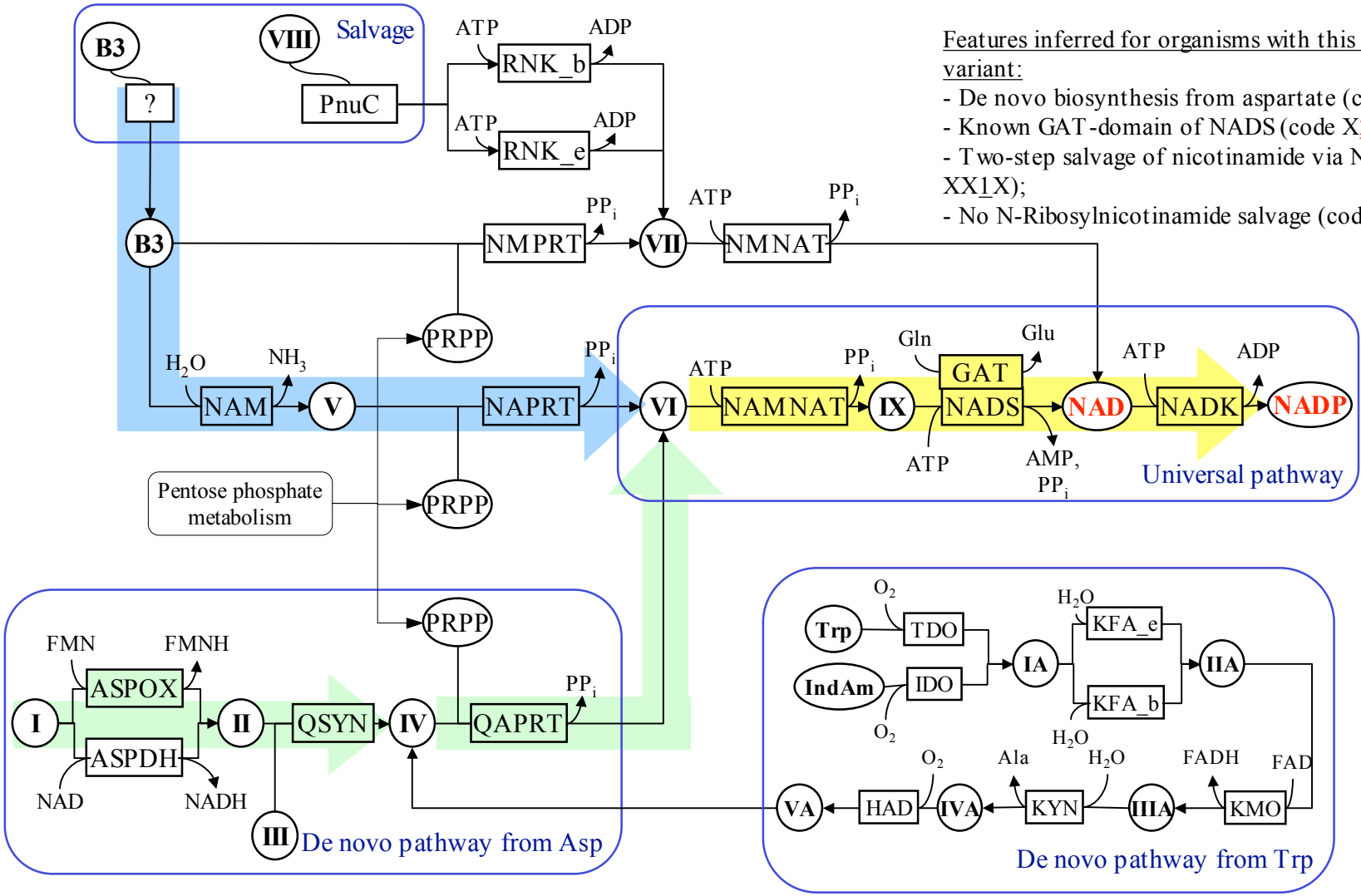
* - Functional roles present in the genome are highlighted using the same colors as in Panel 1- 3.

Examples of subsystem variants

Variant 1218: *Aquifex aeolicus*
(and 34 other genomes)

Organism	Variant Code	*TDO	*KFA	KMO	KYN	HAD	*ASPOX	QSYN	QAPRT	NAMNAT	NMNAT	NADS	GAT	NADK	NAM	NAPRT	NMPRT	PNUC	*RNK
Aquifex aeolicus VF5 [B]	12180						549-8	1313	605	27		669	669	632	695	733			

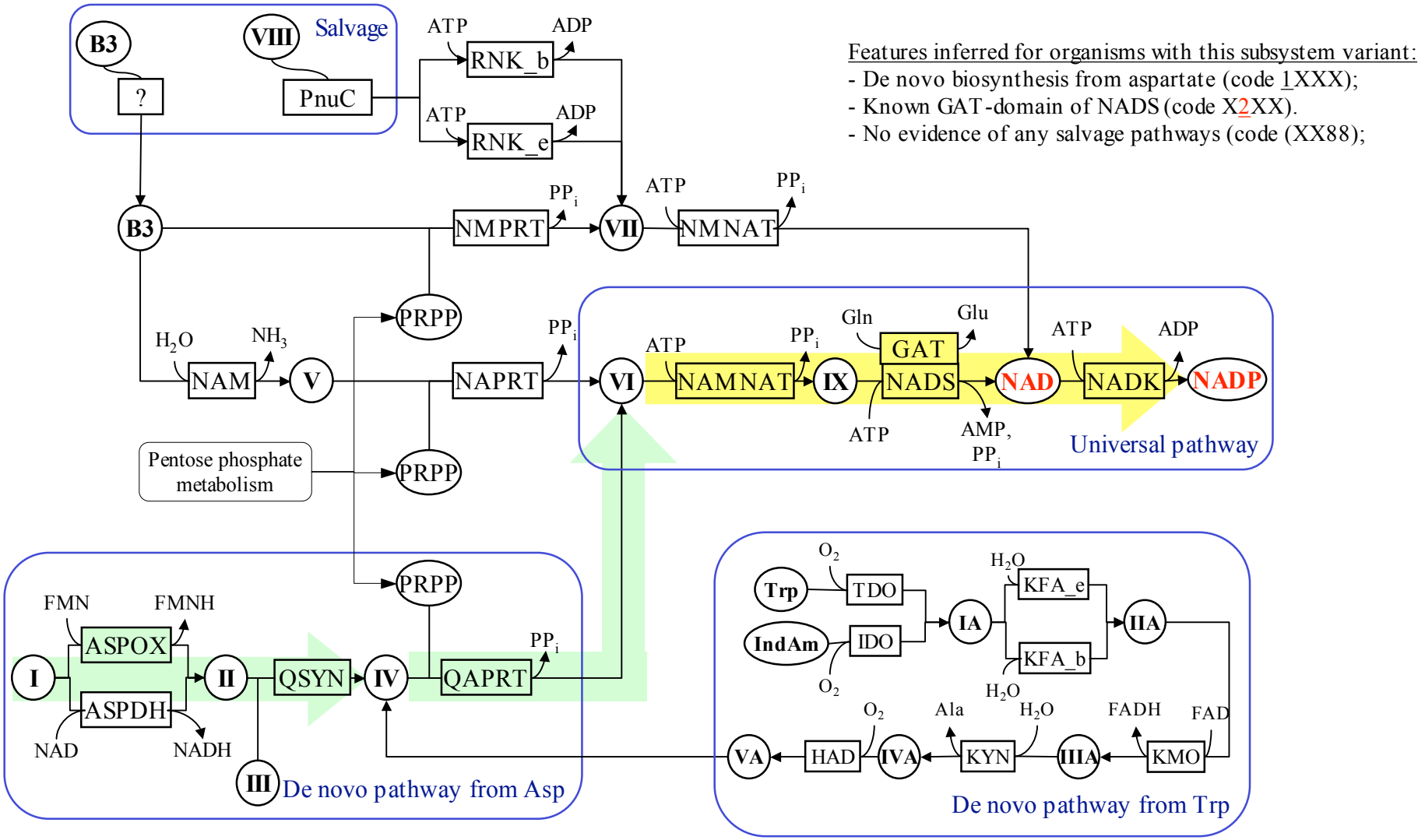


Features inferred for organisms with this subsystem variant:
- De novo biosynthesis from aspartate (code 1XXX);
- Known GAT-domain of NADS (code X2XX).
- Two-step salvage of nicotinamide via NaMN (code XX1X);
- No N-Ribosylnicotinamide salvage (code XXX8);

Variant 1288: *Prochlorococcus marinus MED4*

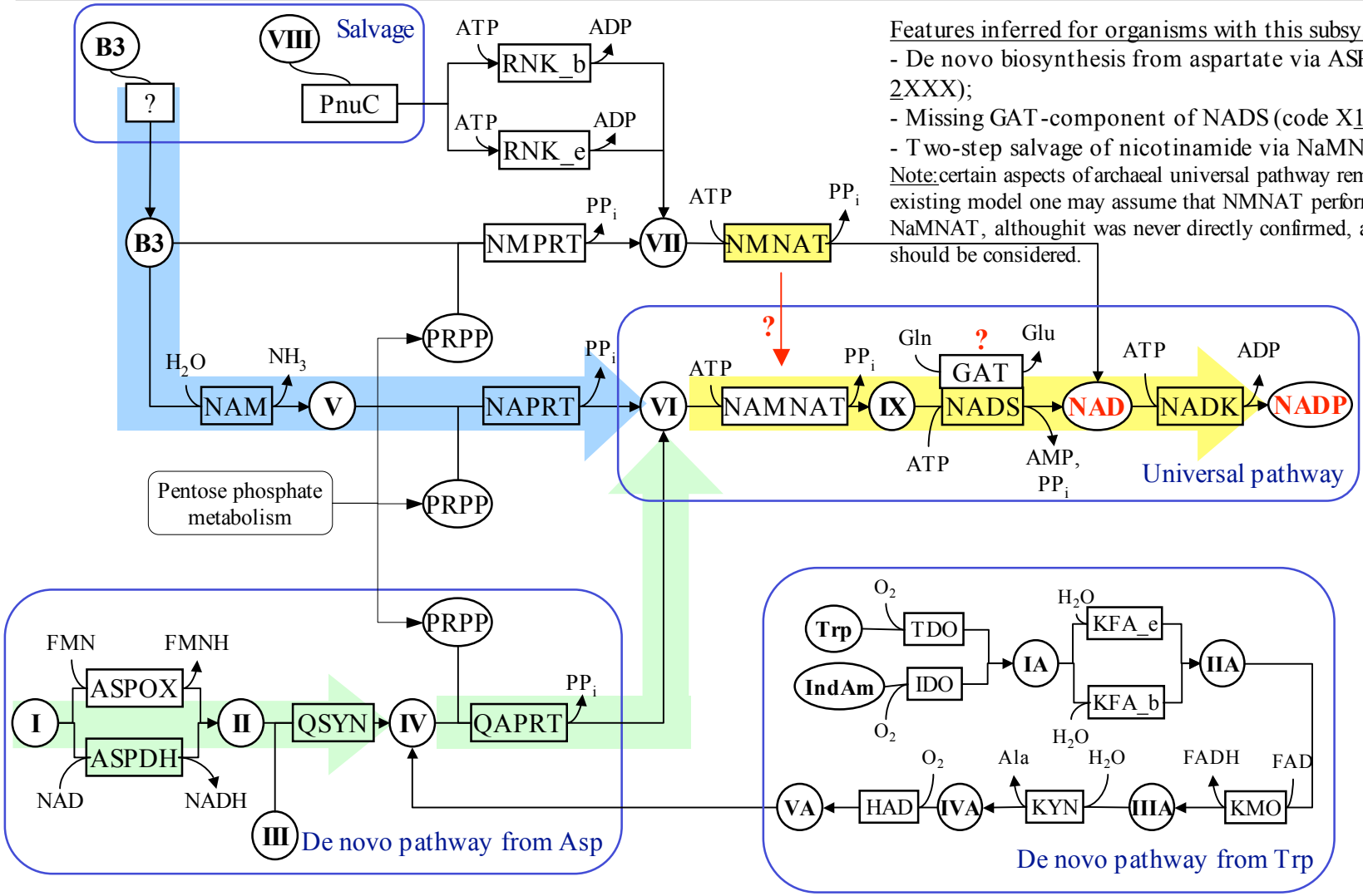
(and 20 other genomes)

Organism	Variant Code	*TDO	*KFA	KMO	KYN	HAD	*ASPOX	QSYN	QAPRT	NAMNAT	NMNAT	NADS	GAT	NADK	NAM	NAPRT	NMPRT	PNUC	*RNK
Prochlorococcus marinus MED4 [B]	12880						350-8	1670	259	727		726	726	292, 957					



Variant 2118: *Pyrobaculum aerophilum*
(and *Archaeoglobus fulgidus*)

Organism	Variant Code	*TDO	*KFA	KMO	KYN	HAD	*ASPOX	QSYN	QAPRT	NAMNAT	NMNAT	NADS	GAT	NADK	NAM	NAPRT	NMPRT	PNUC	*RNK
Pyrobaculum aerophilum str. IM2 [A]	21180						2190-9	2191	2190		995, 1576	841		2192, 410	2109	2101			



Features inferred for organisms with this subsystem variant:
- De novo biosynthesis from aspartate via ASPDH* (code 2XXX);
- Missing GAT-component of NADS (code X1XX);
- Two-step salvage of nicotinamide via NaMN (code XX1X).

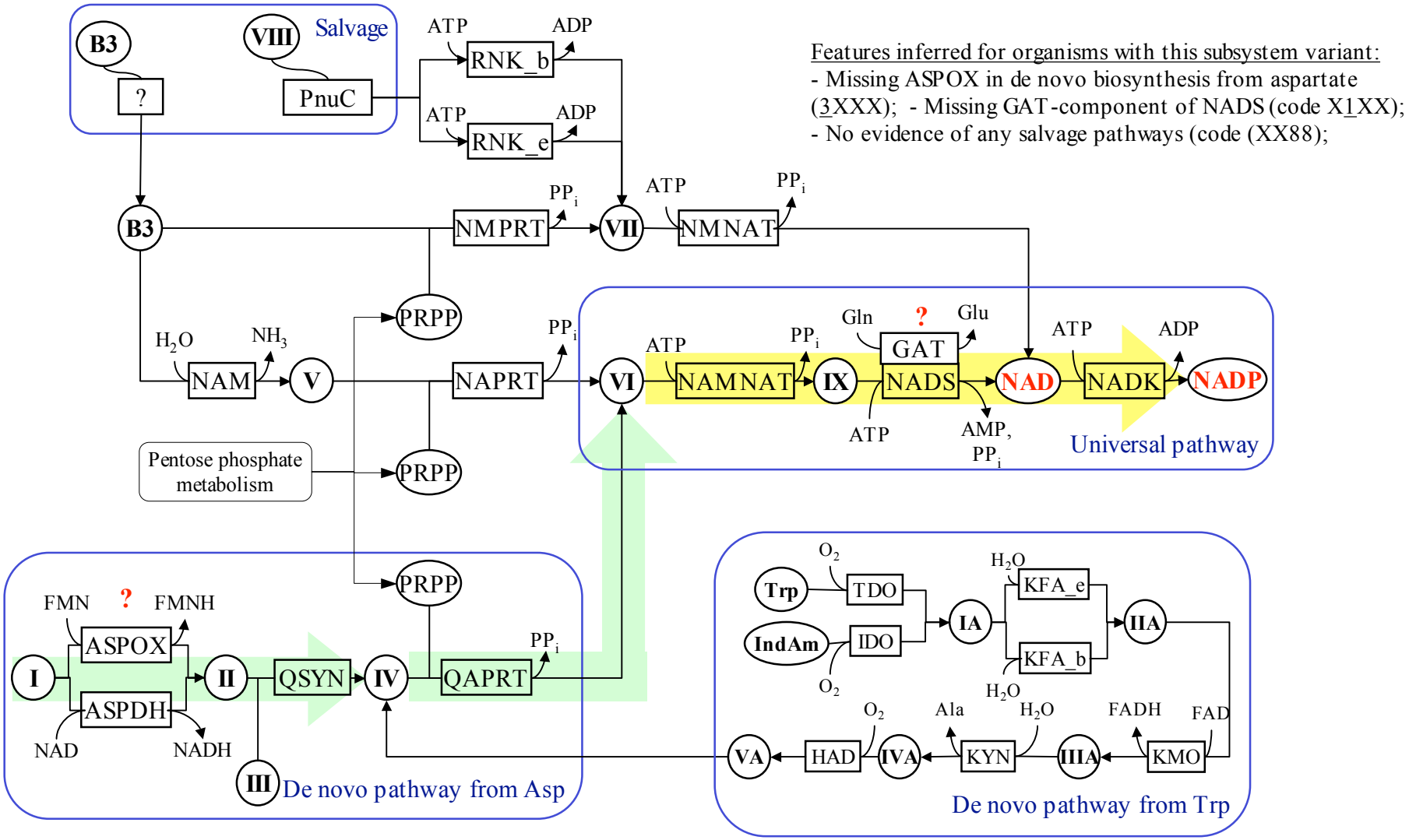
Note: certain aspects of archaeal universal pathway remain unclear. Under existing model one may assume that NMNAT performs the role of NaMNAT, althoughit was never directly confirmed, and other possibilities should be considered.

* - recently discovered in Thermotoga maritima [10]

Variant 3188: *Helicobacter pylori J99*

(and 2 other genomes)

Organism	Variant Code	*TDO	*KFA	KMO	KYN	HAD	*ASPOX	QSYN	QAPRT	NAMNAT	NMNAT	NADS	GAT	NADK	NAM	NAPRT	NMPRT	PNUC	*RNK
Helicobacter pylori J99 [B]	31880							1270	1269	1252		309		1429					

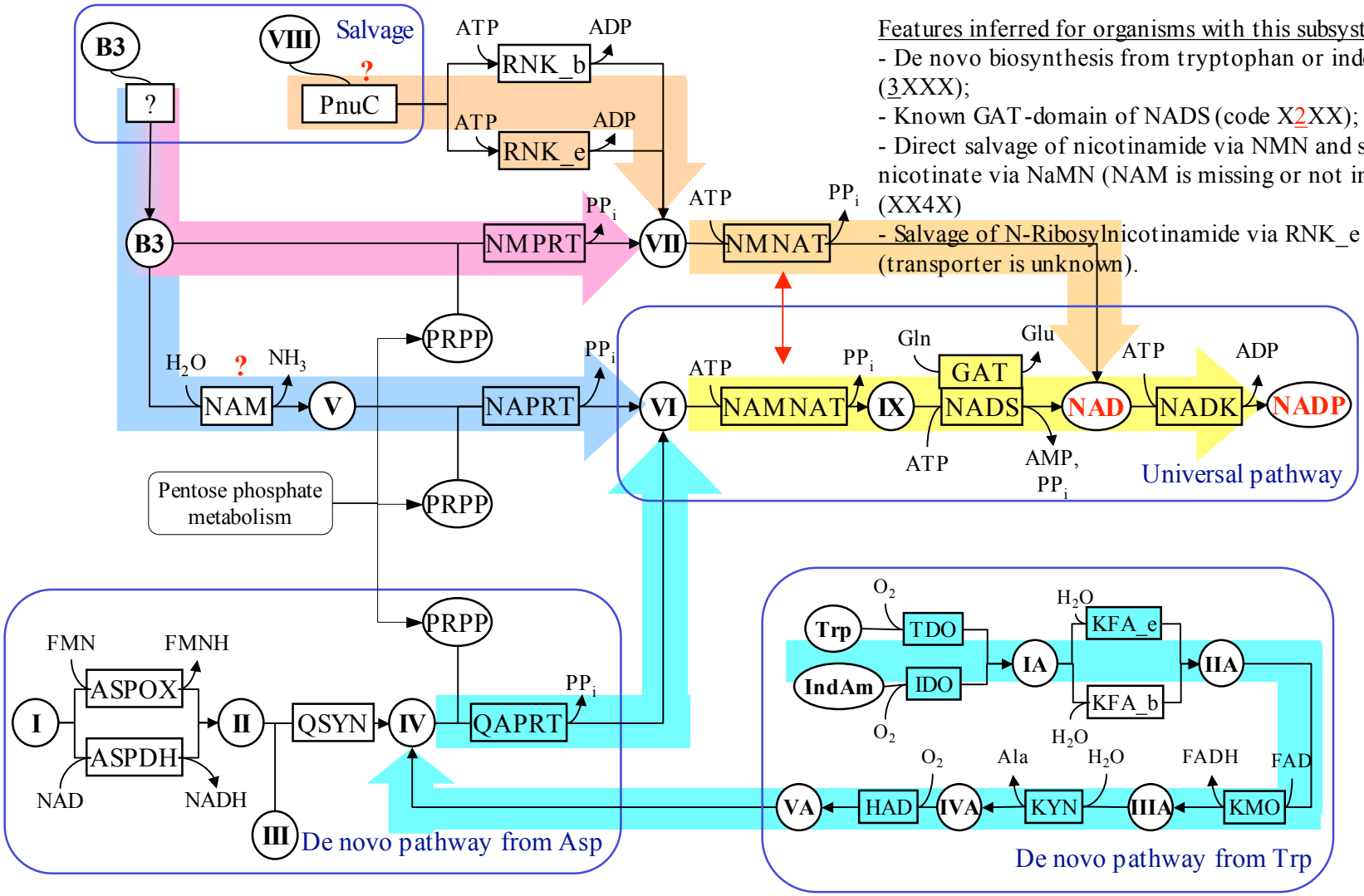


Features inferred for organisms with this subsystem variant:
- Missing ASPOX in de novo biosynthesis from aspartate (3XXX); - Missing GAT-component of NADS (code X1XX);
- No evidence of any salvage pathways (code (XX88);

Examples of subsystem variants

Variant 4243: *Homo sapiens*
(and *Mus musculus*)

Organism	Variant Code	*TDO	*KFA	KMO	KYN	HAD	*ASPOX	QSYN	QAPRT	NAMNAT	NMNAT	NADS	GAT	NADK	NAM	NAPRT	NMPRT	PNUC	*RNK
Homo sapiens (Human) [E]	42430	24524-1, 29968-2	14251-3	3138	18474	17562			11798	232, 2579, 2580	232, 2579, 2580	6020	6020	17183, 24887, 84, 86		30577	29005, 29006, 3598		15176-22, 15177-22, 31182-22



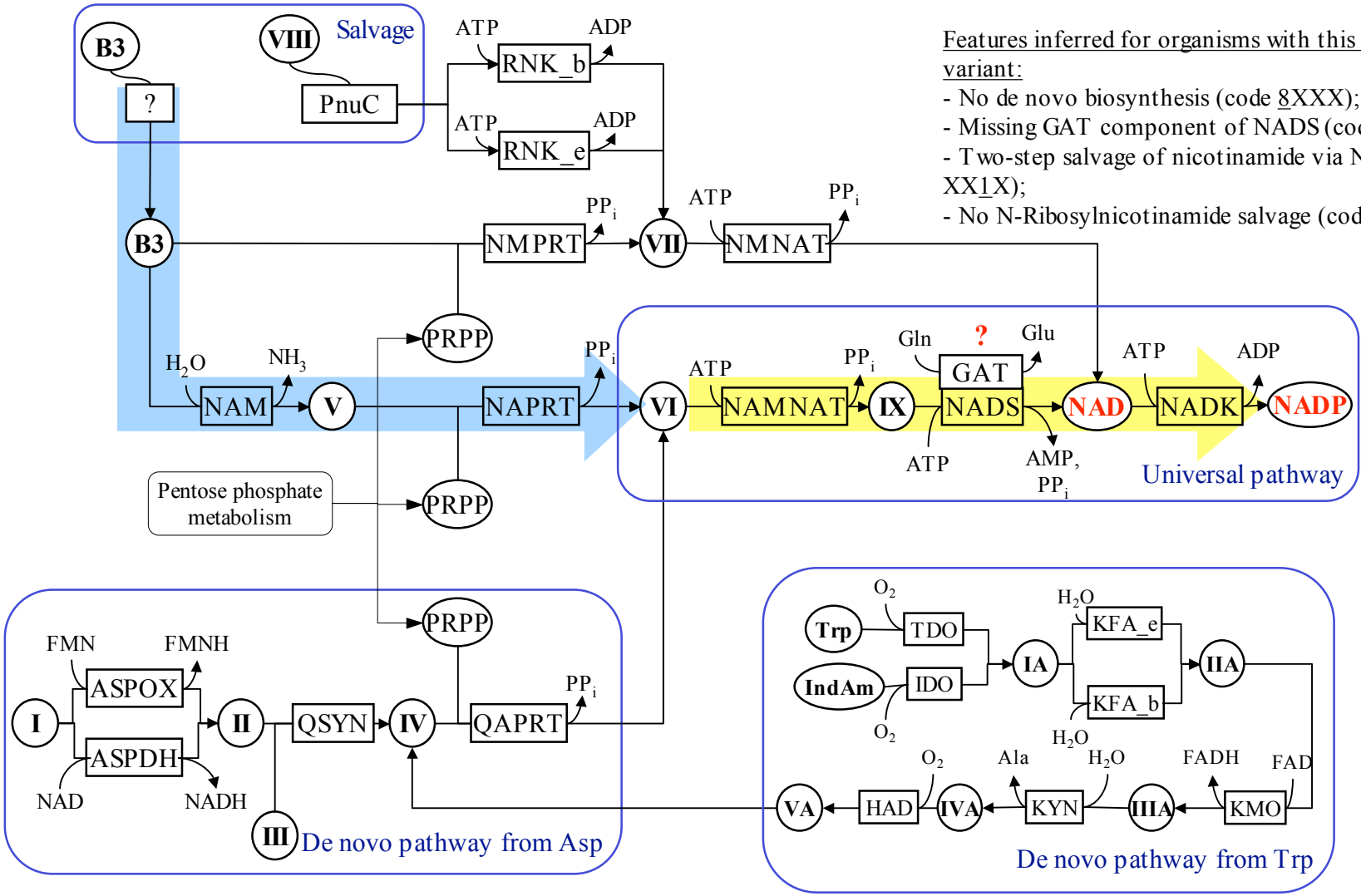
Features inferred for organisms with this subsystem variant:
- De novo biosynthesis from tryptophan or indoleamine (3XXX);
- Known GAT-domain of NADS (code X2XX);
- Direct salvage of nicotinamide via NMN and salvage of nicotinate via NaMN (NAM is missing or not involved) (XX4X)
- Salvage of N-Ribosylnicotinamide via RNK_e [11] (XXX3), (transporter is unknown).

Note, that both NMNAT and NaMNAT are performed by the same bi-functional enzyme. Three isoforms were experimentally characterized [12].

Variant 8118: *Staphylococcus epidermidis*

(and 40 other genomes)

Organism	Variant Code	*TDO	*KFA	KMO	KYN	HAD	*ASPOX	QSYN	QAPRT	NAMNAT	NMNAT	NADS	GAT	NADK	NAM	NAPRT	NMPRT	PNUC	*RNK
Staphylococcus epidermidis ATCC 12228 [B]	81180									1280		1596		696	1601	1597			



Features inferred for organisms with this subsystem variant:

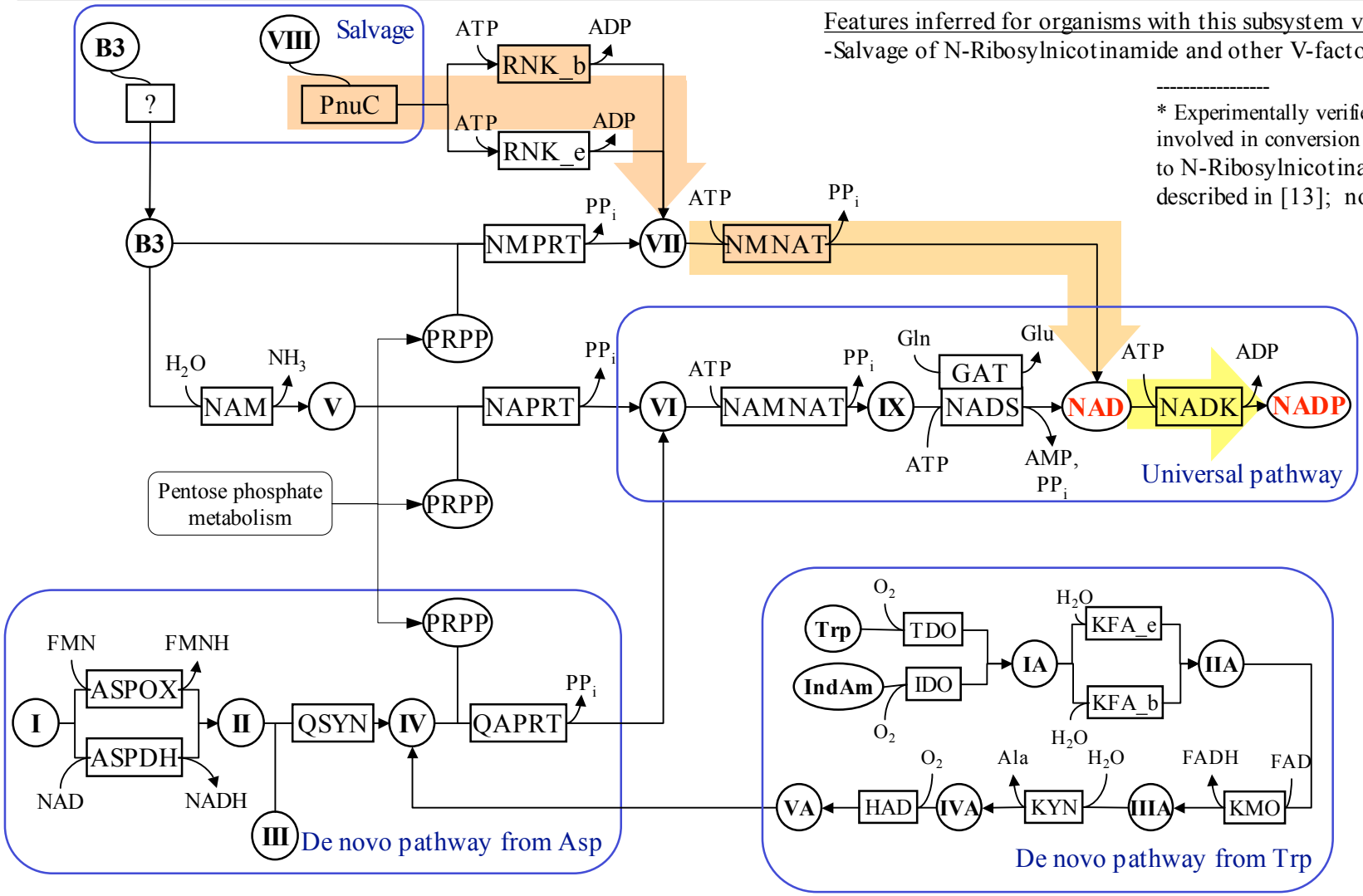
- No de novo biosynthesis (code 8XXX);
- Missing GAT component of NADS (code X1XX).
- Two-step salvage of nicotinamide via NaMN (code XX1X);
- No N-Ribosylnicotinamide salvage (code XXX8);

Examples of subsystem variants

Variant 1111: *Haemophilus influenzae Rd*

(and 3 other genomes)

Organism	Variant Code	*TDO	*KFA	KMO	KYN	HAD	*ASPOX	QSYN	QAPRT	NAMNAT	NMNAT	NADS	GAT	NADK	NAM	NAPRT	NMPRT	PNUC	*RNK
Haemophilus influenzae Rd KW20 [B]	88810								1404		731			70					731-21



Features inferred for organisms with this subsystem variant:

-Salvage of N-Ribosylnicotinamide and other V-factors* (code XXX1);

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* Experimentally verified in [9]. Enzymes involved in conversion of exogenous NAD to N-Ribosylnicotinamide were described in [13]; not shown.

IV. References

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