Subsystem: Fatty Acid Biosynthesis FASII

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Introduction

Fatty acid biosynthesis is one of the most conserved components of bacterial biosynthetic machinery, (for reviews see [1.2]). With a few exceptions (such as in Mycoplasmas) Fatty Acid Synthase (FAS) multienzyme complex is present in all bacteria and eukaryotes, but it is absent in archaea. Saturated and unsaturated fatty acids participate in a number of cellular processes, most importantly in building the cell envelope. **Most of the FAS-related genes are indispensable for cell growth and survival**, as demonstrated in a number of gene essentiality studies in model (*E.coli, B.subtilis*) and pathogenic (*H.influenzae, S.aureus, S.pneumoniase*) bacteria.

Eukaryotic and bacterial FAS drive fundamentally the same sequence of biochemical transformations, although structurally they are highly divergent. In eukarytotic (non-dissociable) FAS I, most of the catalytic domains are encoded in one gigantic modular polypeptide chain, In a typical bacterial (dissociable) FAS II, individual components (enzymes and carrier proteins) are encoded by separate genes. Most (but not all) of these components are clearly homologous to functional domains of eukaryotic FAS I.

FASII is a complex system with significant variations and peculiarities, which are relatively poorly understood beyond a handful of model organisms. In this example we will limit a discussion of FASII by a group bacterial pathogens, which are in focus of the NMPDR project (see www.nmpdr.org). We will illustrate possible applications of a subsystems approach for the analysis of a possible phenotype, including susceptibility to a particular type of antibiotics. Expansion of this subsystem over a wide variety of bacterial species is a project in development, and we will continue updating this subsystem on a publicly available SEED web-site.

Subsystem Notes
(focusing on NMPDR bacterial pathogens)

One of the important variations in this subsystem (see Panel 1) is the existence of alternative (nonorthologous) forms of Enoyl-ACP-reductase. A classic NAD dependent form (FabI, as in E.coli), which is present in most bacterial species, is a proven target for popular antibiotics, such as triclosan. Recently, it was shown that enoyl-ACP-reductase in *S. pneumoniae* (lacking a classic *fabI*), is encoded by a nonhomologous gene *fabK* [3,4]. FabK is very distantly related to the FMN-dependent enoyl-ACP-reductase domain of eukaryotic FASI, and it is completely insensitive to the inhibitors of bacterial FabI, providing a rational for the known triclosan-resistance of *S. pneumoniae* and other bacteria with the same gene pattern.

The analysis of functional variants (ocurrence of FabK/FabI) over a subset of bacterial pathogens provides a possibility to tentatively rationalize and even predict the triclosan sensistivity/resistance (see Panel 2,3,4). However, *Vibrio* ssp represent a case of an open problem. None of them have strong candidates for FabI, although they contain several paralogs with relatively low scores. Most of the species in this group (except Vibrio parahaemolyticus) also lack strong FabK candidates. Additional studies are required to elucidate this aspect of FAS in Vibrio ssp.

Another type of conjectures can be made by considering the presence of one of the two described branching pathways of Unsaturated Fatty Acids (UFA) biosynthesis (see Panel 2,3,4). *Vibrio* ssp seem to be capable of producing UFAs using the same two-step fabB/fabA branching pathway as in *E.coli*. An alternative UFA branching pathway involving a universally conserved *fabF* gene and a relatively rare *fabM* gene was described in *S.pneumoniae* [5] , which does not have *fabA*/*fabB* homologs. This alternative UFA pathway is relatively rare, and it is apparently not present in *Staphylococci, Listeria* and *Campylobacter* ssp. Genetics and enzymology of UFA synthesis in these organisms remain to be explored.

Finally, the analysis of a chromosomal cluster in *S.pneumoniae* and a number of related species (see illustration) reveals the presence of an uncharacterized transcriptional regulator of MarR family (see Paneel 5). It is tempting to speculate that this protein may be involved in the regulation of FAS operon in these species.

Variant codes:

Basic variants:

#1 as in *E.coli*, including FabI and UFAI (FabA/FabB)

#2 as inS.pneumoniae, including FabK and FabM-driven UFAII

Subvariants and hybrid (examples):

#10: Variant #1 with 1 missing gene (as in *V cholerae*)

#11: Variant # 1 w/o UFA (as in *Campylobacter*)

#12: Hybrid of #1 and #2 (as in *V.parahaemolyticus*): FabI with UFAI

#112: Hybrid of #11 and #2: with both FabI and FabK

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

Subsystem: Fatty Acid Biosynthesis FASII

Column	Abbrev	Functional Role
1	BCCP	Biotin carboxyl carrier protein of acetyl-CoA carboxylase
2	BC	Biotin carboxylase of acetyl-CoA carboxylase (EC 6.3.4.14)
3	AccA	Acetyl-coenzyme A carboxyl transferase alpha chain (EC 6.4.1.2)
4	AccD	Acetyl-coenzyme A carboxyl transferase beta chain (EC 6.4.1.2)
5	ACP	Acyl carrier protein
6	ACPS	Holo-[acyl-carrier protein] synthase (EC 2.7.8.7)
7	PPT	4'-phosphopantetheinyl transferase (EC 2.7.8.-)
8	FabD	Malonyl CoA-acyl carrier protein transacylase (EC 2.3.1.39)
9	FabF	3-oxoacyl-[acyl-carrier-protein] synthase, KASII (EC 2.3.1.41)
10	FabH	3-oxoacyl-[acyl-carrier-protein] synthase, KASIII (EC 2.3.1.41)
11	FabG	3-oxoacyl-[acyl-carrier protein] reductase (EC 1.1.1.100)
12	FabZ	(3R)-hydroxymyristoyl-[acyl carrier protein] dehydratase (EC 4.2.1.-)
13	FabI	Enoyl-[acyl-carrier-protein] reductase [NADH] (EC 1.3.1.9)
14	FabK	Enoyl-[acyl-carrier-protein] reductase [FMN] (EC 1.3.1.9)
15	FabL	Enoyl-[acyl-carrier-protein] reductase [NADPH] (EC 1.3.1.10)
16	FabB	3-oxoacyl-[acyl-carrier-protein] synthase, KASI (EC 2.3.1.41)
17	FabA	3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase (EC 4.2.1.60)
18	FabM	Trans-2,cis-3-Decenoyl-ACP isomerase

Alternative forms

Subsets of roles

Subset	
*ACPS	6,7
*FAB_FH	9,10
*FAB_IKL	13,14,15
Acetyl_CoA_Carboxylase	1,2,3,4

2. Subsystem spreadsheet and inferred phenotype

Subsystem: Fatty Acid Biosynthesis FASII

Organism	Variant Code	BCCP	BC	AccA	AccD	ACP	ACPS	FabD	FabF	FabH	FabG	FabZ	FabI	FabK	FabB	FabA	FabM	Triclosan	UFA type
Escherichia coli K12 [B]	1	3197	3198	185	2290	1079	2533	1077	1080	1076	1078	180	1276		2297	939		S	I
Vibrio cholerae O1 biovar eltor str. N16961 [B]	10	292	291	2216	987	1995	2427	1997	1994	1998	1996	2221				1465		S?	I
Vibrio parahaemolyticus RIMD 2210633 [B]	12	2880	2881	2302	2189 3875	2053	2568	2055	2052	2056	2054	2307	?	3376	2194	1592		R?	I
Vibrio vulnificus CMCP6 [B]	1	1132	1131	1713	1826 4021	2770	1428	2772	2768	2773	2771	1708			1821	2412		S?	I
Vibrio vulnificus YJ016 [B]	1	3201	3202	2608	2491 5001	1344	2895	1342	1345	1341	1343	2614			2497	1730		S?	I
Campylobacter jejuni subsp. jejuni NCTC 11168 [B]	11	1215	1214	411	116	409	1328	105	410	297	403 746	245	1319					S	?
Staphylococcus aureus EMRSA-16 (Str. 252) [B]	11	2357	2356	2521	2522	1037	835	1039	926	925	1038	863	2131					S	?
Staphylococcus aureus MSSA (Str. 476) [B]	110	146	532	620 621	622	498	1747	496	?	1207	497	1028	424					S	?
Staphylococcus aureus NCTC 8325 [B]	110	1280	1281	2156	2157	1599		1597	150	1029	1598	127	720					S	?
Staphylococcus aureus subsp. aureus MW2 [B]	11	1480	1479	1643	1644	1115	1995	1113	866	865	1114	2023	892					S	?
Organism	**Variant Code**	**BCCP**	**BC**	**AccA**	**AccD**	**ACP**	**ACPS**	**FabD**	**FabF**	**FabH**	**FabG**	**FabZ**	**FabI**	**FabK**	**FabB**	**FabA**	**FabM**		
Staphylococcus aureus subsp. aureus Mu50 [B]	11	1527	1526	1700	1701	1232	2071	1230	984	983	1231	2098	1011					S	?
Staphylococcus aureus subsp. aureus N315 [B]	11	1403	1402	1572	1573	1110	1941	1108	867	866	1109	1968	893					S	?
Staphylococcus epidermidis ATCC 12228 [B]	11	1208	1207	1375	1376	907	1675	905	678	677	906	1697	712					S	?
Listeria innocua Clip11262 [B]	112	1382	1383	1596	1597	1907	878	1909	2289	2290	1908	2650	959	2258 805				R?	?
Listeria monocytogenes EGD-e [B]	112	1348	1349	1564	1565	1798	878	1800	2192	2193	1799	2514	962	2161 808				R?	?
Listeria monocytogenes str. 4b F2365 [B]	112	1358	1359	1579	1580	1814	894	1816	2211	2212	1815	2472	978	2179 820				R?	?
Streptococcus agalactiae 2603V/R [B]	2	348	350	352	351	343	1640	345	347	342	346	349		344			340	R	II
Streptococcus agalactiae NEM316 [B]	2	336	338	340	339	331	1694	333	335	330	334	337		332			328	R	II
Streptococcus equi subsp. zooepidemicus [B]	20	1556	1554	1552	1416 1417 1553	2117	784	2119	?	2116	2120	1555		2118			2114	R	II
Organism	**Variant Code**	**BCCP**	**BC**	**AccA**	**AccD**	**ACP**	**ACPS**	**FabD**	**FabF**	**FabH**	**FabG**	**FabZ**	**FabI**	**FabK**	**FabB**	**FabA**	**FabM**		
Streptococcus mitis NCTC 12261 [B]	21	252	254	256	255	247	1029	249	251	2027	250	253		248			2029	R	II
Streptococcus mutans UA159 [B]	2	1578	1576	1574	1575	1583	1665	1581	1579	1584	1580	1577		1582			1586	R	II
Streptococcus pneumoniae 23F [B]	200	985	987	989	988	980	1795	982	984	?	983	986		981				R	?
Streptococcus pneumoniae R6 [B]	2	383	385	387	386	378	1540	380	382	377	381	384		379			375	R	II
Streptococcus pneumoniae TIGR4 [B]	2	393	395	397	396	388	1584	390	392	387	391	394		389			385	R	II
Streptococcus pyogenes M1 GAS [B]	2	1347	1345	1343	1344	1352	1389	1350	1348	1353	1349	1346		1351			1355	R	II
Streptococcus pyogenes M5 [B]	2	279	281	283	282	274	235	276	278	273	277	280		275			271	R	II
Streptococcus pyogenes MGAS315 [B]	2	1521	1519	1517	1518	1526	1564	1524	1522	1527	1523	1520		1525			1529	R	II
Streptococcus pyogenes MGAS8232 [B]	2	1503	1501	1499	1500	1508	1544	1506	1504	1509	1505	1502		1507			1511	R	II

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 " ?".. Susceptibility to triclosan inferred from the FabI/FabK occurrence is marked as " R" (resistant) and " S" (sensitive). Presence of UFA pathways inferred from the occurrence of FabA/FabB vs FabF/FabM is shown by " I" and " II", respectovely. Some of the examples are further illustrated by projection on a subsystem diagram.

3. Example: *C. jejuni* (variant #11)

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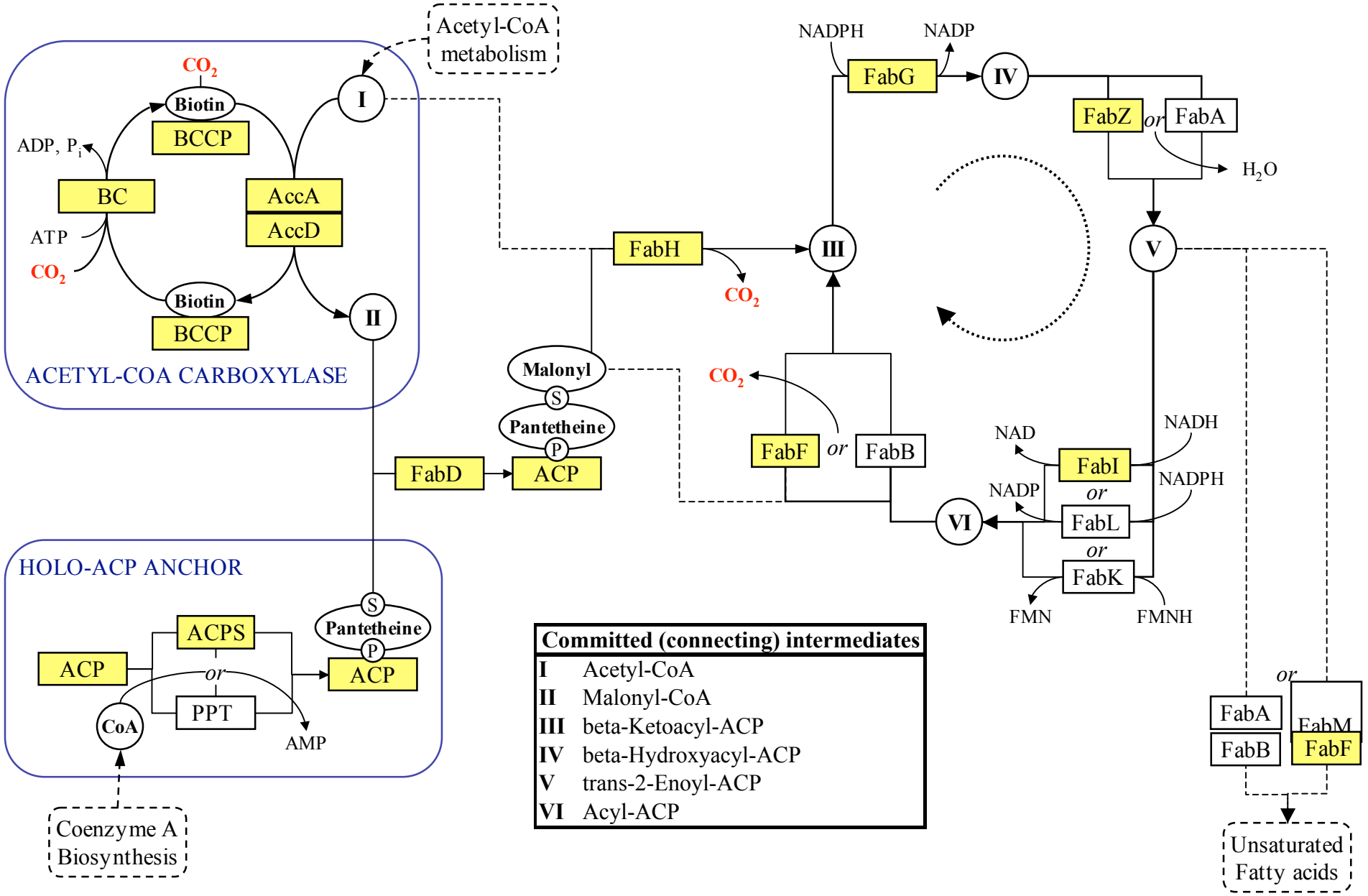
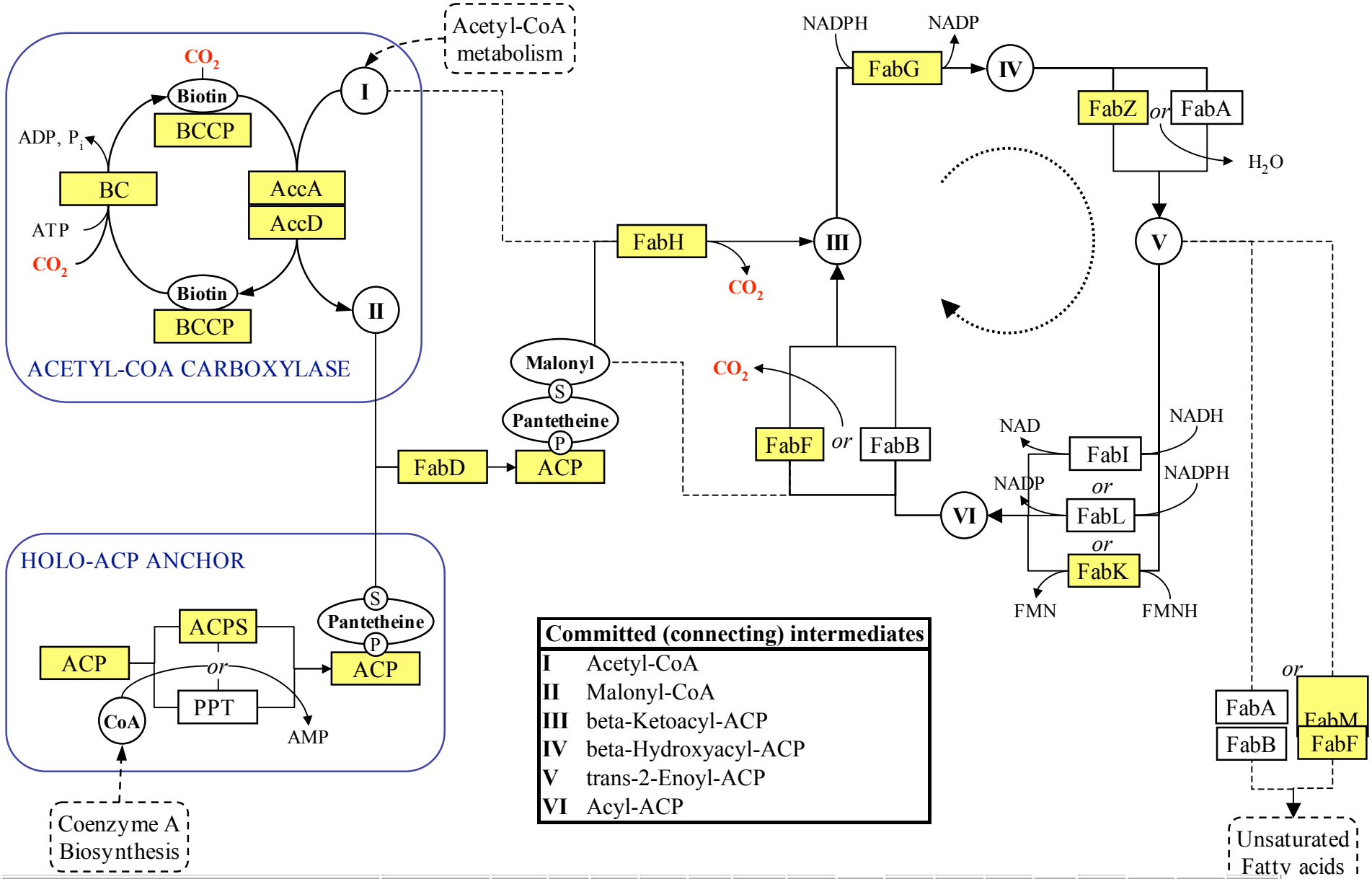


Committed (connecting) intermediates	
I	Acetyl-CoA
II	Malonyl-CoA
III	beta-Ketoacyl-ACP
IV	beta-Hydroxyacyl-ACP
V	trans-2-Enoyl-ACP
VI	Acyl-ACP

Organism	Variant Code	BCCP	BC	AccA	AccD	ACP	ACPS	FabD	FabF	FabH	FabG	FabZ	FabI	FabK	FabB	FabA	FabM
Campylobacter jejuni subsp. jejuni NCTC 11168 [B]	11	1215	1214	411	116	409	1328	105	410	297	405 746	245	1319				

4. Example: *S. pneumoniae* (variant #2)

Subsystem: Fatty Acid Biosynthesis FASII

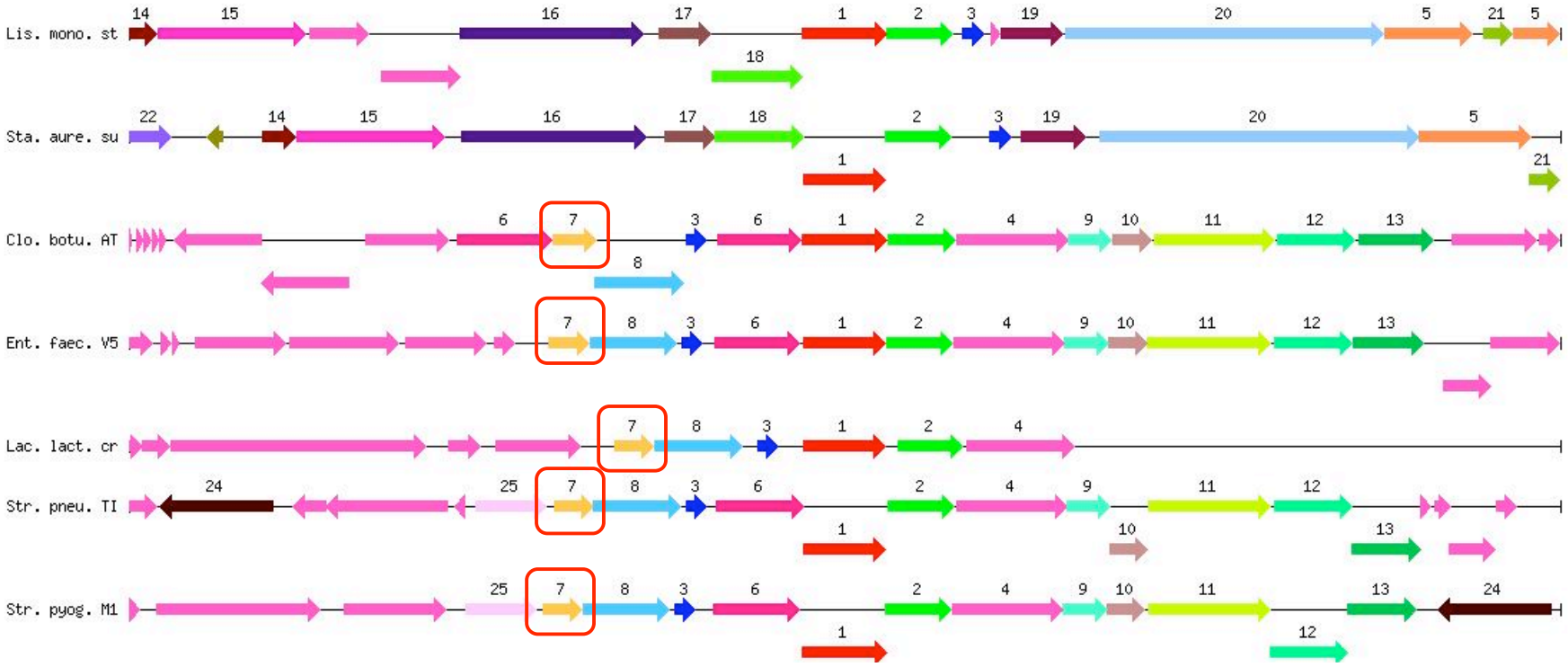


Committed (connecting) intermediates	
I	Acetyl-CoA
II	Malonyl-CoA
III	beta-Ketoacyl-ACP
IV	beta-Hydroxyacyl-ACP
V	trans-2-Enoyl-ACP
VI	Acyl-ACP

Organism	Variant Code	BCCP	BC	AccA	AccD	ACP	ACPS	FabD	FabF	FabH	FabG	FabZ	FabI	FabK	FabB	FabA	FabM
Streptococcus pneumoniae R6 [B]	2	383	385	387	386	378	1540	380	382	377	381	384		379			375
Streptococcus pneumoniae TIGR4 [B]	2	393	395	397	396	388	1584	390	392	387	391	394		389			385

5. A conserved chromosomal cluster in *S. pneumoniae* and related species

allows to propose a candidate for FAS transcriptional regulator_



Set	Protein in S.pneumoniae	Annotation
1	fig|170187.1.peg.390	Malonyl CoA-acyl carrier protein transacylase (EC 2.3.1.39)
2	fig|170187.1.peg.391	3-oxoacyl-[acyl-carrier protein] reductase (EC 1.1.1.100)
3	fig|170187.1.peg.388	Acyl carrier protein
4	fig|170187.1.peg.392	3-oxoacyl-[acyl-carrier-protein] synthase, KASII (EC 2.3.1.41)
6	fig|170187.1.peg.389	Enoyl-[acyl-carrier-protein] reductase [FMN] (EC 1.3.1.9)
7	fig|170187.1.peg.386	*Transcriptional regulator, MarR family, predicted regulator of FAS
8	fig|170187.1.peg.387	3-oxoacyl-[acyl-carrier-protein] synthase, KASIII (EC 2.3.1.41)
9	fig|170187.1.peg.393	Biotin carboxyl carrier protein of acetyl-CoA carboxylase
10	fig|170187.1.peg.394	(3R)-hydroxymyristoyl-[acyl carrier protein] dehydratase (EC 4.2.1.-)
11	fig|170187.1.peg.395	Biotin carboxylase of acetyl-CoA carboxylase (EC 6.3.4.14)
12	fig|170187.1.peg.396	Acetyl-coenzyme A carboxyl transferase beta chain (EC 6.4.1.2)
13	fig|170187.1.peg.397	Acetyl-coenzyme A carboxyl transferase alpha chain (EC 6.4.1.2)
18	fig|265669.1.peg.1817	Fatty acid/phospholipid synthesis protein plsX
25	fig|170187.1.peg.385	Trans-2,cis-3-Decenoyl-ACP isomerase

prediction →

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