Transcriptional regulation in bacterial membrane lipid synthesis

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Abstract This review covers the main transcriptional mechanisms that control membrane phospholipid synthesis in bacteria. The fatty acid components are the most energetically expensive modules to produce; thus, the regulation of fatty acid production is very tightly controlled to match the growth rate of cells. Gram-negative and Gram-positive bacteria have evolved different structural classes of regulators to control the genes required for fatty acid biosynthesis. Also, there are other transcriptional regulators that allow the cells to alter the structure of fatty acids in existing phospholipid molecules or to modify the structures of exogenous fatty acids prior to their incorporation into the bilayer.

A major thrust for future research in this area is the identification of the ligands or effectors that control the DNA binding activity of the transcriptional regulators of fatty acid biosynthesis. With the exception of malonyl-CoA regulation of FapR from Bacillus subtilis and long-chain acyl-CoA regulation of FadR from Escherichia coli and DesT from Pseudomonas aeruginosa, the identity of these intracellular regulators remains unknown.—Zhang, Y-M., and C. O. Rock. Transcriptional regulation in bacterial membrane lipid synthesis. J. Lipid Res. 2009. 50: S115–S119.

Supplementary key words bacteria • fatty acid • phospholipid • desaturase • type II fatty acid synthase • membrane homeostasis

Bacterial membranes consist of proteins that are embedded in a lipid matrix that closely approximates a phospholipid bilayer. Bacterial survival depends on membrane lipid homeostasis and on an ability to adjust lipid composition to acclimate the bacterial cell to optimize growth in diverse environments (1). The most energetically expensive membrane lipid components to produce are the fatty acids. These phospholipid acyl chains also determine the viscosity of the membrane, which in turn influences many crucial membrane-associated functions, such as the passive permeability of hydrophobic molecules, active solute transport and protein–protein interactions. Thus, bacteria have evolved sophisticated mechanisms to finely control the expression of the genes responsible for the formation of fatty acids and to modify existing fatty acyl chains to adjust the biophysical properties of the fatty acids to maintain a stable membrane. There is not a single system or paradigm that is used by all bacteria to regulate this important aspect of intermediary metabolism. Rather, different groups of bacteria employ different types of transcription factors that control different subsets of genes to affect regulation of gene expression. This review covers the principal transcriptional mechanisms used by bacteria to adjust the rate of fatty acid synthesis and to modify existing fatty acid chains to adapt to changing environmental conditions.

GRAM-NEGATIVE BACTERIA

Coordination of fatty acid synthesis and degradation by FadR in Escherichia coli

The fatty acid degradative and biosynthetic pathways are coordinated in E. coli to take advantage of the changing availability of fatty acids in their environment through a bifunctional transcription factor, FadR (2). FadR belongs to the GntR family of transcription factors that function as dimers consisting of a C-terminal ligand binding domain and an N-terminal DNA binding helix-turn-helix domain (3). The cellular process by which this occurs has been best characterized in E. coli. Early genetic data had demonstrated that the fatty acid degradative enzymes encoded by the fad genes are inducible, and it was subsequently shown that FadR controls the transcription of the entire fad regulon that consists of all enzymes required to completely degrade fatty acids to acetyl-CoA plus the enzymes of the glyoxylate bypass. FadR operates as a classical bacterial repressor in controlling transcription of the β-oxidation genes (Table 1). In the absence of fatty acids, it binds at a site downstream of the fad gene promoters and represses transcription by interfering with the progress of RNA polymerase. When fatty acids are available, they are converted...
TABLE 1. Transcriptional factors for bacterial fatty acid metabolism

<table>
<thead>
<tr>
<th>Transcription Factor</th>
<th>Organism</th>
<th>Activation</th>
<th>Repression</th>
<th>Regulator</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FadR</td>
<td>Escherichia, Salmonella, Vibrio, Shigella, Haemophilus, Klebsiella, Yersinia</td>
<td>fabA, fabB, iclR</td>
<td>fadR, fadD, fadBA, fadE, fadF, fadFF (ycfYX)</td>
<td>Acyl-CoA</td>
<td>(4, 7, 10)</td>
</tr>
<tr>
<td>FabR</td>
<td>Escherichia, Salmonella, Vibrio, Shigella, Haemophilus</td>
<td>None</td>
<td>fabA, fabB</td>
<td>Unknown</td>
<td>(17)</td>
</tr>
<tr>
<td>FapR</td>
<td>Bacillus, Staphylococcus, Clostridium, Desulfovibrio, Carboxidothermus</td>
<td>None</td>
<td>fabHHE, fabH2, yhdO, fapR, fabA, fabB, fabG, plcX</td>
<td>Malonyl-CoA</td>
<td>(20, 21)</td>
</tr>
<tr>
<td>FabT</td>
<td>Streptococcus, Enterococcus, Lactococcus</td>
<td>None</td>
<td>fabT, fabH, fabK, fabD, fabZ, fabG, fabE, fabZ, AccABCD</td>
<td>Unknown</td>
<td>(33, 37)</td>
</tr>
<tr>
<td>DesT</td>
<td>Pseudomonas</td>
<td>None</td>
<td>desCB</td>
<td>Acyl-CoA</td>
<td>(18, 39)</td>
</tr>
<tr>
<td>DesR</td>
<td>Bacillus</td>
<td>None</td>
<td>desA</td>
<td>Unknown</td>
<td>(29, 32, 40)</td>
</tr>
</tbody>
</table>

classic winged-helix motif, and the C-terminal domain is a
bundle of α-helices. The structure of the FadR dimer is vir-
tually unchanged when bound to cognate DNA, illustrating
that the high affinity of the protein for DNA in the absence
of ligand. There is no interaction between the C-terminal
domain ligand binding domain and the DNA. The CoA
thioester binds exclusively within the C-terminal domain.
Compared with the structure of the ligand-free protein,
the binding of the CoA thioester requires that several amino
acids in the binding pocket change their conformation to
accommodate the ligand. This generates a conformational
“switch” that is transmitted to the N-terminal DNA binding
domain. The end result is that the sequence-specific recog-
nition helices in the N-terminal domain move apart, drasti-
cally lowering the affinity of FadR for DNA.

Control of unsaturated fatty acid synthesis by FabR in
E. coli

FabR is a second transcriptional regulator of type II FAS
in E. coli (17). This factor is a transcriptional repressor of
fabA and fabB, the two essential genes for unsaturated fatty
acid formation (Table 1). FabR belongs to the TetR family
of transcription regulators, and homologs of FabR are
found exclusively in γ-proteobacteria. Deletion of fabR in
E. coli leads to significantly elevated levels of unsaturated
fatty acids, specifically cis-vaccinol due to substantial in-
creases in fabB transcription coupled with somewhat lower
levels of fabA induction (17). FabR repressor binding site
is downstream of the FadR activator binding site on both the
fabA and fabB promoters. The binding sites are adjacent to
each other, and it is likely that the two factors cannot bind
to the promoter simultaneously. Thus, these two key unsat-
urated fatty acid biosynthetic genes are controlled by a
combinatorial lock that involves the interplay between a
transcriptional activator and a repressor. Unfortunately,
the ligand that controls the binding of FabR to DNA is un-
known, and the structure of this protein or its complexes
with DNA have not been determined.

Regulation of a Δ9 acyl desaturase in
Pseudomonas aeruginosa

P. aeruginosa has two mechanisms for the oxidative
desaturation of existing fatty acid chains. The most well-
characterized system, DesBC, is responsible for introducing
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system is inducible and is regulated by DesT, a transcriptional regulator that has the unusual property of being able to sense the fatty acid composition of the acyl-CoA pool to adjust the expression of the desaturase (Table 1). DesT binds tightly to the desCB promoter to repress transcription in the presence of unsaturated acyl-CoA but is released from the promoter to induce synthesis of DesB when bound to a saturated acyl-CoA (19). The ability of DesT to respond to differences in fatty acid structure provides a mechanism that allows cells to substitute environmental fatty acids for energy-intensive de novo synthesized fatty acids and at the same time maintain membrane homeostasis by controlling the degree of unsaturation in the membrane phospholipid fatty acids. One aspect of this regulatory system that needs to be defined is how the de novo fatty acid biosynthetic pathway, particularly the fabAB operon, is controlled by exogenous fatty acids.

The second desaturase in P. aeruginosa is a Δ9 phospholipid acyl desaturase similar to the B. subtilis DesR/DesA system described below (18). Pseudomonas DesA introduces the double bond at the Δ9-position into saturated fatty acids attached to membrane phospholipids, as opposed to the Δ5-position by Bacillus DesA. The existence of DesA permits the growth of P. aeruginosa in the absence of cis-unsaturated fatty acid production by de novo biosynthesis in fabA mutants (18). The system also allows for the transformation of the existing membrane biophysical properties following abrupt changes in the environment (i.e., temperature). However, in the case of P. aeruginosa, it is not known how the expression or activity of the membrane-associated phospholipid DesA desaturase is regulated.

**GRAM-POSITIVE BACTERIA**

**Global regulation of fatty acid synthesis by FapR in B. subtilis**

In contrast to their Gram-negative cousins, many Gram-positive bacteria coordinately control the expression of the genes in fatty acid biosynthesis through the action of a single transcription factor (Table 1). This system is most carefully studied in the model organism B. subtilis by the de Mendoza laboratory (20, 21). FapR is conserved in many Gram-positive bacteria along with its consensus DNA binding site and is clearly predicted to be relevant to the metabolism of important pathogens, such as Staphylococcus aureus. These investigators discovered a protein named FapR that acted as a genetic repressor of the genes of fatty acid biosynthesis and some of the first steps in phospholipid synthesis, such as pkbX. Deletion of FapR leads to the overexpression of the genes in the fatty acid biosynthesis system that are scattered throughout the chromosome (Table 1). The notable exceptions to this general rule are the four genes encoding the subunits of acetyl-CoA carboxylase. Malonyl-CoA was first proposed to be the regulatory of FapR–DNA interactions based on the activation of fab regulon transcription when fatty acid synthesis was inhibited with antibiotics and the repression of the regulon when the expression of acetyl-CoA carboxylase was blocked. The crystal structure of the FapR effector binding domain was solved in complex with malonyl-CoA, supporting a role for this ligand in regulating FapR function (21). The FapR effector binding domain has a “hot dog” fold characteristic of thioesterases and the β-hydroxylacyl-ACP dehydrases of fatty acid synthesis. The binding of malonyl-CoA to this domain is postulated to trigger a conformational change that is predicted to alter the DNA binding properties of the FapR protein. Mutants designed to block malonyl-CoA binding based on the structural data inhibited bacterial growth when expressed in cells, suggesting that they are superrepressors that prevent the expression of the essential enzymes of fatty acid biosynthesis. Taken together, these data place FapR as a master repressor of the genes of fatty acid biosynthesis that is regulated by the intracellular concentration of malonyl-CoA. Presumably, the expression of the acetyl-CoA carboxylase genes that in turn controls the malonyl-CoA production is under control of other factors that tie fatty acid formation to macromolecular synthesis.

Global regulation of fatty acid synthesis by FapR in B. subtilis

The DesR regulation of a Δ5 phospholipid desaturase in B. subtilis is a unique example of membrane biophysical properties being controlled by a membrane-associated sensor (27–29). B. subtilis does not produce unsaturated fatty acids via the de novo biosynthetic pathway, but rather forms iso- and anteiso-branched chain fatty acids. However, when the bacteria are subject to an abrupt shift to a lower growth temperature, existing phospholipids are desaturated to increase the fluidity of the membrane bilayer. This adaptive reaction is carried out by DesA, a membrane-bound acyl desaturase that introduces a double bond at the 5-position of the fatty acid chain on intact phospholipids (30). This gene was first identified in a genomic B. subtilis clone that was able to complement the E. coli fabH (Ts) growth phenotype (31). The desA gene is tightly regulated by a two-component regulatory system encoded by the desK desR genes located in an operon adjacent to the
unsaturated fatty acids coupled with a corresponding regulon. Thus, the upregulation of the genes of fatty acid ratio of unsaturated to saturated fatty acids is controlled in the formation of unsaturated fatty acids (37), and the fabM dimerization of all the genes in fatty acid biosynthesis except for some compounds is known (36). FabT controls the expression of the acetyl-CoA carboxylase genes. FabT is distinctly different from the other transcription factors in this review in that it belongs to the MarR superfamily of regulators. The MarR-type of regulator has a DNA binding winged helix-turn-helix domain of ~135 amino acids and exist as dimers (34, 35). Most family members regulate genes involved in resistance to multiple antibiotics, household disinfectants, organic solvents, oxidative stress agents, and virulence factors in pathogens. In most cases, the specific ligands that control their DNA binding properties are not known, although regulation of their binding activity by aromatic compounds is known (36). FabT controls the expression of all the genes in fatty acid biosynthesis except for fabM. In *S. pneumoniae*, FabM catalyzes the key branch point in the formation of unsaturated fatty acids (37), and the ratio of unsaturated to saturated fatty acids is controlled by competition for substrates between FabM and FabK, an enoyl-ACP reductase (38) that belongs to the FabT regulon. Thus, the upregulation of the genes of fatty acid biosynthesis in FabT knockout strains leads to a decrease in unsaturated fatty acids coupled with a corresponding increase in saturated fatty acids and overall carbon number. This imbalance in membrane lipid homeostasis leads to increased sensitivity to low pH.

The unsolved question with regard to FabT-like transcription factors is the identity of the ligand that regulates its DNA binding activity. Malonyl-CoA immediately comes to mind based on the FapR paradigm, but the upregulation of acetyl-CoA carboxylase by high levels of its product, malonyl-CoA, seems an unlikely regulatory scenario. Other potential ligands that are being explored are long-chain acyl-ACP, fatty acylphosphate, lysophosphatic acid, and global regulators of bacterial metabolism, such as cAMP, ppGpp, etc. Identifying the ligand will be a key piece of information required to understand the functioning of the FabT regulatory circuit in this important group of pathogens.

**PERSPECTIVES**

Membrane lipid homeostasis is of critical importance to bacterial physiology. The large investment in energy for fatty acid biosynthesis means that bacteria have evolved multiple mechanisms to control pathway activity and precisely match fatty acid production to growth rate. Although some of the major players in these regulatory networks have been identified, we are just beginning to understand how their DNA binding activity is controlled. FapR regulation by malonyl-CoA suggests that the other transcription factors may be regulated by this or other intermediates in the membrane lipid biosynthetic pathway, such as acyl-ACP, acyl-PO₄, etc. However, the diversity of transcriptional regulator structures and the groups of genes they control (Table 1) suggests the existence of multiple control mechanisms that are adapted for the niche in which the particular bacterium lives. The understanding of these multiple mechanisms is clearly relevant to the development of new therapeutics because the inactivation of these regulatory networks leads to a loss of virulence. Defining how the transmembrane domain of DesK detects the biophysical properties of the phospholipid bilayer and transmits this signal to the kinase domain is both a fascinating and difficult problem. Ligand-induced conformational changes are well known in protein structure, but how meaningful conformational changes are induced by small changes in the lipid environment are not obvious, and a final definition of the regulatory cycle of DesK awaits the development of more robust structural biology tools to examine integral membrane proteins. Finally, more research is required to determine if the ligands are uniform pathway intermediates or whether some of the factors respond to global intracellular regulators, such as cAMP, ppGpp, etc.

**REFERENCES**


