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.**

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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 acclimatize 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

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TABLE 1. Transcriptional factors for bacterial fatty acid metabolism

Transcription Factor	Organism	Activation	Repression	Regulator	Reference
FadR	*Escherichia, Salmonella, Vibrio, Shigella, Haemophilus, Klebsiella, Yersinia*	*fabA, fabB, iclR*	*fadI, fadD, fadBA, fadE, fadF, fadIJ(yfcYX)*	Acyl-CoA	(4, 7, 10)
FabR	*Escherichia, Salmonella, Vibrio, Shigella, Haemophilus*	None	*fabA, fabB*	Unknown	(17)
FapR	*Bacillus, Staphylococcus, Clostridium, Desulfitobacterium, Carboxidothermus*	None	*fabH1F, fabH2, yhdO, fapR, fabI, fabD, fabG, plsX*	Malonyl-CoA	(20, 21)
FabT	*Streptococcus, Enterococcus, Lactococcus*	None	*fabT, fabH, fabK, fabD, fabZ, fabG, fabF, fabZ, AccABCD*	Unknown	(33, 37)
DesT	*Pseudomonas*	None	*desCB*	Acyl-CoA	(18, 39)
DesR	*Bacillus*	None	*desA*	Unknown	(29, 32, 40)

to CoA thioesters, which then bind to FadR and induce a conformational change that releases FadR from its DNA binding sites and derepresses *fad* gene expression (4–6). When the FadR DNA binding sequence was characterized, the surprising discovery was made that there is a FadR binding site at the −40 region of the *fabA* fatty acid biosynthetic gene promoter (7, 8). The −40 region is typically associated with transcriptional activators, and this finding suggested that DNA-bound FadR functions not only as a repressor of β-oxidation regulon, but also is an activator of the *fabA* gene of fatty acid biosynthesis (Table 1). The *fabA* gene encodes the β-hydroxydecanoyl-acyl carrier protein (ACP) dehydratase that is an essential step in the formation of unsaturated fatty acids. FadR was confirmed as an activator of *fabA* transcription and was one of the first examples of a transcriptional regulator playing a dual role as both a repressor and an activator. The dependence of *fabA* on FadR explained an earlier observation that *fadR* deletion mutants contained higher levels of saturated fatty acids and were more sensitive to growth inhibition by a FabA inhibitor (9). More recently, FadR was demonstrated to also regulate the *fabB* gene expression (10), a second protein that is essential for unsaturated fatty acid synthesis in *E. coli*. Not all FadR proteins have the same mode of regulation and spectrum of target genes (11). Although the N-terminal DNA binding domain is highly conserved, the C-terminal ligand binding domain is not and reflects a broad range of sensitivity of the FadRs from various bacterial species to the structure of the acyl-CoA regulator (11). In some bacteria, such as *Bacillus subtilis*, it is clear that the regulator of the fatty acid β-oxidation regulon does not coregulate fatty acid biosynthetic genes. In fact, a comparative genomics approach (12) suggests that the dual regulation of β-oxidation and biosynthetic genes is not common in bacteria. Although these predictive data are a good guide, they have not been validated by direct experiment in most examples. Recent work shows that FadR is essential for *Vibrio vulnificus* to produce an infection in mice, suggesting that the regulatory circuits controlled by FadR are related to virulence (13).

Structural studies from two laboratories have fully characterized how *E. coli* FadR operates at the molecular level, and crystal structures are available of the three relevant states of the transcription factor: FadR (14), the FadR•DNA complex (15), and the FadR•acyl-CoA complex (16). FadR has the typical two-domain structure of a bacterial repressor, and it functions as a dimer. The N-terminal domain has a classic winged-helix motif, and the C-terminal domain is a bundle of α-helices. The structure of the FadR dimer is virtually 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 recognition helices in the N-terminal domain move apart, drastically 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*-vaccinate due to substantial increases 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 unsaturated 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 unknown, 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 double bonds at the 9-position into acyl-CoAs that are derived from exogenous fatty acids (18). This desaturase

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 *plsX*. 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 regulator 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. In *E. coli*, expression of the four acetyl-CoA carboxylase genes is under both growth rate (22) and autoregulatory control (23). Although direct experiments are not available in the *Bacillus* system, acetyl-CoA carboxylase also may be subject to these forms of regulation.

One seemingly counterintuitive aspect of FapR regulation is its control by the first metabolite in the pathway rather than the end product(s), such as acyl-ACP. A biochemical regulatory loop involving long-chain acyl-ACP inhibition of acetyl-CoA carboxylase may explain how transcriptional regulation is connected to the supply of acyl-ACP for membrane formation. In *E. coli*, inhibition at the first step in phospholipid synthesis leads to the accumulation of long-chain acyl-ACP (24), which in turn acts as a feedback inhibitor of the initiation steps, FabH (25) and acetyl-CoA carboxylase (26). Thus, the accumulation of pathway end-products may lead to a reduction in malonyl-CoA via the inhibition of acetyl-CoA carboxylase that would increase the FapR occupancy on the *fab* promoters and downregulate the expression of the pathway enzymes.

A membrane thermosensor controls a Δ5 desaturase 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* *fabA*(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

desA gene. The DesK protein has four transmembrane segments and an intracellular domain that specifies a histidine kinase and has the histidine that is the site for autophosphorylation (32). Autophosphorylated DesK is a phosphodonor to DesR, a transcriptional regulator that accepts the phosphate on a conserved aspartate residue. The phosphorylated form of DesR binds to a DNA sequence upstream of *desA* and activates the transcription of the Δ5-desaturase (Table 1).

Although the DesKR system has many features in common with the large family of bacterial two-component regulators, there are distinct differences. In the typical two-component system, sensor kinase activity is controlled by the binding of a ligand to the transmembrane domain to activate histidine autophosporylation. In DesK, the transmembrane domain of the kinase is thought to directly sense the fluidity of the membrane bilayer, which in turn regulates its kinase activity and *desA* expression. In this model, DesA desaturation increases the fluidity of the membrane leading to reduced DesK kinase activity and reduced *desA* expression. The major challenge in this area is to determine how the transmembrane sensor domain detects changes in phospholipid biophysical properties and transmits this signal to the kinase domain.

Global regulation of fatty acid synthesis by FabT in *Streptococcus pneumoniae*

Transcriptional regulation of the genes in type II fatty acid synthesis in *S. pneumonia*, and other related pathogens such as *Enterococcus* and *Clostridium*, is carried out by a protein called FabT (33). The genes for fatty acid biosynthesis in *S. pneumonia* are arranged in a row in the genome, and there appears to be only two binding sites for FabT. One site controls the expression of FabH and FabT itself. The second binding site is located in front of the *fabK* gene and controls the transcription of all the other genes in the biosynthetic pathway organized into one giant regulon (Table 1). Unlike *B. subtilis* FapR regulon, 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. pnemoniae*, 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-PO_4, 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.

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