Long-Chain Acyl-CoA–Dependent Regulation of Gene Expression in Bacteria, Yeast and Mammals

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ABSTRACT Fatty acyl-CoA thioesters are essential intermediates in lipid metabolism. For many years there have been numerous conflicting reports concerning the possibility that these compounds also serve regulatory functions. In this review, we examine the evidence that long-chain acyl-CoA is a regulatory signal that modulates gene expression. In the bacteria *Escherichia coli*, long-chain fatty acyl-CoA bind directly to the transcription factor FadR. Acyl-CoA binding renders the protein incapable of binding DNA, thus preventing transcription activation and repression of many genes and operons. In the yeast *Saccharomyces cerevisiae*, genes encoding peroxisomal proteins are activated in response to exogenously supplied fatty acids. In contrast, growth of yeast cells in media containing exogenous fatty acids results in repression of a number of genes, including that encoding the Δ9-fatty acid desaturase (OLE1). Both repression and activation are dependent upon the function of either of the acyl-CoA synthetases Faa1p or Faa4p. In mammals, purified hepatocyte nuclear transcription factor 4α (HNF-4α) like *E. coli* FadR, binds long-chain acyl-CoA directly. Coexpression of HNF-4α and acyl-CoA synthetase increases the activation of transcription of a fatty acid–responsive promoter, whereas coexpression with thioesterase decreases the fatty acid–mediated response. Conflicting data exist in support of the notion that fatty acyl-CoA are natural ligands for peroxisomal proliferator-activated receptor α (PPARα). J. Nutr. 130: 305S–309S, 2000.

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membrane protein, FadL, and a cytoplasmic fatty acyl-CoA synthetase, FadD. The concerted activities of each of these proteins result in the production of fatty acyl-CoA, rendering this process unidirectional. The transport and activation of fatty acids are coupled directly to transcriptional regulation of a large number of structural genes involved in fatty acid metabolism through the transcription factor FadR (Fig. 2).

Wild-type E. coli show a distinctive pattern of growth when fatty acids are provided as the sole carbon and energy source (DiRusso et al. 1999). Only fatty acids with acyl chain length >14 C (i.e., long chain) normally support growth. Mutations in the regulatory gene fadR allow for growth on both medium- (C:8–C:12) as well as long chain (≥14 C) fatty acids. This is due to the fact that only long-chain compounds inhibit FadR and prevent repression of genes encoding proteins required for growth on fatty acids. The genes regulated negatively by FadR include the following: the membrane-bound fatty acid transport protein (fadL) (DiRusso et al. 1993, Nunn et al., 1986); the activating enzyme (fadD) (Black et al. 1992); the enzymes of the β-oxidation cycle (fadBA, fadE, fadF, fadG, fadH) (DiRusso et al. 1992); and a protein of unknown function that is highly expressed during stress response, uspA (DiRusso and Nyström 1998, Farewell et al. 1996). FadR also activates the expression of at least three genes, i.e., fabA and fabB, required for unsaturated fatty acid biosynthesis (DiRusso et al. 1993), and iclR, a repressor of the aceBAK operon (Gui et al. 1996).

There are at least four distinct functions associated with the FadR protein as follows: 1) DNA binding; 2) transcriptional repression; 3) transcriptional activation involving direct FadR-RNA polymerase interactions; and 4) long-chain acyl-CoA binding. FadR-long-chain acyl-CoA binding results in a conformational change that inhibits or prevents FadR-DNA binding (DiRusso et al. 1992 and 1998).

The DNA binding of FadR to regions within the promoters of responsive genes and operons is inhibited by long-chain acyl-coenzyme A thioesters but not medium-chain acyl-CoA (C:8 or C:10), free fatty acids or coenzyme A (DiRusso et al. 1999, Raman and DiRusso 1995). The concentrations of long-chain acyl-CoA required to inhibit DNA binding of the purified protein are in the nanomolar range, whereas fatty acids and detergents require micromolar to millimolar amounts (DiRusso et al. 1998). Thus, the FadR-ligand binding domain distinguishes both the CoA moiety and the acyl chain length of the ligand. To localize and characterize amino acids in FadR required for binding of long-chain acyl-CoA, nonin...
ducible mutations in the FadR gene were selected after chemical mutagenesis of plasmid DNA (Raman and DiRusso 1995). These fadR alleles, called superrepressors, encode proteins that are able to bind to DNA to repress transcription of the fadB gene or activate fabA, but are not inactivated by long-chain acyl-CoA. As a result, cells carrying these fadR alleles are unable to grow on fatty acids of any chain length. One super-repressor FadR6XHis was overexpressed, purified and characterized in vitro. DNA binding of FadR6XHis was unaffected, whereas acyl-CoA binding was reduced 10-fold. Alanine substitution of amino acid residues adjacent to S219 identified Y179, Y193, G216, E218, W223 and K228 as also required for maximal derepression of fadB by long-chain fatty acids (Raman and DiRusso 1995). These preliminary studies led to the prediction that the acyl-CoA binding region was localized in a carboxyl-terminal domain of the protein. This conclusion was supported further by the phenotypic analyses of protein fusions between the DNA binding domain of LexA (amino acids 1–87) and amino acids 102–239 of FadR (Raman et al. 1997). The resulting protein fusion retained the DNA binding specificity of LexA and was inducible by long-chain fatty acids demonstrating the ligand binding function contributed by FadR.

The acyl-CoA binding domain of FadR was further localized by affinity labeling of the full length protein and an amino terminal deletion derivative, FadRΔ167, with a palmitoyl-CoA analog, 9-p-azidophenoxyl-9-3Hjnonanoic acid-CoA ([3H]APNA-CoA) (DiRusso et al. 1998). After labeling, the full length FadR and the deletion derivative were each digested with trypsin and tryptic peptides separated by HPLC. One labeled peptide common to both the full-length protein and the deletion derivative was identified. The amino terminal sequence of the labeled peptide was SLALGFYHK, which corresponds to amino acids 187–195 in FadR (DiRusso et al. 1998).

Isothermal titration calorimetry was used to estimate affinity of the wild-type full-length FadR, a HIS-tagged derivative of FadR (FadR6XHis) and FadRΔ167 for acyl-CoA (DiRusso et al. 1998). The binding was characterized by a large negative ΔH° (−16 to −20 kcal/mol). The binding specificity, as expected, was for compounds >12 C in length, and no binding was detected for the medium-chain ligand C8-CoA. Full-length wild-type FadR and FadR6XHis bind oleoyl-CoA and myristoyl-CoA with similar affinities (Kd = 45 and 63 nmol/L and 68 and 59 nmol/L, respectively). The Kd for palmitoyl-CoA binding was higher (about fivefold) despite the fact that palmitoyl-CoA is 50-fold more efficient in inhibiting FadR binding to DNA than myristoyl-CoA (DiRusso et al. 1992). These apparently conflicting data indicate that the interaction of acyl-CoA with FadR is complex; although the shorter-chain compounds C12 and C14 bind with high affinity, they are not expected to result in a change in protein conformation required to either prevent DNA binding or release the protein from the DNA.

**Alteration of gene expression in Saccharomyces cerevisiae by exogenous fatty acids**

Yeast are a valuable model system with which to study fatty acid transport, activation and gene regulation because they can grow on long-chain fatty acids as a sole carbon and energy source. Yeast also require exogenous unsaturated fatty acids in the natural environment when growing anaerobically because the O2-dependent fatty acid desaturase (Ole1p) is inactive (Walenga and Lands 1975). The sole site of fatty acid degradation for energy production is the peroxisome. Biosynthesis and proliferation of this organelle occurs when yeast are grown on fatty acids as a carbon and energy source (Elgersma and Tabak 1996). In stationary phase, yeast accumulate fatty acids and store them as triacylglycerides in a lipid body. Thus, unlike bacteria, yeast modulate not only their metabolism but also organelle structure and function in response to fatty acids.

In yeast, imported long-chain fatty acids are converted to CoA thioesters by the fatty acyl CoA synthetases Faa1p and Faa4p (Johnson et al. 1994). Recent evidence indicates that, similar to E. coli, FadD, either Faa1p or Faa4p, is required for import of fatty acids (Black and DiRusso, unpublished data). Thus transport is coupled to activation. The fatty acyl-CoA may be incorporated into phospholipids or triglycerides, used as a substrate in protein acylation or can be used as a carbon and energy source.

Regulation of transcription by fatty acids in yeast has been the subject of intense research in recent years. Growth on long-chain fatty acids causes induction of the genes encoding structural proteins and enzymes of peroxisomes (Elgersma and Tabak 1996, Igual et al. 1992, Kos et al. 1995). Two fatty acid–responsive transcription factors are essential for peroxisome biogenesis, Oaf1p/Oaf2p and Oaf2p/Pip2p (Karpichev and Small 1998, Rottensteiner et al. 1996). Oaf1p and Oaf2p form a heterodimer, which interacts specifically with promoter DNA containing an oleate response element (ORE), which is CGGNNNTNA(N6$_{15}$)CGG (Luo et al. 1996, Rottensteiner et al. 1997). Karpichev and Small (1998) recently conducted a database search for yeast genes and identified 40 that contained a putative ORE. Northern hybridization analysis confirmed that 22 are induced by oleate and regulated by either Oaf1p or Oaf2p or a heterodimer of Oaf1p and Oaf2p. Most, but not all of the genes encode peroxisomal proteins. OAF2 transcription is itself increased when cells are grown in oleate, and the increase in expression is dependent upon Oaf1p (Rottenstein et al. 1997). The expression of one gene encoding a protein of undefined function, YOR002c, is dependent upon Oaf1p and Oaf2p whether oleate is or is not provided in the growth media, thus demonstrating the complexity of Oaf1p/Oaf2p–dependent gene regulation. The gene encoding Δ9-fatty acyl-CoA desaturase in yeast, OLE1, is repressed by monounsaturated and polyunsaturated fatty acids (PUFA) in any Oaf1p/Oaf2p–independent fashion (Choi et al. 1996, McDonough et al. 1992). At this time, the transcription factor(s) mediating repression have not been identified, although two groups have reported the isolation of mutations that eliminate repression (Fujimori et al. 1997, McHale et al. 1996). Characterization of the products of these mutant alleles should help to define their function in fatty acid–mediated repression of OLE1.

Fatty acid–dependent gene regulation (both activation and repression) in yeast requires the activity of fatty acyl-CoA synthetases Faa1p or Faa4p (McDonough et al. 1992). At this time, it is not known whether the reduction of transcriptional control is due to an inability to form acyl-CoA, the natural ligand, or to a defect in fatty acid import.

**Evidence that long-chain acyl-CoA effect changes in gene expression in mammals**

It has been recognized for many years that fatty acids have a significant effect on RNA abundance of genes encoding proteins involved in fatty acid metabolism in mammals. In general, fatty acids suppress fatty acid synthetic enzymes, including acetyl-CoA carboxylase, fatty acid synthase, ATP-citrate lyase and apolipoprotein (apo) A-I expression, whereas they increase expression of genes involved in peroxisomal biogenesis, β-oxidation and several fatty acid transport proteins including CPT 1 and FATP (regulatory protein for SMDP1998). These effects can be traced to the activities of the nuclear orphan receptor family members called peroxisomal proliferator-activated receptors (PPAR). The PPAR factors function as heterodimers with reti-
noic acid receptor (RXR) family members. There are at least three isoforms of PPAR (α, γ and δ), which exhibit different patterns of expression and regulation of target genes (see Clarke et al. 1997, Klewier and Willson 1998 and Reginato et al. 1998). PPARα is most highly expressed in liver, intestine, kidney and brown adipose tissue and is highly activated by the synthetic compounds known collectively as peroxisomal proliferators. Treatment of animals with peroxisomal proliferative drugs results in the proliferation of hepatocytes and a 10-fold increase in peroxisomes. Chronic administration of these compounds also results in liver tumors. The natural ligands for the PPAR family have been difficult to discern (Krey et al. 1997, Lin et al. 1999, Wolf 1998). This is due in part to the fact that each responds in varying degrees to a broad range of natural compounds, including long-chain saturated, unsaturated and PUFA. PPARγ and PPARδ are each highly expressed in adipose tissue but in a different developmental sequence. Although PPARγ appears to be adipocyte specific, PPARδ (also known as PPARβ), FAAR and NUC1 is expressed in a number of tissues, particularly neuronal tissue. PPARγ and PPARδ are activated to a limited extent by synthetic peroxisomal proliferators by comparison to PPARα (Reginato et al. 1998, Staels et al. 1998).

Recently, transgenic mice deficient in PPARα have been generated. Surprisingly, the mice were essentially asymptomatic (Gonzalez 1997). They exhibited normal numbers of peroxisomes and levels of systemic lipids and lipoproteins. However, peroxisomes do not proliferate in response to synthetic peroxisomal proliferators nor do animals develop tumors upon chronic administration of those compounds (Aoyama et al. 1998). In contrast, animals deficient in peroxisomal acyl-CoA oxidase, the first enzyme in the peroxisomal β-oxidation pathway, exhibit a mimicking of the effects of chronic administration of synthetic peroxisomal proliferative drugs (Fan et al. 1998). The animals have elevated numbers of peroxisomes and develop steatohepatitis and liver tumors by 15 mo of age. This is suggested to be the result of sustained activation of PPARα that is assumed to be due to the accumulation of a natural ligand (Aoyama et al. 1998). Candidates for the proximal ligand include long-chain acyl-CoA and very long-chain fatty acids (VLCSFA), which accumulate to high levels in transgenic animals. There is no direct evidence to distinguish either the CoA thioester or the free acid form as the natural ligand. However, in other conditions in which VLCFA accumulate, e.g., in AOX mice such as in X-linked adrenoleukodystrophy or in mice lacking VLCFA-CoA synthetase, peroxisomes do not proliferate nor do liver tumors develop.

In eukaryotes, there is only one case providing compelling evidence for regulation of a transcription factor’s activity by long-chain acyl-CoA. Recently, Bar-Tana and co-workers evaluated DNA binding and transcriptional activation of hepatocyte nuclear factor 4α (HNF-4α) by long-chain acyl-CoA (Hertz et al. 1998). HNF-4α is a member of a transcription factor family involved in hepatocyte differentiation and cellular metabolism (Duncan et al. 1998, Fraser et al. 1998). Mutations in HNF-4α cause two forms of diabetes, maturity-onset diabetes of the young (MODY1) and MODY3 (Funuta et al. 1997, Gragnoli et al. 1997, Sladek et al. 1998). Binding of palmitoyl-CoA to purified HNF-4α is saturable with an apparent K_d of 1.2–3.4 μM/l and is specific for the CoA thioester of the long-chain fatty acid (Hertz et al. 1998). The measured affinities are much lower than that estimated for purified E. coli FadR but within the normal physiologic range of liver cytosolic long-chain acyl-CoA. Palmitic acid and free coenzyme A (CoASH) had no effect on binding using purified protein in a direct filter-binding assay. Coexpression of thioesterase in transfected cells inhibited activation of a CAT reporter construct under the control of the apo.

Is the free acid or acyl-CoA the regulatory molecule?

As summarized above, there is clear evidence that exogenous administration of fatty acids to bacteria, yeast or mammals results in alterations in mRNA synthesis such that fatty acid synthesis is reduced, and fatty acid transport and degradation are increased. In most cases, it is also clear that β-oxidation is not required to form the proximal natural ligand because administration of 2-bromo-palmitate and other substituted fatty acids result in a response similar to free fatty acids. These compounds are substrates for acyl-CoA synthetase and may be activated to a CoA thioester like the natural fatty acids. Therefore, as long as pools of both free fatty acid and acyl-CoA are present within a cell, it is not easy to distinguish which class of compounds is the proximal effector. Additionally, it appears that activation in many cell types occurs concomitantly with import; thus, eliminating acyl-CoA dependence may be to control dimerization of the transcription factor, which in turn controls the protein’s DNA binding activity. In the same experiments demonstrating direct binding and regulation of HNF-4α by long-chain acyl-CoA, activity and binding of acyl-CoA to PPARδ was evaluated. In these experiments, PPARδ activity was stimulated by 18:0 and 18:3 acyl-CoA. However, when acyl-CoA synthetase was cotransfected with PPARα, activation was inhibited. These results appear to contradict the suggestion above that increases in intracellular long-chain acyl-CoA stimulate PPARα-dependent gene activity in the acyl-CoA oxidase-deficient mice (Aoyama et al. 1998).

Although PPARα and HNF-4α are the most highly visible candidates for transcription factors regulated directly by fatty acids, a substantial body of evidence has been accumulated that indicates other unidentified factors may be involved in the regulation of some genes. PUFA and peroxisomal proliferators have different and separable effects on genes such as fatty acid synthase (Bing et al. 1997) SCD1 (Miller and Ntambi 1996) and the rat S14 gene (Bing et al., 1997). Clarke et al. (1997) monitored the change in expression of peroxisomal acyl-CoA oxidase mRNA abundance upon administration of PUFA and a peroxisomal proliferative compound to rats. They found that increased peroxisomal acyl-CoA oxidase mRNA abundance upon treatment with a potent PPAR activator, 5,8,11,14-eicosatetraynoic acid; however, PPAR activation did not reduce fatty acid synthase, whereas PUFA was effective. The results from experiments such as these point to the complexity of fatty acid–dependent control of transcription in mammals. Two candidate transcription factors include steroid receptor element binding proteins (Thewke et al. 1998) and thyroid hormone receptor (Thurmond et al. 1998). Additionally, they indicate that other factors and/or mechanisms of regulation have yet to be uncovered.
strains deficient in acyl-CoA binding protein (ACBP1) (Scherling et al. 1996). Similarly, the alterations in response noted in cells that overexpress thioesterase in E. coli (Cronan 1997) and mammalian cells (Hertz et al. 1998) each give valuable, albeit circumstantial evidence for a role for acyl-CoA.

**LITERATURE CITED**

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