My Word

Repressors

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To understand a thing it often helps to first understand its opposite…Anon

Jacques Monod and Francois Jacob, our heroes, described a gene-regulatory world run exclusively by repressors. The activities of repressors, their abilities to bind DNA as we later learned, would be controlled — helped or hindered — by small molecules that bind these proteins. They showed [1] that networks of considerable complexity could be created with such a regulatory system. There was no need for gene 'activators' and, indeed, invoking such regulators (strongly resisted by Monod, it is said) would only confuse a coherent picture. This picture has been turned on its head. Almost all genes — especially in eukaryotes — are controlled by the combined effects of activators and repressors. What happened?

To get from there to here we need invoke two ideas, one implicit in the original picture of Monod and Jacob. It helps to start with bacteria to understand this predominant mode of gene regulation — control by both activators and repressors. First, without making a point of it, Monod and Jacob assumed that bacterial RNA polymerase is constitutively active — it will automatically, as it were, transcribe any gene whose promoter is accessible (not blocked by a repressor). Second, something the French scientists did not realize, promoters vary widely in their affinities for the active polymerase. One of the promoters they studied, from phage lambda, turns out to be exceptional: its affinity for polymerase is so high that control by repressor suffices: the gene is essentially 'off' in the presence of lambda repressor, which binds DNA and excludes polymerase; or it is fully 'on' (transcribed at a high rate) in the absence of repressor.

But it turns out that most promoters are 'weak': in the absence of its repressor, and at the concentration of polymerase found in cells, the corresponding genes are transcribed at only low (basal) levels. Such promoters require the helping effect of an activator for efficient transcription of the gene. As this description implies, all a specific activator must do, when working on this kind of promoter, is to recruit, using a simple binding reaction, the active polymerase to a promoter to elicit a higher degree of transcription [2]. But, as this description also suggests, such promoters also require specific repressors: in the absence of an activator there would otherwise be a basal level of expression (usually only 10–100 fold lower than the fully activated level). The requirement for a repressor along with an activator is a happy coincidence, as it were, because now the gene is subject to two regulatory inputs, one activating, the other repressing.

All this might seem complicated, but actually it is a simple (even crude) system, easily evolvable. Imagine a gene subject to no regulation. Such a gene, our discussion implies, would be expressed at some low, but possibly physiologically important level. Adding an activator — easy to do as I have emphasized previously [2] — would cause higher expression when required. Then adding a repressor would eliminate the basal level expression and, in addition, would allow a new signal to be conveyed to the gene. In both steps a system that works will be made to work better. The *lac Z* gene of *Escherichia coli* is a famous example (Figure 1). In the absence of any regulator the gene is expressed at a low level, thereby allowing the bacterium to grow, albeit slowly, using as its sole carbon source the sugar lactose. Thanks to the Lac repressor the gene is not expressed at all in the absence of lactose. And thanks to an activator the gene is expressed at a high level only if glucose (the preferred carbon source) is absent. The activator is CAP, and it is drawn on the figure in dotted lines because Monod and Jacob did not know it existed.

These matters are brought into focus by considering a form of gene regulation I have thus far ignored. Certain bacterial promoters, which differ in base sequence from the more common variety, bind a form of RNA polymerase that, unlike the form of the enzyme we have been discussing, is not constitutively active (one of the subunits of the enzyme, sigma 70, has been replaced by another, sigma 54). The polymerase–promoter complex thus formed is inert; a fancier kind of activator is required in this case, one that bears an enzymatic function that stimulates transcription, not merely by recruiting the polymerase, but by literally activating the bound polymerase in a reaction that

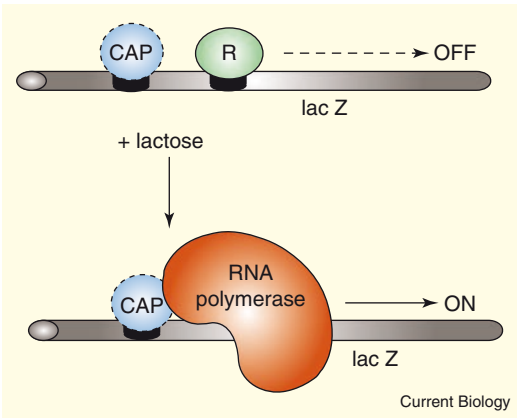

Figure 1. Control of the *LacZ* gene in *E. coli*.

In the absence of lactose the gene is off because the Lac repressor binds and excludes polymerase. A derivative of lactose causes the repressor to change shape and fall off the DNA. Full expression is achieved thanks to the activator known as CAP (catabolite activator protein) which, in the absence of repressor, recruits [2] polymerase. Glucose diminishes this activation in part by decreasing the concentration of a small molecule (cAMP) which helps CAP bind to DNA

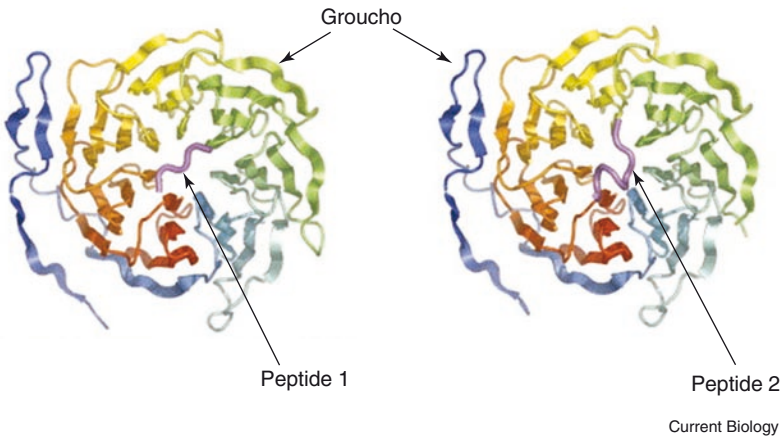

Figure 2. Recruiting the repressor complex Groucho.

The ribbons represent the so-called WD domain of Groucho (the mammalian version of which is known as TLE). The two peptides bind to this region in different conformations and orientations as shown. These peptides are found as parts of two different DNA-binding proteins that recruit Groucho and thereby repress transcription. (Adapted with permission from [6]).

requires utilization of energy in the form of ATP. Because the enzyme–promoter complex is so stable in the absence of the activator, there is essentially no basal level expression and hence no requirement for a repressor. Nor, perhaps, could any ordinary repressor bind tightly enough to exclude formation of this polymerase–enzyme complex. Not surprisingly, therefore, no repressor has been found for such genes. Such a form of regulation, to my knowledge, has not been found in eukaryotes. Rather, all eukaryotic genes are regulated by the opposing effects of activators, that work by recruitment, and by repressors — in a maneuver not found in bacteria so far as we know — that also work by recruitment.

The typical eukaryotic repressor binds specific sites on DNA just as does the typical activator. But whereas the activator recruits the transcribing machinery [2] the repressor recruits one or another 'repressing complex'. These recruiting reactions are, in some cases, well understood. In *Drosophila*, for example, various DNA-binding proteins recruit the repressing machine called Groucho. As with activators interacting with their targets, there seem to be a variety of simple ways to effect this recruitment. Figure 2 shows, for example, two different modes of protein binding to Groucho and thereby recruiting it to DNA. In certain mammalian cases, specific peptides have been described that relieve or prevent repression by interfering with the kind of binding reaction shown in Figure 2 [3].

What is less clear is how 'repressing machines' like Groucho work once recruited to DNA. There is evidence suggesting both histone modifications (which might directly or indirectly interfere with recruitment of the transcribing complex) and direct negative effects on the transcriptional complex play a part. Groucho, recruited to specific sites on DNA, can counter the effects of activators working on near-by genes. The effects do not seem to be all or none: the stronger the activator (the higher its affinity for the transcription complex) the less the degree of repression, and so on. Nor, evidently, is repression 'memorized': wherever tested, repression requires the continual presence of the recruiter [4].

It is not surprising, given the alternative 'activation only' form of gene regulation discussed above, that evolution chose the recruitment method for controlling transcription of eukaryotic genes. The combination of activators and repressors allows for multiple inputs; the positive and negative effects can be implemented separately; and merely positioning binding sites for the recruiters expands or changes patterns of gene regulation. As with so many other regulatory processes — ubiquitylation, RNA splicing, proteolysis, and so on — evolution's strategy seems to have been to evolve an active machinery (which, for example, transcribes genes or opposes that transcription) and then, as it were, implement its specific use by recruitment [5].

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