Activators, deactivators and deactivated activators

The discovery of transcriptional activators regulated by small molecule ligands that block their binding to DNA fills a blank in the set of real examples of possible types of transcriptional regulation.

Studies of prokaryotic gene control have been a fertile source of surprises. It was recently found, for example, that several replication proteins of bacteriophage T4 double as transcriptional activators whilst bound at a moving replication fork [1]. As another example of a recent surprise, it turns out that activation of the *Escherichia coli malK* gene, which encodes an enzyme of maltose metabolism, by the transcriptional activator MalT requires that several MalT monomers at the promoter interact with MalT monomers bound at upstream sites, with the intervening DNA forming a loop. These interactions result in a repositioning of MalT at the *malK* promoter that potentiates activation [2]. If history is any indication, these and other recent discoveries on the mechanisms of transcriptional control in prokaryotes probably foreshadow the discovery of similar control mechanisms in eukaryotes.

A new addition to the list of prokaryotic control mechanisms, which as yet lacks a eukaryotic counterpart, comes in part from studies of the FadR protein, a regulator of operons encoding products involved in fatty acid metabolism in *E. coli* [3]. FadR activates transcription of *fabA*, which encodes an enzyme involved in the synthesis of unsaturated fatty acids. FadR also turns off transcription of *fadL* and *fadBA*, which encode products involved in the catabolism of fatty acids. FadR binds specifically to sites overlapping the RNA polymerase binding sites at the negatively-controlled promoters of *fadL* and *fadBA*. FadR bound at these sites probably exerts negative control simply by blocking RNA polymerase binding, a common mechanism of negative control employed, for example, by the phage λ *cI* repressor. At the positively-controlled *fabA* gene, FadR binds adjacent to the RNA polymerase binding site, at the − 40 position with respect to the start site of transcription. Many prokaryotic transcriptional activators bind around − 40, such as CAP (catabolite activator protein) at the *gal* operon [4] and λ repressor (in its guise as a transcriptional activator) at P_{RM} [5]. At − 40, the activator is positioned to contact RNA polymerase bound at the promoter and thereby increase the rate of transcription initiation.

In these respects, FadR resembles other DNA-binding proteins that serve as both activators and repressors of transcription, such as λ repressor and CAP. The distinctive feature of gene control by FadR is its response to small molecule regulators. Activation of *fabA* was found to be antagonized by certain metabolites of fatty acids, one the the first examples of a feedback system in which a small molecule regulator acts by blocking the function of a transcriptional activator.

Thus, in the presence of exogenous fatty acids, the cell stops making its own fatty acids, and the imported fatty acids can be degraded for use as a carbon and energy source, or used directly for phospholipid synthesis. This control system may seem odd in light of the fact that *E. coli* requires both saturated and unsaturated fatty acids for membrane synthesis, but the presence of saturated fatty acids deactivates transcription of *fabA*, which encodes an enzyme required for the synthesis of unsaturated fatty acids. Two observations resolve this paradox: unsaturated fatty acids are better deactivators than saturated fatty acids, and the *fabA* gene has a second promoter that provides a basal level of *fabA* product. Thus, even in the presence of high quantities of saturated fatty acids, enough *fabA* product is synthesized to provide adequate amounts of unsaturated fatty acids.

Henry and Cronan [3] present convincing evidence that fatty acids turn off *fabA* and turn on *fadBA* by blocking binding of FadR to its DNA sites. Promoter activity was monitored *in vivo*, by fusing the promoters of *fabA* and *fadBA* to *lacZ* and then measuring the effects of various fatty acids on synthesis of β-galactosidase in cells containing each fusion. Each type of fatty acid displayed a characteristic potency for regulating these promoters. Fatty acids containing carbon atom chains of 14–18 atoms reduced activity of FadR, whereas fatty acids with chains of 10 atoms or less did not. For fatty acids of a given chain length, mono-unsaturated derivatives were more potent regulators than the corresponding saturated fatty acids. Significantly, when the relative effects of the different fatty acids were compared, the percent regulation of *fabA* and *fadBA* (loss of activation or derepression) varied in parallel, consistent with a model in which different fatty acids antagonize FadR binding with different efficiencies.

The effects of fatty acids on FadR binding were revealed directly by binding studies *in vitro*. Henry and Cronan reasoned that the physiological modulators of FadR activity were probably acyl-CoA thioesters, because fatty acids are rapidly converted to their acyl-CoA derivatives following import into *E. coli* [6]. As expected from this view, acyl-CoA derivatives of the fatty acids that had the greatest effects *in vivo* were most potent at blocking DNA binding by FadR *in vitro*, as measured in a standard 'band shift' assay. The unmodified fatty acids had no effect. A potential concern in the binding studies was that the acyl-CoA derivatives, which are known to have detergent properties, might have blocked binding *in vitro* by denaturing the FadR protein. Henry and Cronan demonstrated, however, that the antagonism of FadR binding was readily reversible by addition of acyl-CoA thioesterase, which

converts the inducing acyl-CoA derivative into the non-inducing fatty acid plus free CoA. As it is expected that unfolding of FadR (or any protein) will not be efficiently reversible, this finding indicates that denaturation of FadR was probably not the reason it fails to bind to its sites in the presence of the acyl-CoA derivatives.

These studies on the control of FadR fill a blank in the picture of small molecule effects on repressors and activators. For repressors, examples of small molecules that relieve repression (inducers) have been found, as in the *lac* and *gal* operons, as well as small molecules that promote repression (corepressors), as in the *trp* operon. As pointed out by Henry and Cronan, in the case of activators, only small molecules that promote activation (inducers, confusingly given the same name as small molecules that relieve repression), have been described, as in the cAMP effect on CAP. FadR is the first example of a transcriptional activator that is inactivated by a small molecule ligand (Fig. 1).

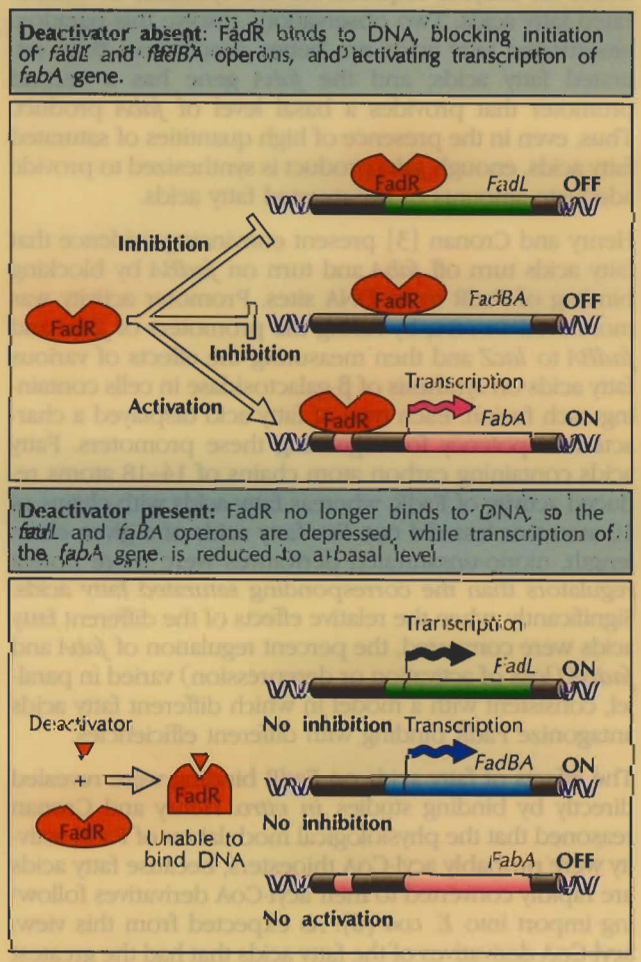

Fig. 1. Transcriptional regulation by FadR.

Actually, it turns out that there is precedence for this type of regulation in *E. coli*, the title of the paper by Henry and Cronan notwithstanding. The leucine-responsive regulatory protein (Lrp) is also an example of a transcriptional activator that is functionally blocked at the level of DNA binding by a small molecule ligand, in this case leucine [7]. Another transcriptional activator, AsnC, is similar in sequence to Lrp, and its function is blocked by the presence of asparagine, though in this case the ligand has not yet been shown to exert its effect at the level of DNA binding [8]. The paper by Henry and Cronan usefully draws attention to the finding that transcriptional activators can be prevented from acting by binding small molecules, but results of studies on Lrp must be credited with making this point also.

These discoveries complete the symmetry between activator and repressor systems, but also raise some novel problems in nomenclature. Lewin [9], considering the possible existence of such systems, proposed that the unliganded protein be called the 'active apoinducer', the ligand the 'corepressor' and the ligand-bound protein the 'inactive apoinducer'. Savageau [10] has also proposed a nomenclature for such systems. Perhaps the simplest is the nomenclature proposed by Henry and Cronan, in which the protein is the 'activator', the usual name, the ligand is the 'deactivator' and the ligand-bound protein is the 'deactivated activator'.

These findings raise a variety of further questions. The sequences of FadR, Lrp and AsnC contain reasonable matches to the 'helix-turn-helix' motif [11], as do the *lac*, *gal* and *trp* repressors, but the ligands of FadR are much larger than the ligands of the other members of this protein family. The ligands of the three repressors, allolactose, galactose (or some related compound) and tryptophan, are 3–5 fold lighter per mole than the acyl-CoA ligands of FadR. The binding data *in vitro*, however, imply that both the fatty acid and CoA parts of the ligands must be recognized by FadR. How are these relatively large molecules specifically recognized, and how are the effects of ligand binding communicated to the DNA-binding unit? Another set of questions center on how FadR, Lrp, and AsnC activate transcription. Do they appose a negatively-charged region of the protein surface to RNA polymerase, as seems to be the case for activation by the phage repressors [12,13]? Mutants in the activation function could be isolated by demanding loss of activation but continued DNA binding. In addition to defining the activation surface, such 'positive control' mutants could be useful in reversion studies to identify the targets of these activators. Furthermore, the fact that these proteins can be deactivated at will may be useful in experiments *in vitro* to define the mechanistic step at which they act. Lastly, one might ask the question that arises from each discovery of a new prokaryotic control mechanism: do these discoveries foreshadow the discovery of similar control mechanisms in larger organisms?

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