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FabT, a Bacterial Transcriptional Repressor That Limits Futile Fatty Acid Biosynthesis

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► **To cite this version:**

Clara Lambert, Claire Poyart, Alexandra Gruss, Agnes Fouet. FabT, a Bacterial Transcriptional Repressor That Limits Futile Fatty Acid Biosynthesis. *Microbiology and Molecular Biology Reviews*, 2022, 86 (3), pp.00029-22. 10.1128/membr.00029-22 . hal-03815204

HAL Id: hal-03815204

<https://hal.science/hal-03815204v1>

Submitted on 25 Oct 2022

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1 FabT, a bacterial transcriptional repressor that limits futile fatty acid biosynthesis

2

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4

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8

9 Running Head: FabT, a unique FASII transcriptional repressor

10

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31 **SUMMARY**

32 Phospholipids are vital membrane constituents that determine cell functions and interactions
33 with the environment. For bacterial pathogens, rapid adjustment of phospholipid composition
34 to changing conditions during infection can be crucial for growth and survival. Fatty acid
35 synthesis (FASII) regulators are central to this process. This review puts the spotlight on
36 FabT, a MarR-family regulator of FASII characterized in *Streptococci*, *Enterococci* and
37 *Lactococci*. Roles of FabT in virulence, as reported in mouse and non-human primate
38 infection models, will be discussed. We present FabT structure, the FabT regulon, and
39 changes in FabT regulation according to growth conditions. A unique feature of FabT
40 concerns its modulation by an unconventional co-repressor, acyl-ACP. Some bacteria express
41 two ACP proteins, which are distinguished by their interactions with endogenous or
42 exogenous fatty acid sources, one of which causes strong FabT repression. This system seems
43 to allow preferred use of environmental fatty acids, thereby saving energy by limiting futile
44 FASII activity. Control of *fabT* expression and FabT activity link various metabolic pathways
45 to FASII. The various physiological consequences of FabT loss summarized here suggest that
46 FabT has potential as a narrow range therapeutic target.

47

48 Keywords: Fatty acid synthesis, FabT, repressor, feed-back regulation, acyl-ACP, binding
49 sites

50

51 INTRODUCTION

52 All cells are delimited by lipid membranes, which form a selective permeable barrier
53 that regulates the transport of nutrients and waste, and receives and transmits environmental
54 signals that adjust metabolic processes according to environmental conditions. Cell
55 membranes commonly comprise a phospholipid bilayer composed of a polar head and an
56 apolar body comprising two fatty acid (FA) chains per glycerol-phosphate backbone, whose
57 features contribute to membrane composition and dynamics. FA structure and length are
58 decisive for membrane topology and properties such as fluidity, permeability and integrity.
59 These features are crucial for the adaptation of bacteria to various environments (1).

60 Phospholipids comprise two main FA classes, saturated (straight and branched chain
61 FA) and unsaturated (including mono- and poly-unsaturated FA). The FA synthesis pathway
62 (FASII) comprises an initiation phase followed by a recursive elongation cycle, which
63 produces acyl-Acyl Carrier Protein (acyl-ACP) (Fig. 1A). Various bacteria including
64 *Streptococci*, *Enterococcus faecalis*, and *Lactococcus lactis* produce monounsaturated FAs,
65 which are synthesized *via* a shunt within the cycle (Fig. 1A). Many bacteria also generate
66 cyclic FA *via* an enzyme that acts post-synthetically on membrane phospholipids (2). The
67 process of cyclopropanation generates a three membered ring at the site of an unsaturation on
68 the carbon chain. The numerous possible FA products (different chain lengths, saturated or
69 unsaturated, cyclized) may facilitate membrane adjustments according to shifts in
70 environmental conditions.

71 FASII is widespread among bacteria, but genetic organization and regulation of FASII
72 genes are not that conserved. However, these features are conserved among lactic acid
73 bacteria (Fig. 1B). A gene within the main FASII locus encodes a member of the MarR
74 family of regulators, named FabT (Fig. 1B) (3). FASII regulation plays a crucial role in
75 adjusting FA production under different conditions. Both feedback and feedforward

76 mechanisms of FASII regulation are described for different bacterial species (4, 5). In this
77 review, we summarize the current state of knowledge concerning FabT, a FASII regulator
78 studied in Streptococci, Enterococci, and Lactococci. While Lactococci are mainly known as
79 food-fermenting bacteria, the genus includes a fish pathogen, and commensals of plants,
80 intestinal tracts, and the vagina, where FabT is highly conserved; its characterization in
81 Lactococci thus contributes to understanding FabT functionality. The unique features of
82 FabT-family regulators will be highlighted, and findings linking FabT and virulence will be
83 discussed.

84

85 **FABT INVOLVEMENT IN *STREPTOCOCCUS PYOGENES* VIRULENCE**

86 The natural occurrence and potential consequences of *fabT* mutations during
87 *Streptococcus pyogenes* *in vivo* infections motivated us to write this review. *S. pyogenes*
88 (group A *Streptococcus*, GAS) is an important human pathogen responsible for a large variety
89 of clinical manifestations ranging from mild superficial infections to more life-threatening
90 invasive infections including necrotizing fasciitis (NF) or streptococcal toxic shock syndrome
91 (STSS). GAS infections are also responsible for post-infectious complications such as
92 rheumatic arthritis and glomerulonephritis and, altogether, GAS infections are responsible for
93 517,000 deaths annually worldwide (6, 7). In one study, 74 clinical isolates of the same
94 genotype, from either invasive infections (mainly streptococcal toxic shock syndrome), or
95 non-invasive infections, were analyzed (8). *fabT* point mutations were only detected in non-
96 invasive infections (2 out of 38 isolates). This suggested that spontaneous mutations in *fabT*
97 are counterselected in invasive infections and infrequent in non-invasive ones. Similarly,
98 another study identified only 9 single nucleotide polymorphisms or non-synonymous
99 insertions/deletions in *fabT* when comparing the genomic sequences of more than 3615
100 clinical isolates of *S. pyogenes* of the same genotype from invasive and non-invasive

101 infections (9, 10). In the Nasser *et al* study, *fabT* mutant distribution between invasive and
102 non-invasive infections was not considered. In striking contrast with the low incidence of
103 *fabT* polymorphisms among invasive isolates, their occurrence rose to twelve percent among
104 isolates recovered at the site of infection from non-human primates in necrotizing fasciitis
105 experiments (44 amino acid replacements at 30 different FabT positions were identified) (10).
106 Infection was then compared using a reference strain and its isogenic *fabT* deletion mutant in
107 the non-human primate necrotizing fasciitis model. The mutant strain multiplied less at the
108 inoculation point, and caused significantly less spread, smaller lesions and less tissue
109 destruction than the wild-type strain; it was also more sensitive to killing in blood and in
110 polymorphonuclear neutrophils (10). Another study in a murine subcutaneous infection model
111 used wild-type strains, their *fabT* deleted derivatives, or clinical isolates harboring
112 spontaneous *fabT* point mutations and a *fabT*-complemented strain. Virulence was strongly
113 attenuated for all *fabT* mutant strains (8), in support of the above observations.

114 In conclusion, *fabT* mutants appear spontaneously among non-invasive isolates, and
115 when mutated, may compromise bacterial virulence. Characterization of the genes controlled
116 by FabT, FabT repression mechanisms, and how FabT influences bacterial physiology will
117 shed light on how and why *fabT* mutants emerge in weakly pathogenic isolates.

118

119 **IDENTIFICATION OF FABT AS THE FASII REGULATOR**

120 Bioinformatics analysis revealed a similar genetic organization of the FASII main
121 cluster in three *Streptococcus* species, and in *Clostridium acetobutylicum* (3, 11), *L. lactis*,
122 and *E. faecalis* (Fig. 1B). In *Streptococci*, FASII genes are grouped in a single locus, whereas
123 in *L. lactis* and *E. faecalis*, they are found in two distinct loci. The main Acyl Carrier Protein
124 (ACP) encoded by *acpA* is always present on the main locus, in an operon comprising three or
125 more genes, the first of which is the MarR family regulator FabT (Fig. 1B) (12-14). A second

126 transcript starting with *acpA* may indicate the existence of a second operon, or of a stabilized
127 part of a longer mRNA (12, 15). When present, a second ACP encoded by *acpB* is colocalized
128 with *plsX*, and will be discussed below.

129 FabT was shown in *S. pneumoniae* by the Rock laboratory to be a FASII transcriptional
130 regulator (12, 16). Its role was further demonstrated in *S. pyogenes*, *E. faecalis* and *L. lactis*,
131 and suggested by synteny and sequence similarity analyses in *C. acetobutylicum* (3). FabT is
132 conserved among the Streptococci; the 144 amino acid *S. pneumoniae* FabT protein
133 respectively shares 64%, 62%, 51%, 59% and 45% sequence identity with *S. pyogenes*,
134 *Streptococcus agalactiae*, *E. faecalis*, *L. lactis* and *C. acetobutylicum* FabT (unpublished
135 data). FabT is distinct from FapR-type regulators as described in *Bacillus*, *Staphylococcus*,
136 and *Listeria*, which involve a feed-forward-type regulation (17). In that system, FapR
137 repression is alleviated by binding to FASII precursors malonyl-CoA or malonyl-ACP (4, 5,
138 18).

139

140 **THE FABT REGULON**

141 The roles of FabT and the breadth of its regulon, which includes FASII and non-FASII
142 genes, were reported in different transcriptional studies. For this, expression was compared in
143 wild-type and *fabT* deleted or overproducing mutants, using various growth conditions,
144 including those requiring adjustments in membrane composition (10, 12, 19-21).

145

146 **FASII gene regulation by the FabT repressor**

147 FASII genes are organized in three to five transcriptional units in *Streptococcus*, and
148 four and five in *E. faecalis* and *L. lactis*, respectively (Fig. 1B). Internal transcriptional start
149 sites within the operon beginning with *fabT* were uncovered in *S. pneumoniae* and *S.*
150 *pyogenes*, suggesting that expression may be finely controlled (12, 15). In *E. faecalis*,

151 overlapping RT-PCR demonstrated that the 12-gene cluster is organized as a single operon
152 that accommodates a putative transcription reinitiation site upstream from *fabK* (13).

153 In *S. pneumoniae* and *S. pyogenes*, at 37°C, *fabT* loss of function mutants showed 2- to
154 9- fold increases in FASII gene expression compared to the wild-type strain, although species
155 differences were observed (12, 21). Those studies showed that in *S. pneumoniae*, FabT
156 represses expression of all FASII genes with the exception of *fabM* (required for FA
157 unsaturation) (12). In *L. lactis*, only three genes from the main locus, *fabD*, *fabG1* and *accB*,
158 and two genes in a separate locus, *fabZ1* and *fabI*, displayed increased expression in a *fabT*
159 deleted strain, suggesting that repression was too weak to be detected for other FASII genes
160 in the test condition (19). Indeed, in an *L. lactis* strain overproducing *fabT* by ~135-fold, all
161 FASII genes except *fabH* were repressed by a factor of 2.5 to 5.3, indicating that these genes
162 are controlled by FabT (19). The moderate effects of FabT on FASII gene expression in these
163 extreme conditions, evokes the possibility that FabT activity is modulated by a cofactor (see
164 the FabT co-repressor). In *Streptococcus mutans*, FASII gene derepression in a *fabT* deletion
165 mutant was observed under both neutral and acidic pH conditions (20). In *S. pyogenes*,
166 variations in FASII gene transcription were analyzed between wild-type and *fabT* deletion
167 strains, at different temperatures and growth phases. Authors concluded that FabT controls all
168 FASII pathway genes, although *fabM* was less tightly controlled at 35°C than the other genes
169 (10). Weaker control of *fabM* is reminiscent of results obtained in *S. pneumoniae*, where it
170 was not repressed by FabT (12). In conclusion, FabT represses FASII genes in a coordinated
171 but non-identical way. Differential regulation of fluidifying fatty acids (requiring FabM)
172 might be important for adjustment to stress conditions.

173

174 **Non-FASII gene regulation by the FabT repressor**

175 Transcriptomic studies revealed numerous non-FASII genes whose expression is
176 affected by the FabT status. In *S. pneumoniae*, comparison of transcription profiles between
177 strains grown in laboratory medium showed that *fabT* deletion led to mostly minor (up to 5-
178 fold) and 4 major (12- to 50-fold) expression changes for 85 upregulated and 4 downregulated
179 genes, involved in transport, DNA metabolism and protein synthesis, and proteins of
180 unknown function (12). The likely indirect effects of the *fabT* deletion may reflect bacterial
181 adaptation to the altered ratio of saturated to unsaturated FAs (see 7. FabT and *in vitro*
182 bacterial physiology). In support of this finding, comparing transcriptomes of wild-type and
183 *fabT* deleted strains in *L. lactis* revealed expression changes of only 10 non-FASII genes (19).
184 The use of dairy strains in that study may limit the identified targets when compared to a
185 lactococcal pathogen (*Lactococcus garvieae*), or *L. lactis* plant, vaginal, or intestinal isolates,
186 which express identical FabT proteins. However, *fabT* overexpression affected 125 genes
187 belonging to the same functional categories as those identified in *S. pneumoniae*. It is likely
188 that most expression changes are indirect consequences of FabT control.

189 Interestingly, FabT represses the expression of two non-FASII genes involved in FA
190 and phospholipid metabolism. i- *fakB3* encodes FakB3, which is involved in the incorporation
191 of polyunsaturated FAs, which are not synthesized by Firmicutes (22, 23); and ii- *plsC*
192 encodes PlsC, which catalyzes joining of a second FA to the 1-acyl-*sn*-glycerol 3-phosphate
193 (bearing a single FA) to complete phospholipid acylation. *S. pneumoniae* FabT thus couples
194 regulation of FA and phospholipid synthesis, similar to FapR regulation in *B. subtilis* and
195 *Staphylococcus aureus*, where the regulatory mechanisms are different (24, 25). A direct or
196 indirect role of FabT in regulating *fakB3* and *plsC* expression remains to be shown.

197 In *S. mutans*, transcription profile comparisons of wild-type and *fabT* deleted strains
198 were performed after growth at pH 7 or pH 5 (20). The expression of 25% of *S. mutans* genes
199 was altered at pH 7 and 7% at pH 5. They also belonged to the same functional categories as

200 described in *S. pneumoniae*. Deletion of *fabT* may thus have more consequences at neutral
201 pH, as normally found in the oral cavity, than at acidic pH, as produced during *S. mutans*
202 growth. Alternatively, low pH already creates the membrane perturbations that are similarly
203 generated by FabT mutation, so that the changes are already in place.

204 Transcriptional analyses of a *fabT* deletion strain were also performed in *S. pyogenes*, in
205 which growth phase and temperature were varied. Stationary growth phase and to a lesser
206 extent 40°C induced the highest number of differentially expressed genes, *i.e.*, up to 28.5%,
207 between wild-type and *fabT* strains. The majority of affected genes was less expressed in the
208 *fabT* mutant (10). Significantly, expression of many genes of carbohydrate metabolism and
209 purine and pyrimidine synthesis pathways was modified, particularly during the stationary
210 phase. This suggests that *fabT* mutations may lead to metabolic dysbiosis. Temperature,
211 growth phase, biofilm status, and the nutritional environment, were documented to affect
212 bacterial FA composition and thereby impact membrane fluidity (26-29). Thus, in bacteria
213 using FabT as the FASII regulator, FabT would ensure the maintenance of membrane
214 homeostasis in response to membrane stress.

215

216 Altogether, these studies indicate that FabT likely has similar roles in different
217 streptococcaceae. They show that the consequences of *fabT* mutation on bacterial physiology
218 are numerous; possibly, these pleiotropic effects are triggered by changes in membrane
219 composition. Our previous works showed that FASII transcription is turned off in FA-rich
220 environments (11); in light of current knowledge, this repression involves FabT. The above
221 observations show the far-reaching consequences of FASII regulation by FabT in bacterial
222 lifestyle.

223

224 **FABT MECHANISM OF REPRESSION**

225 Like other MarR-family proteins, the FabT regulator comprises six alpha helices and
226 three beta strands: $\alpha 1$ - $\alpha 2$ - $\beta 1$ - $\alpha 3$ - $\alpha 4$ - $\beta 2$ - $\beta 3$ - $\alpha 5$ - $\alpha 6$. These regulators exist as homodimers both
227 in their free or DNA-bound forms, and each subunit contributes a winged helix-turn-helix
228 DNA-binding motif (Fig. 2A) (30, 31). MarR regulators have varying DNA targets and
229 interact with either an inducer or a co-repressor that modulates their affinity for their targets.
230 Identification of these cofactors has been invaluable for understanding FabT regulatory
231 mechanisms.

232

233 **FabT dimerization and target gene interaction**

234 The crystal structure of the *S. pneumoniae* FabT – DNA complex revealed that FabT
235 structure is similar to that of the MarR prototype protein (32). The FabT α helix 1 on one
236 subunit intertwines with α helices 5 and 6 of the second subunit, creating a very compact and
237 stable dimer; its $\alpha 2$, $\alpha 3$ and $\alpha 4$ helices, together with the hairpin comprising the $\beta 1$ - $\beta 2$
238 strands, compose the DNA binding domain. However, the acyl-Acp - FabT complex binds
239 DNA with more affinity than FabT alone. Weak FabT-DNA binding could be non-specific, as
240 only a single amino acid residue (Arg89) – nucleotide (T) interaction was highlighted (32).
241 Further structural analysis will be needed to determine the biological relevance and molecular
242 interactions involved in an effective FabT - DNA binding.

243 In *S. pneumoniae*, palindromes upstream of FASII operon genes *fabM*, *fabT* and *fabK*
244 were detected (Fig. 1B) (16). Their roles as FabT binding sites were confirmed by
245 electrophoretic mobility shift assays (12). The positions of these binding sequences in *S.*
246 *pneumoniae*, 10 bp and 30 bp from the ribosome binding sites of *fabT* and *fabK* respectively,
247 suggested that FabT inhibits RNA polymerase recruitment. Alternatively, the palindrome
248 close to the *fabK* ribosome binding site could stop elongation of an RNA whose transcription
249 starts upstream of *acpA* (12). FabT binding sites were also identified *in silico* in *L. lactis*, *S.*

250 *mutans*, *S. pyogenes*, and *E. faecalis*, and were confirmed for *L. lactis* and *S. mutans* (10, 19-
251 21, 33), (34). They were found upstream of various FASII genes *fabM*, *fabT*, and *fabK* or
252 *fabD*, further supporting the existence of two or more FabT-regulated transcriptional units in
253 the main cluster (Fig. 1B). A consensus FabT binding motif was proposed, based on 56
254 predicted or confirmed sequences (https://regprecise.lbl.gov/regulog.jsp?regulog_id=3571)
255 (Fig. 2B). Most MarR proteins are sequence-specific, but may still show some degeneracy
256 (31). More data are needed to determine whether FabT binds to degenerate motifs.

257 The presence of FabT binding sites adjacent to transcriptional start sites of specific
258 genes is indicative of direct FabT regulation. Interestingly, FabT binding sites outside the
259 main FASII locus are present in the intergenic regions between divergent FASII genes, such
260 that FabT likely controls both transcriptional units. This organization was described for other
261 MarR targets (31). Hence, while the location of the FabT binding sites appear to vary
262 according to the species, a common feature of FabT is that it regulates all FASII genes, albeit
263 at different levels, and depending on the growth conditions. Moreover, the low number of
264 additional binding sites predicted in different species suggests that the FabT direct target
265 genes are almost exclusively FASII genes.

266

267 **The FabT co-repressor**

268 **The FabT ligand, Acyl-ACP**

269 MarR family regulators generally possess a ligand acting as a co-repressor or inducer.
270 However, screening for known effectors of other MarR regulators failed to identify the FabT
271 ligand (35). FabT was found to bind a unique ligand, acyl-ACP, the FASII end product, to
272 modulate FASII expression. FabT-(acyl-ACP) binds DNA with high affinity. This feedback
273 regulation strategy contrasts with the feed-forward strategy used by other Firmicutes (see
274 IDENTIFICATION OF FABT AS THE FASII REGULATOR). However, it is not a simple

275 feed-back regulation. AcpA is encoded in the FASII main locus and *acpA* expression is
276 controlled by FabT, implying that the excess of acyl-AcpA stops *acpA* synthesis. This in turn
277 leads to lower production of acyl-AcpA-FabT production, and subsequent derepression of the
278 FASII genes. This regulation favors fine tuning of the available pools of Acyl-AcpA.

279 It is notable that FabT-(acyl-ACP) is not a unique molecule, and its binding to DNA
280 targets varies according to the acyl moiety (32). While FabT reportedly has the same affinity
281 for all acyl-ACPs, the affinity of the FabT - acyl-ACP interaction with DNA increases with
282 the length of the acyl carbon chain, with a marked increase beyond 14 carbons in *S.*
283 *pneumoniae* (35). Thus, the length of the acyl-ACP chain is essential for consolidating FabT
284 binding to DNA. Also, FabT - DNA binding is greater when FabT is complexed with C18:1-
285 ACP than with C16:0-ACP. This suggested that chain length, and/or saturation status of the
286 bound fatty acid might determine FabT-DNA binding affinity. In *E. faecalis*, FabT-mediated
287 repression of FA synthesis is similarly exacerbated by C16:1 and C18:1 (*cis*-9), but less so in
288 C18:1 (*trans*-9) and even less so in C16:0 (36). This argues in the favor of *cis* unsaturated FA
289 maximizing FabT-DNA binding affinity.

290 The co-repression mechanism mediated by *S. pneumoniae* FabT and acyl-ACP was
291 characterized by combining crystallographic, biochemical and genetic approaches (32). One
292 acyl-ACP molecule binds to each subunit of the FabT dimer. It was suggested that acyl-ACP
293 more readily binds FabT than does free ACP, and that the acyl-ACP-FabT complex provokes
294 a change in FabT conformation favoring the interaction with DNA (32, 35). Evidence for this
295 interaction was obtained by replacing FabT lysine 97 and ACP aspartate 50 by cysteine
296 residues; protein interactions and DNA binding were observed in the absence but not in the
297 presence of DTT (32). The acyl moiety binds directly within a hydrophobic pocket of FabT,
298 favoring ACP interaction within the FabT $\alpha 5$ helix, leading to formation of a stable FabT -
299 acyl-ACP complex (32). The length of the acyl moiety increases stability of the acyl-ACP -

300 FabT complex; this makes physiological sense, as accumulation of full-length phospholipid
301 precursors should arrest FASII.

302 An increased concentration of 18:1-ACP during electrophoretic mobility shift assays
303 performed with a fixed concentration of FabT protein led to a more intense FabT-DNA band
304 (35). Possibly, the acyl-ACP cofactor could be a limiting factor for interactions between
305 FabT-(acyl-ACP) and DNA in the bacterium (32). This would explain the modest difference
306 in repression of FASII genes in *L. lactis* when FabT was overproduced 135-fold (19).

307

308 **Why are there two ACPs in some species?**

309 Numerous species of the order *Lactobacillales*, which comprise the *Streptococcaceae*,
310 possess two ACPs. AcpA is encoded within the FASII locus; AcpB is encoded in the same
311 operon as PlsX (glycerol-3-phosphate acyltransferase), required for phospholipid synthesis
312 (Fig. 1B) (14, 32, 37). *E. faecalis* and *S. pneumoniae* encode the second ACP, AcpB, while *L.*
313 *lactis* does not. AcpB is also found in *C. acetobutylicum* in the same operon as *plsX*
314 (unpublished result). AcpA and AcpB share approximately 40%, 24%, 30% and 45%
315 sequence identity in *S. pneumoniae*, *S. pyogenes*, *E. faecalis* and *C. acetobutylicum*,
316 respectively (unpublished results; (14, 32, 37)). A phylogenetic tree based on multi-alignment
317 comparison of AcpA and AcpB shows clustering of the four AcpA proteins, but not of the
318 four AcpBs. In *C. acetobutylicum*, AcpB is more closely related to the AcpA proteins than to
319 the other AcpB proteins (Fig. 2C). Closer homology between AcpA proteins suggests that it
320 may have more functional constraints than AcpB. Multi-alignment comparison of AcpB
321 sequences highlights clusters of similarity (Fig. 2D). However, BLASTP analysis indicates
322 that AcpB is absent or severely truncated in over half of sequenced *S. agalactiae* strains
323 available in NCBI (unpublished results). These observations raise questions about the need
324 for a second acyl carrier protein in some species.

325 Distinguishing features of AcpA and AcpB with respect to FASII and to interactions
326 with FabT were investigated in *E. faecalis* (14). Whereas the *acpA* gene is essential, *acpB* is
327 not. Furthermore, the *acpB* mutant grows without exogenous FA supplementation, unlike
328 FASII mutants (13). AcpA and AcpB involvement in FA synthesis was assessed by studying
329 their interactions with FabI and FabK enoyl-ACP reductases, which catalyze the last step in
330 FASII synthesis to produce acyl-ACP (38) (Fig. 1A). FabI was reportedly more effective than
331 FabK in reducing the acyl-AcpA precursor; conversely, FabK reduced the acyl-AcpB
332 precursor more effectively than FabI (14). However, interactions of AcpA and AcpB were not
333 studied in *Streptococci*, which possess only FabK. These results suggested that FabI and
334 AcpA are main actors in FA *de novo* synthesis in *E. faecalis*, whereas the role of AcpB in FA
335 synthesis remains in question. To define the involvement of AcpA and AcpB in the
336 phospholipid synthesis step, authors then followed the activity of PlsX, which catalyzes
337 reversible transfer of the acyl moiety between ACP and a phosphate group. Acyl transfer from
338 AcpA proved much more efficient than from AcpB. In contrast, in the presence of exogenous
339 FAs and PlsX, acyl transfer to AcpB (the reverse reaction) was the more efficient. This
340 indicated that acyl-AcpA is the preferred PlsX substrate to synthesize acyl-phosphate, which
341 then initiates phospholipid production *via* PlsY. In contrast, free AcpB would be a PlsX
342 substrate, which produces Acyl-AcpB in the reverse reaction. Acyl-AcpB would then act as
343 corepressor of FabT, signaling availability of the exogenous FA sources. Acyl-AcpB could
344 additionally be a precursor for phospholipid synthesis that favors entry of exogenous fatty
345 acids (14). Altogether, this suggested that AcpA is the main FASII enzyme needed for FA *de*
346 *novo* synthesis, but may also regulate FASII repression in the presence of exogenous FA;
347 AcpB which is encoded in the same operon as *plsX* and is nonessential, may only have a role
348 in facilitating exogenous FA utilization for phospholipid synthesis (14, 36). However, part of
349 these conclusions were recently revised ((36); see below).

350 Although some features of AcpB have been elucidated, our understanding of its role in
351 fatty acid metabolism remains incomplete: a comparison of membrane FA composition and
352 FASII gene repression in the presence of exogenous FAs indicated that *acpB* and *fabT* mutant
353 strains had the same phenotypes, suggesting that AcpB is a corepressor of FabT (14). In
354 keeping with this observation, acyl-AcpB, but not AcpB alone, increased FabT-(acyl-ACP)
355 binding to DNA in *S. pneumoniae* (32). *In silico* comparison of AcpB sequences shows that
356 amino acid residues involved in interactions with FabT are only partially conserved in *S.*
357 *pyogenes*. AcpB Asp⁵⁰ interacts with the FabT Lys⁹⁷; however, the spatially adjacent AcpB
358 Glu⁵⁵ is not conserved, which may weaken the interaction (Fig. 2D) ((unpublished results);
359 (32)). Moreover, AcpB is absent in about half the *S. agalactiae* strains and in *L. lactis*.
360 Consistent with the above observations, a recent report clarified that the role of AcpB in
361 controlling FabT repression was minor, compared to earlier conclusions that the *acpB* mutant
362 exerted a dominant effect on FabT repression; a secondary mutation in *fabT* was identified,
363 which accounted for this revised explanation (14, 36). In a reconstructed *E. faecalis acpB*
364 deletion strain, addition of exogenous FA showed just partial repression of *fabT* promoter
365 expression (36). Also, and in contrast to the situation in *S. pneumoniae*, in *E. faecalis*, acyl-
366 AcpA enhances FabT binding to the *fabT* promoter *in vitro* (32, 36). This confirms that in *E.*
367 *faecalis*, AcpA is also a FabT corepressor. Altogether, these results suggest that AcpA and
368 AcpB as studied in *E. faecalis* and *S. pneumoniae* do not have totally distinct functions, and
369 that their properties may not be conserved in all related strains and species (14). The
370 differential roles of the two ACP proteins merit further study.

371 Since AcpB is constitutively synthesized in exponential growth (10, 12, 21), the
372 concentration of acyl-AcpB and consequently its repression efficiency may depend primarily
373 on the amounts and composition of exogenous FAs. A regulatory model was proposed based
374 on the above considerations (Fig. 3). It is likely that the repressive effects of exogenous FA

375 on FASII gene transcription, as we previously described (11), are carried out at least in part
376 *via* AcpB.

377

378 **REGULATION OF *FABT* TRANSCRIPTION AND *FABT* ACTIVITY**

379 FabT regulates FASII synthesis and concomitantly, exogenous FA utilization and
380 phospholipid synthesis. To understand how this regulon responds to bacterial metabolism, we
381 must first know how FabT itself is regulated.

382 In *S. mutans*, in-depth analysis of genes affected by deletion of *fabT* suggested that
383 catabolite control protein CcpA, a master regulator of catabolic repression in Gram-positive
384 bacteria, also controls their expression. CcpA DNA binding motifs were identified upstream
385 of different *fab* genes and confirmed to be active. Importantly, *fabT* is regulated by CcpA
386 (20). By controlling *fabT* expression, CcpA links carbon and FA metabolism. Whether CcpA
387 exerts similar roles in *fabT* regulation in species related to *S. mutans* remains to be confirmed.

388 In *S. pneumoniae*, *fabT* expression is regulated by a two-component system called
389 VicKR (also known as WalKR), which responds to parietal stress. Upon membrane stress, the
390 VicK kinase autophosphorylates and then phosphorylates the VicR regulator, leading to
391 repression of VicRK regulon genes within 10 minutes (39, 40). In the absence of parietal
392 stress, the non-phosphorylated VicR regulator binds to the *fabT* promoter region, thus directly
393 repressing its transcription (41). *fabT* repression in the non-stress state leads to upregulation
394 of the *fabK* operon, which results in longer FA chains (39). These findings indicate that
395 bacterial membranes would comprise a higher proportion of long-chain FAs in the absence
396 than in the presence of parietal stress; this regulation would contribute to bacterial adaptation
397 to each condition. This adjustment of membrane composition at the *fabT* transcriptional level
398 may bypass the need for FASII regulation *via* (acyl-Acp)-FabT during parietal stress.

399 In *S. pyogenes*, Stk is the kinase of a two-component histidine kinase/regulator system
400 (33). Transcriptomic analysis of the *stk* deleted strain suggested that Stk indirectly activates
401 expression of FASII pathway genes. Two hypotheses were proposed. The simplest is that Stk
402 phosphorylates FabT, preventing it from exerting its role as a transcriptional repressor. The
403 second postulates that Stk phosphorylates FASII enzymes, and that a loss of function leads to
404 feedback activation of FASII gene expression. Kinases may have important roles in
405 modulating FASII activity that warrant further studies.

406 Taken together, FabT is subject to various selective pressures and types of regulation,
407 which seem to differ according to the *Streptococcus* species. Whether regulatory controls on
408 *fabT* cross species boundaries remains to be tested. However, these reports underscore the
409 importance of FabT in coordinating FASII-mediated membrane biogenesis with other
410 metabolic processes to optimize bacterial fitness.

411

412 **FABT AND BACTERIAL PHYSIOLOGY**

413 Invasive *S. pyogenes* infections cause >150 000 deaths / year, thus calling for new
414 treatment targets and strategies. We discussed above that emergence of *fabT* variants
415 correlated with non-invasive infections (see section 2). Mutations in *fabT* may thus be
416 counterselected in invasive infections due to lower virulence of these mutants. Assessing the
417 consequences of mutating *fabT* on bacterial physiology and on potential penalties and/or
418 benefits during infection could give insight into the use of FabT as a therapeutic target to
419 reduce severity of infection.

420 The extent to which FabT affects bacterial survival *in vitro* varies with the species, and
421 possibly with test conditions. In *E. faecalis* and *L. lactis*, *fabT* deleted strains are viable,
422 although the *E. faecalis* mutant displays a growth delay (14, 19). An *S. pneumoniae fabT*
423 mutant containing a stop codon early in the *orf* is viable, with no growth defects reported in

424 rich medium (12). In contrast, construction of *S. pyogenes fabT* deletion mutants, as attempted
425 in three separate studies, suggested that *fabT* has an important role: *fabT* mutants could not be
426 obtained without deleting *clpX* (a protease ATP-binding subunit), or *gdpP* (encoding a c-di-
427 AMP phosphodiesterase) (21). In a second study, a *S. pyogenes fabT* deleted strain displayed
428 highly altered growth: longer latency time, slower growth and lower bacterial yield in rich
429 medium (8). Growth was restored in medium supplemented with unsaturated FAs (*cis*-
430 vaccenic acid, C18:1, or *cis*-eicosenoic acid, C20:1). Unlike *fabT* deletion mutants, *fabT* point
431 mutants were obtained with no effects on *in vitro* growth. In a third study, a *S. pyogenes fabT*
432 deletion mutant displayed no major doubling time defect but did show a longer latency period
433 (10). All the *S. pyogenes* constructs respected the integrity of *fabH*, the gene directly
434 downstream of *fabT* (Fig. 1B); hence, the discrepancies between reports can best be explained
435 by wild-type strain differences and/or variations in test conditions. Despite these phenotypic
436 differences, a common feature of the *fabT* point mutant and deleted strains was that both
437 displayed attenuated virulence (8, 10).

438 Since FabT regulates the quasi-ensemble of FASII pathway genes (see THE FABT
439 REGULON), its inactivation should have direct consequences on membrane FA composition.
440 In *S. pneumoniae* and *L. lactis*, the proportion of long-chain FAs is higher in the *fabT* mutant
441 than in the wild-type strain, in keeping with derepression of FASII (12, 19). A greater
442 proportion of saturated FAs was also reported. Interestingly, *fabT* inactivation leads to greater
443 up-regulation of *fabK* than *fabM*. FabM (required for unsaturated FA synthesis) and FabK
444 compete for the same substrate (Fig. 1A); the relative increase in FabK expression compared
445 to FabM accounts for this ratio variation. Moreover, in *S. pneumoniae*, FabT also controls
446 phospholipid synthesis gene *plsC* (see THE FABT REGULON). These observations are
447 consistent with findings described above, that *fabT* repression results in longer FA chains (39)
448 (see REGULATION OF *FABT* TRANSCRIPTION AND *FABT* ACTIVITY).

449 Membrane permeability studies were performed to determine how FabT mutation-
450 induced alterations of FA ratios impact membrane properties. Measurements of ethidium
451 bromide incorporation in *L. lactis* indicated that permeability of the *fabT* membrane is greater
452 than that of the wild-type strain (19). Membrane rigidity is known to depend in part on the
453 ratio of saturated / unsaturated FAs. Different ratios in wild-type and *fabT* strains can explain
454 the reported differences in membrane permeability.

455 Osmotic homeostasis is critical for all cells, and membrane FA composition is a key
456 determinant of bacterial survival under osmotic pressure. Interestingly, *fabT* mutations arose
457 when *S. agalactiae* was submitted to osmotic stress conditions: in that work, strains were
458 grown in a minimal medium supplemented with 0.5 mM potassium (42). One could speculate
459 that higher relative amounts of saturated FAs in the *fabT* mutant, and consequent modified
460 permeability may be responsible for this natural selection. Alternatively, but not exclusively,
461 altered membrane protein composition may contribute to the response to potassium excess
462 (see below).

463 Modifying membrane composition by environmental FAs that are not normally present
464 may affect bacterial growth or survival. For example, ~20µg/ml free linoleic acid (C18:2), a
465 main FA constituent of soy bean oil, inhibits growth of lactobacilli. Increased consumption of
466 soybeans in the USA and Europe was suggested to correlate with a decrease of *L. reuteri* and
467 *Lactobacillus johnsonii* prevalence in the human intestinal microbiota (43). Di Rienzi *et al*
468 considered a possible link between this decrease and enriched linoleic acid in the diet.
469 Isolation of linoleic acid-resistant *L. reuteri* mutants identified mutations in *fabT*. In
470 hindsight, the selection of *fabT* mutations would lead to increased proportions of membrane
471 saturated FAs, which could equilibrate FA composition in linoleic-acid-rich environments.

472 During a search for a narrow-spectrum antibiotic, the alkylated dicyclohexyl carboxylic
473 acid 2CCA-1, a polyunsaturated FA mimetic, was identified as targeting *S. pneumoniae*.

474 Investigation of its bactericidal effect led to the isolation of resistant mutants (23). One
475 mutation mapped to *fakB3*, encoding a FA binding protein involved in the uptake of
476 polyunsaturated FAs. The second mapped to *fabT*. *fakB3* is less expressed in the *fabT* mutant
477 than in the wild-type strain. Consequently, in both mutant strains, 2CCA-1 incorporation
478 would be reduced, rendering the strain resistant to the FA analog.

479 *S. pyogenes* strains were selected for resistance to polymyxin B, an antibiotic targeting
480 anionic lipids (21). Sequencing showed that *fabT* point mutants displayed high polymyxin B
481 resistance. In line with this result, *fabT* deleted strains are more resistant to polymyxin B (10,
482 19). Thus, changes in FA synthesis associated with *fabT* mutations may alter lipid
483 composition, with consequences on antibiotic resistance, particularly to antibiotics targeting
484 membrane constituents.

485 Deletion of *fabT* impacts *S. pneumoniae* adaptation to different known stresses (pH,
486 reactive oxygen species (ROS), detergents) (12). The *fabT* mutant proved to be more sensitive
487 than the wild type strain to acidic pH and detergents such as deoxycholate, suggesting that
488 FabT may have specific roles in bacterial adaptation to these conditions. Resistance to
489 ampicillin, vancomycin, and ROS was unchanged. Mutations in the FabT partner AcpB,
490 would expectedly give similar effects as the *fabT* mutant, and could further strengthen these
491 findings.

492 Membrane modifications may also affect structure, function, and abundance of
493 membrane, parietal, and secreted proteins. Indeed, some membrane protein complexes, such
494 as the general secretion system Sec or sortases, are involved in the secretion and/or surface-
495 anchoring of numerous proteins (44). Changes in phospholipid characteristics could affect
496 both expression levels and activities of membrane proteins, and thereby impact bacterial
497 adaptation to environmental changes. Studies based on exogenous FA incorporation
498 capacities of *E. faecalis* confirm that disturbance of the membrane FA ratio modifies bacterial

499 morphology, growth and adaptation capacities (45, 46). Current studies in our laboratory will
500 monitor the effects of *S. pyogenes fabT* mutations (which alter the membrane FA ratio) on
501 protein expression and bacterial fitness. This study might help explain the emergence of
502 mutations under some stress conditions.

503 Studies thus far show that FASII deregulation when FabT is mutated has direct and
504 indirect consequences on membrane composition, and modifies bacterial adaptation to its
505 environment. Selective advantages driven by *fabT* mutations during non-invasive infection
506 remain to be assessed. Accumulation of pathogen mutations during infection raises the
507 possibility that *fabT* revertants might be selected because they restore bacterial invasive
508 capacity.

509

510 **CONCLUSIONS**

511 The emergence of multi-resistant bacteria and growing awareness of the need to preserve the
512 microbiota have spurred development of therapeutic targets specific to bacterial pathogens.
513 FASII was thought to be essential for bacterial survival, which drove the development of
514 numerous FASII inhibitors. The understanding that Gram-positive bacteria, such as *S. aureus*,
515 *E. faecalis* and *Streptococci*, incorporate exogenous FAs that allow these bacteria to bypass
516 FASII inhibition has modified the rationale of monotherapy, as anti-FASII reaches its targets,
517 but fails to block streptococcal, staphylococcal and enterococcal growth in FA-rich host
518 environments (11, 13, 47-50). Thus, anti-FASII are ineffective as stand-alone *in vivo*
519 antibacterial agents against these bacteria.

520 Here we summarized the roles of FabT as a major regulator that acts as repressor in the
521 presence of long chain acyl-ACP. Whether acyl-ACP comprises AcpA or AcpB, which are
522 stakeholders of a feedback or an exogenous FA-sensing mechanism, respectively, FabT-acyl-
523 Acp-mediated repression limits futile FASII activity. FabT mutants arise spontaneously when

524 bacteria undergo osmotic stress, and seem to emerge in non-invasive infections, suggesting
525 that *fabT* mutations may improve bacterial fitness in certain conditions. Since FabT controls
526 FA length and the saturated to unsaturated FA ratio, the state of FabT is a key factor in
527 bacterial fitness and adaptation. Development of FabT inhibitors, such as FAs leading to acyl-
528 Acp toxic analogues, could pave the way for new narrow-spectrum therapeutic tools.

529 **ACKNOWLEDGEMENTS**

530 We thank Dr A. Grove for permission to reproduce and adapt figures from her
531 manuscript (Gupta *et al.* 2019. MarR Family Transcription Factors from *Burkholderia*
532 Species: Hidden Clues to Control of Virulence-Associated Genes. *Microbiol Mol Biol Rev*
533 83:e00039-18).

534 Research of authors on the topic of this review was funded by the following French
535 granting agencies: the regional DIM-Malinf, DIM-OneHealth Projet 00002247, the Agence
536 Nationale de la Recherche (StaphEscape project ANR-16-CE15-0013), and the Fondation
537 pour la Recherche Medicale ([DBF20161136769](#)), the Université de Paris (CL).

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688

689 **BIOGRAPHICAL SKETCHS**

690 **Clara Lambert** is a fourth-year PhD student in Pr. Claire Poyart and Dr. Agnès Fouet's
691 laboratory at Institut Cochin, Paris, France. She received her bachelor's degree in biology
692 from Université Paris Saclay in 2016 and in 2018 her master's in fundamental microbiology
693 from Université Paris Cité in collaboration with Institut Pasteur. She is currently investigating
694 the role of FabT in *Streptococcus pyogenes* invasive infections. She characterizes the link
695 between the deregulation of fatty acid synthesis and the virulence defect in *S. pyogenes*. Using
696 various approaches, her project provides a holistic view of the consequences of FabT
697 mutations on bacterial environmental adaptation. She has expertise in bacterial physiology,
698 omic techniques such as transcriptomic, proteomic, lipidomic and metabolomic, and *in vivo*
699 and *ex vivo* analysis of bacteria-host interaction. She will defend her thesis in December 2022.
700 After graduating, she would like to investigate the importance of the bacterial cell-wall in
701 bacterial adaptation.

702

703 **Prof Claire Poyart**, MD,PhD, studied medicine at the Universities Pierre&Marie Curie and
704 Paris Descartes and her PhD at Institut Pasteur.

705 In 2003, nominated Professor at Hospital Cochin, she heads the Clinical bacteriological
706 laboratory. Since 2006, she heads the National Reference Center for Streptococci and co-
707 directs an INSERM Team at the Institut Cochin affiliated to Université Paris Cité.

708 Her research combines clinical and fundamental approaches towards characterizing the
709 virulence factors of Group A and B Streptococcus. The main fundamental research topics are:
710 surface proteins, environmental adaptation and expression of virulence genes, genetic
711 variability and virulence. She showed, together with Alexandra Gruss, that fatty acid
712 synthesis is not a suitable antibiotic target for Gram-positive pathogens. As a clinical
713 microbiologist her clinical research aims at the epidemiological characterization of GAS and

714 GBS strains responsible for invasive infections and colonization, the development of
715 diagnostic tools for the detection and the identification of Gram-positive bacteria.

716

717 **Alexandra Gruss** obtained her PhD in Microbiology from the New York University Medical
718 Center, where she worked on plasmid replication determinants in *Staphylococcus aureus*. She
719 then established her lab at the French National Institute for Agriculture, Food and
720 Environment (INRAE, Jouy en Josas). Her research team identified the recombination
721 ‘crossover hotspot instigator’ motif Chi in various Gram-positive bacteria; it also isolated
722 thermosensitive plasmids currently used for genetic manipulation. Her subsequent research on
723 dairy bacteria uncovered that exogenous heme activated respiratory metabolism, greatly
724 improving lactococcal biomass and stability; this fundamental observation was successfully
725 applied to industrial starter culture production. Fatty acid utilization has been a research focus
726 of authors’ labs since 2006. A major finding was that environmental fatty acids, as used by
727 essentially all Firmicutes, can overcome antibiotics targeting the fatty acid synthesis (FASII)
728 pathway. A current focus is on combinatorial therapy approaches that block *S. aureus*
729 development.

730

731 **Agnes Fouet** obtained her PhD at the Pasteur Institute, Paris, on the regulation of genes
732 encoding proteins involved in sucrose transport and catabolism in *Bacillus subtilis*. She then
733 moved to Tufts Medical School, Boston, as a post-doc where she studied the relationship
734 between catabolic repression and repression of sporulation initiation in *B. subtilis*. In 1990,
735 she established her group at the Pasteur Institute, where her research focused on surface
736 components and regulation of synthesis of virulence factors in *Bacillus anthracis*. In 2011,
737 she moved to the Institut Cochin, Paris, and initiated an entirely new project, investigating the
738 mechanisms involved in initial steps of *Streptococcus pyogenes* infection. In this context, she

739 uncovered a pivotal role of fatty acid metabolism in *S. pyogenes* - host cell interaction. She
740 collaborates with Alexandra Gruss at the Micalis Institute, Jouy en Josas, France, on fatty acid
741 metabolism in other Firmicutes, including *Enterococcus faecalis*.

742

743 **FIGURE LEGENDS**

744

745 **FIG 1. Fatty acid synthesis pathway (FASII) in food and pathogenic Lactobacillales.** (A)

746 FASII comprises a first initiation phase for precursor synthesis, followed by the recursive
747 elongation cycle. The mature acyl-ACP products supply fatty acids for phospholipid
748 synthesis. Products of FabM/FabN are unsaturated (in gold), while those of FabI/FabK are
749 saturated. Proteins performing the same functions may vary according to species. (B) FASII-
750 genes are clustered in *Streptococci* (*S. pneumoniae*, *S. agalactiae*, *S. pyogenes*), *E. faecalis*,
751 and *L. lactis*, and share a similar genetic organization. Proteins (in A), or coding genes (in B)
752 are in green for initiation functions, red for elongation functions, brown for FA-modifying
753 functions, and grey for phospholipid synthesis functions. *fabF1* is also named *fabO*. In A) the
754 dashed arrow represents the shunt leading to unsaturated FA synthesis; in B) dark blue, *fabT*;
755 purple and light blue, *acpA* and *acpB*, respectively. Bent arrows, transcription start sites (12,
756 15); dashed bent arrow, suggested transcription reinitiation site (11); * FabT binding sites; a
757 single star represents one or two closely localized consensus sequences.

758

759 **FIG 2. Structural features of FabT, the ACP corepressor, and the FabT-DNA binding**

760 **motif.** (A) Overall structure of MarR family member dimer (30); one monomer is represented
761 multicolored and the other in blue. (B) FabT DNA binding motif; top, calculated from 56
762 FabT DNA binding sequences (https://regprecise.lbl.gov/regulog.jsp?regulog_id=3571). The
763 relative size of letters corresponds to the relative frequency at which each nucleotide is
764 present. (C) Phylogenetic Neighbor-joining tree obtained from a multiple sequence alignment
765 carried out using Clustal W (1.83). Branch lengths correspond to: *C. acetobutylicum* AcpA,
766 0.28257; *E. faecalis* AcpA, 0.19187; *S. pneumoniae*, AcpA 0.0325; *S. pyogenes*, AcpA
767 0.04858; *C. acetobutylicum* AcpB, 0.25594; *E. faecalis* AcpB, 0.29157; *S. pneumoniae*, AcpB,

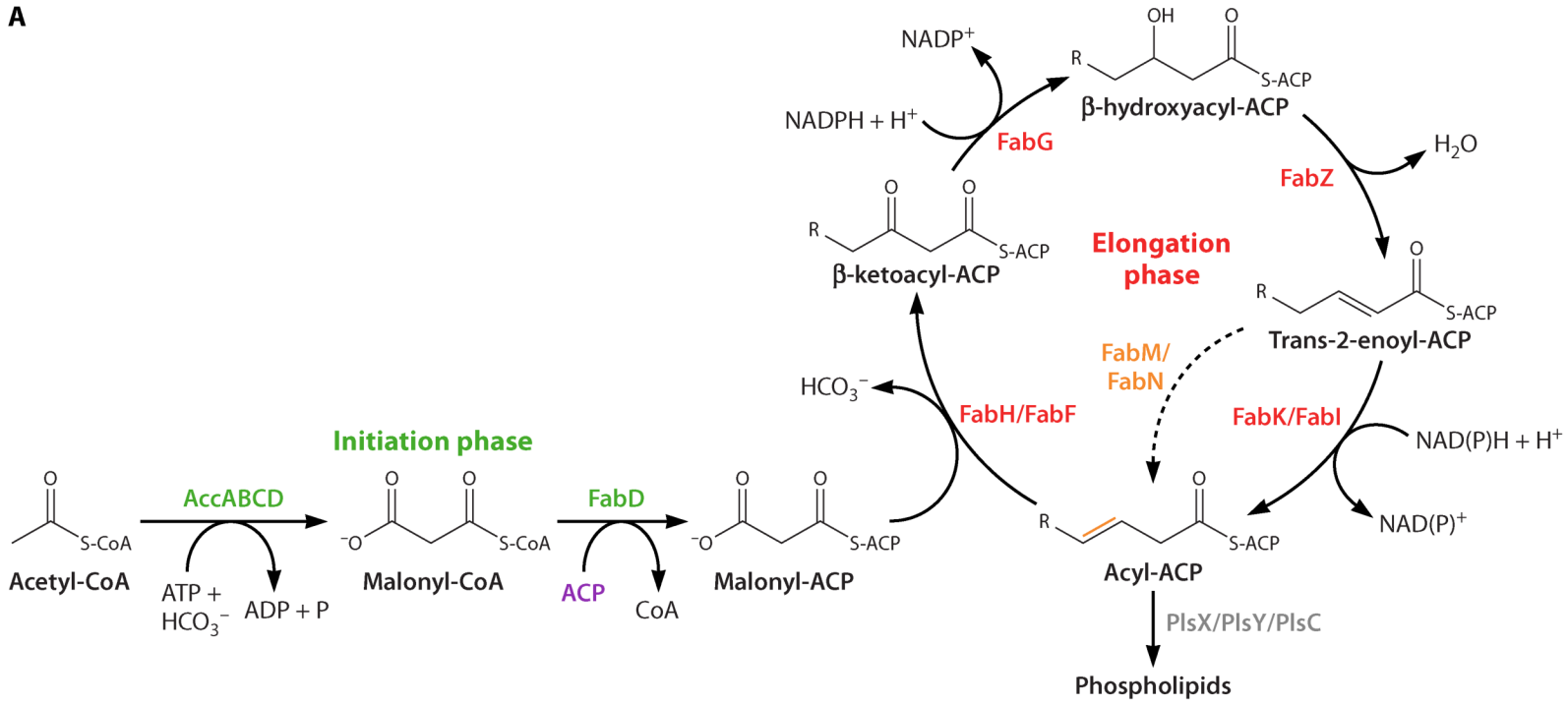
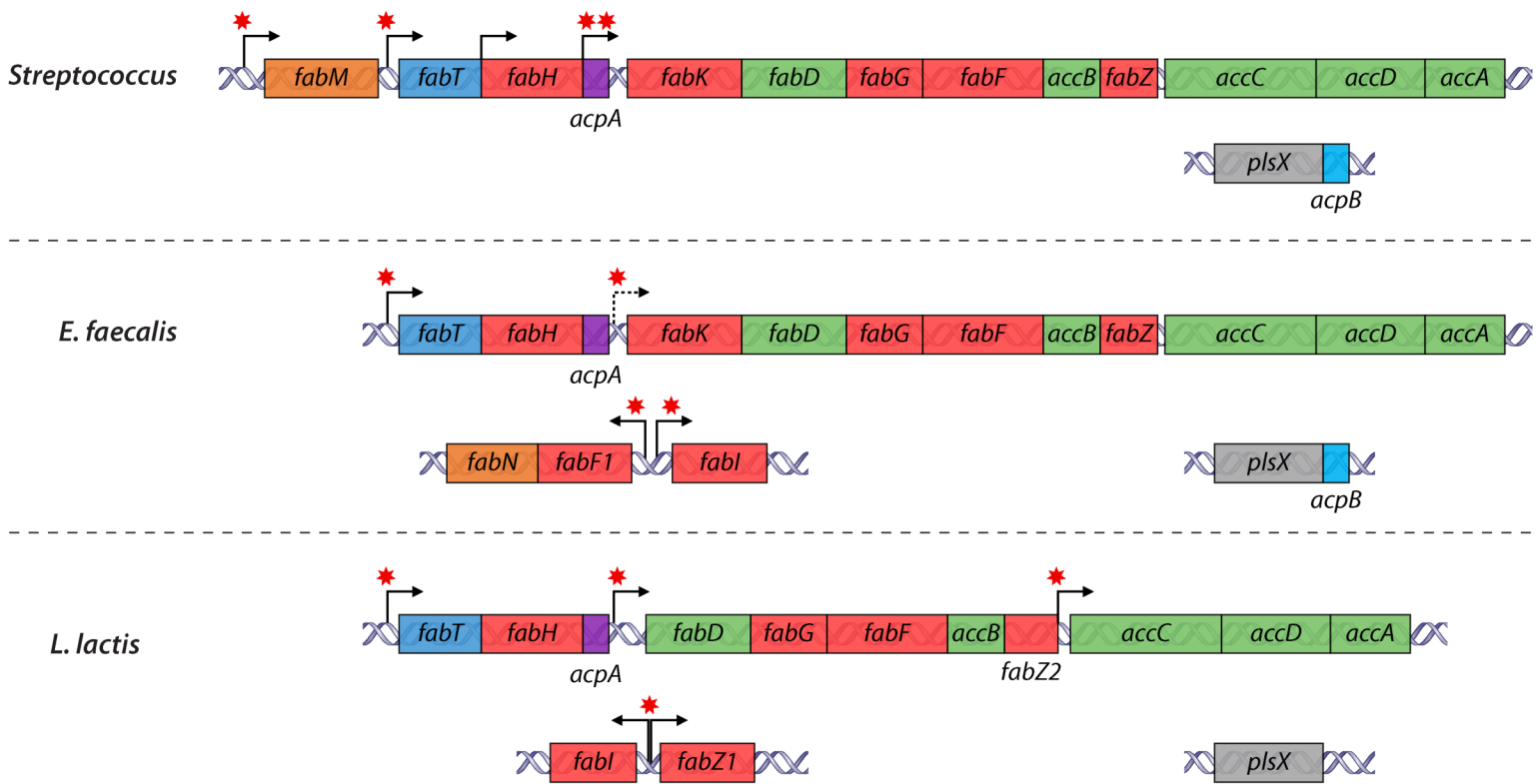
768 0.25143; *S. pyogenes*, AcpA, 0.35383. (D) AcpB alignment; multiple sequence alignment was
769 carried out using Clustal W (1.83) (unpublished results), color code follows functional
770 classification; ‘*’ identical in all four species; ‘:’ the same structural class; vertical arrow
771 highlights amino acid residue 55, which is implicated in FabT – AcpB binding (32)) but is not
772 conserved in *S. pyogenes*.

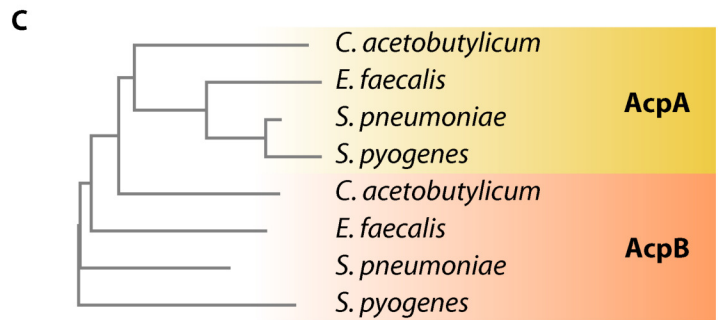
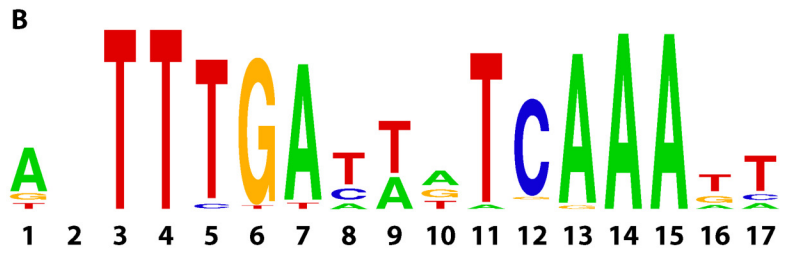
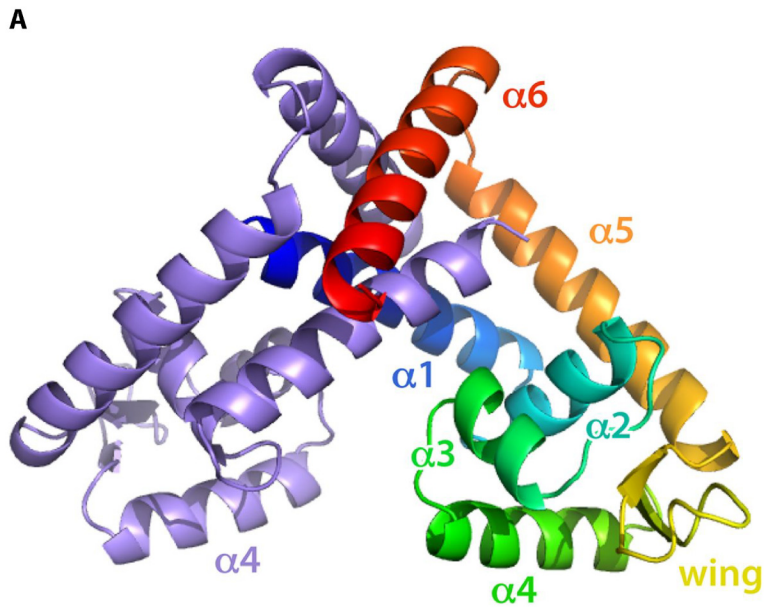
773

774 **FIG 3. Model of the FabT repression mechanism in *S. pneumoniae* and *E. faecalis*.** (A)

775 Endogenous FA synthesis: An excess of FA synthesis generates acyl-AcpA, which binds to
776 FabT; the complex binds with low affinity to the promoter regions of its target transcriptional
777 units, leading to weak repression of FabT directly controlled genes, including *acpA*. Acyl-
778 AcpA is also a substrate for the bidirectional glycerol-3-phosphate acyltransferase PlsX,
779 leading to phospholipid synthesis (not shown). The minor apparent regulatory role of FabT
780 when interacting with acyl-AcpA contrasts with its prominent role when exogenous FAs are
781 present, *e.g.*, as is common *in vivo*. (B) Exogenous FA utilization: Entering FAs may be
782 phosphorylated by fatty acid kinase complex FakA coupled to one of the FA-binding FakB
783 subunits, each of which has FA preferences (22). Acyl-P is then converted to acyl-AcpB or
784 AcpA (36) by a reverse PlsX reaction. Acyl-ACP binds to FabT, and the complex binds with
785 high affinity to the promoter regions of its transcriptional unit targets, strongly repressing
786 gene expression. The information contained in this figure corresponds to results shown in at
787 least one species. Grey arrows under the genes represent the various transcriptional units
788 described; their thickness symbolizes the level of transcription. Black arrows above or below
789 the genes represent the binding of acyl-Acp to promoter regions; their thickness indicates
790 repression efficiency; dotted arrows indicate that the repression level is species dependent.
791 Note that only FabT-related functions are indicated. eFAs: exogenous FAs; acyl-P, acyl-

792 phosphate. Note that acyl-AcpA is the FASII product; both acyl-AcpB and acyl-AcpA appear
793 to be involved in eFA incorporation and reverse activity of PlsX.

A**B**

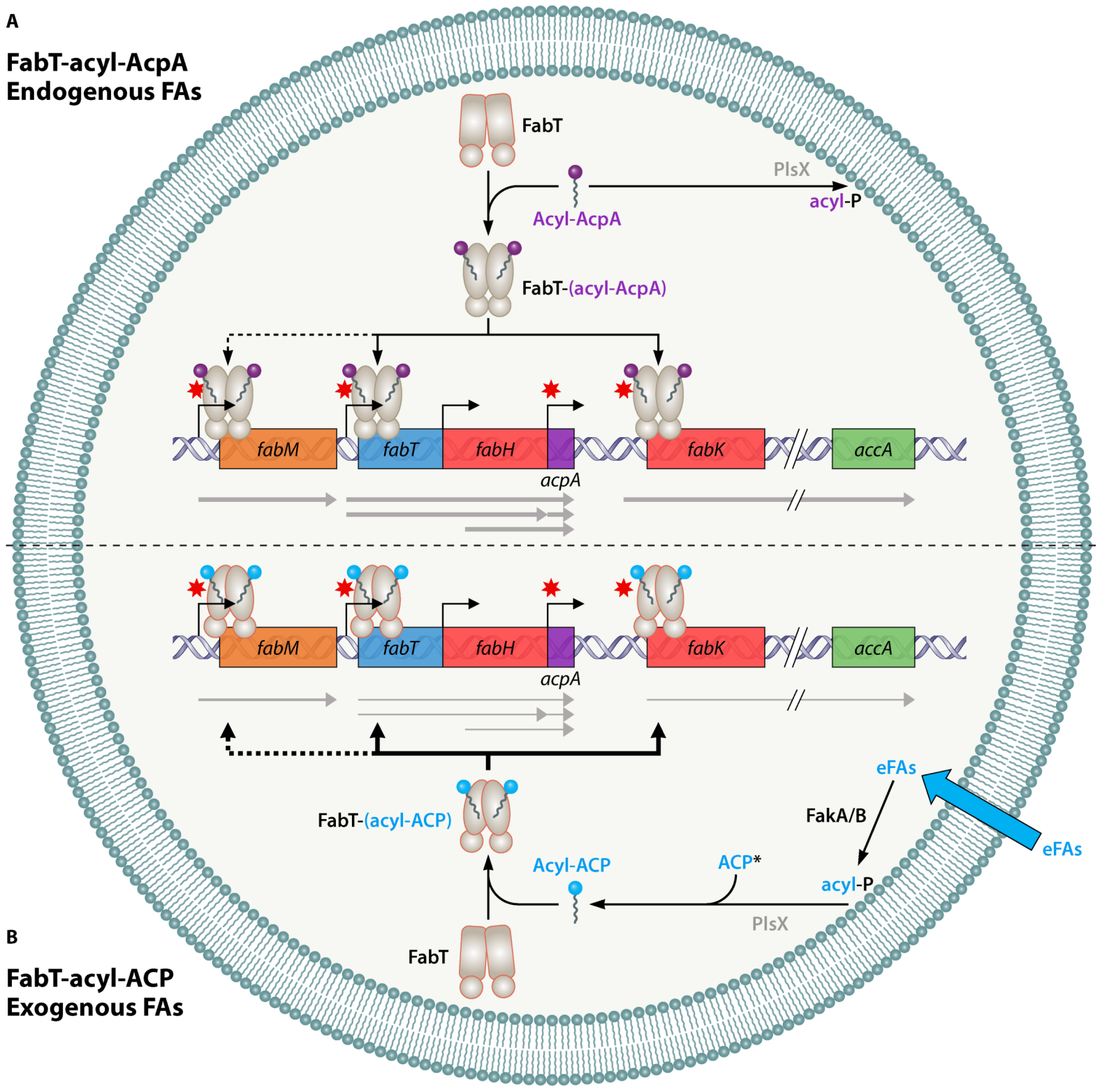


D

	1		40
<i>C. acetobutylicum</i>	M	---	VFEKVKDIIADQLGIDATEIKMESSFIDDLGADSLDIVELIMALE
<i>E. faecalis</i>	M	TREEVLQKVAKIISNHFDIEADQVTDQLNIKDDLNADSLVMEFVLELE	
<i>S. pneumoniae</i>	M	TEKEIFDRIVTIIQERQGE-DFVVTESLSLKDDLADSVLMEFILTLE	
<i>S. pyogenes</i>	M	TRQEIFERLINLIQQRSYLSVAITEQTHLKNDLAVDSIELVEFIINVE	
	*	: : : : : *	: . . : : ** . ** : : : : : : *
		55	
<i>C. acetobutylicum</i>		EEFDIEMPDEEA	EKVSSVGDVVNYIKAHTEE
<i>E. faecalis</i>		DEFGTEISDEDAEKIETVGA	AVDYIVSN--S
<i>S. pneumoniae</i>		DEFSIEISDEEIDQLQNVGDVVKIIQ	G---K
<i>S. pyogenes</i>		DEFHIAIPDEDVEDMVFMEDVLDYLVQRL	-T
		***	↑ : : ** : : : : : . . . :

A

**FabT-acyl-AcpA
Endogenous FAs**



B

**FabT-acyl-ACP
Exogenous FAs**

