My Word

Words

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"*Every word is a prejudice*"

Turn on a TV news channel in the US and you are likely to see some well-spoken, attractive personality referring to opponents of the Iraq war as members of the 'hard left'. A large fraction of the American public opposes the war, of course, but we are inured to this prejudicial use of language. At least one network speaks quite purposefully, but one wonders about the others — are the announcers themselves, along with the rest of us, being manipulated by their own words?

Scientists think of themselves as being in control of the words they use rather than the other way around. Nietzsche's comment above might give us pause. I have discussed in a previous communiqué unwitting problems engendered by too-facile use of the scientific term 'epigenetic' [1]. Here my concern is with four rather ordinary words — 'activation', 'cooperativity', 'recruitment' and 'regulation'. We have gotten so used to using these words in one context — thereby engraining our 'prejudices' — that we can be fooled when they are used in another. In the context I will emphasize, these words are interrelated, and understanding one requires understanding all.

Activation

The yeast protein Gal4 is called a 'transcriptional activator'. It binds to specific sites on DNA and causes the adjacent genes to be transcribed, at a high level, into mRNA. We say that Gal4 'activates' transcription of these genes. Some years ago, just when we had figured out how Gal4 works, I showed a *Scientific American* article describing this mechanism to a physicist friend who, after a puzzlement, said that he simply couldn't understand it: there was no 'activation' that he could see. Only later did I realize the problem: the word 'activation' suggests just what does *not* happen when Gal4 (like many other so-called transcriptional activators in bacteria and in higher organisms) works.

The word 'activation' suggests a conversion from an inert to an active state – like what happens when you turn a key and start a Ferrari engine. Some activators — cyclins, for example — cause their targets (certain kinases in this case) to undergo a transition from an inert to an enymatically active state. But Gal4 doesn't literally 'activate' anything, neither the enzyme (RNA polymerase) nor the gene (whatever that might mean). Rather, it simply recruits (a word discussed more fully below) the transcriptional machinery (which includes RNA polymerase) to the gene. This apposition causes the gene to be transcribed at a higher rate than that observed in the absence of the activator. The mechanism is so simple as to be elusive.

This problem with the word activation applies to a wide array of biological control processes because the relevant mechanism in these disparate cases is essentially the same. In all of these cases the enzyme (for example, RNA polymerase) has multiple possible targets (for example, genes), and 'activation' means apposing the enzyme with one or another specific substrate. Just as a transcriptional activator apposes the polymerase with a specific gene, so does, for example, a subunit of an 'E3 ligase' appose a specific protein with the ubiquitylating machinery (that which attaches ubiquitin to specific proteins and so marks them for proteolysis). There are many such subunits (for example, F-box proteins) just as there are many transcriptional activators, and their modes of action are strictly analogous. To say that an E3 ligase (in itself a misleading name) 'activates' ubiquytylation is as misleading as to say that Gal4 'activates' transcription.

If there is a short alternative word to use in place of 'activate' for these various cases I don't know it. 'Transactivate', a term sometimes used, adds nothing but fog.

Cooperativity

Here a problem can arise from exposure to a classical scientific education. A famous example of cooperativity involves the binding of O_2 to haemoglobin. As we learned once, haemoglobin binds four O_2 molecules, and binding of each O_2 increases the affinity of the haemoglobin for the next O_2. Binding of O_2 changes the shape of the protein (or if one prefers, traps the haemoglobin in a conformation different from the O_2-free form) and this alternative form of haemoglobin binds subsequent O_2 molecules with increased affinity. We say that the binding of one O_2 'helps' another to bind to haemoglobin. We are so used to this description that sometimes the word 'cooperativity' is automatically associated with a required conformational change.

But there is another example of cooperativity, widely used in Nature, that requires no changes in the shapes of the components. The formal description of the reaction is the same as that of the haemoglobin case: one ligand 'helps' another bind to a common target. But here we are dealing with macromolecules, for example, two proteins (the ligands) binding to their affined sites on DNA. In this case, the helping effect requires simply that the two proteins touch one another when bound to DNA. More than once I have failed to explain this idea, only later to realize that the listener associated 'cooperativity' with 'conformational changes' . The idea here sometimes seems too simple to grab the serious person's attention.

The source of the cooperativity is easier to see in the case involving three macromolecules than in the case of haemoglobin and O_2. For example, for the case of two proteins binding cooperatively to DNA, to a first approximation one simply adds together three binding energies: two protein-DNA and

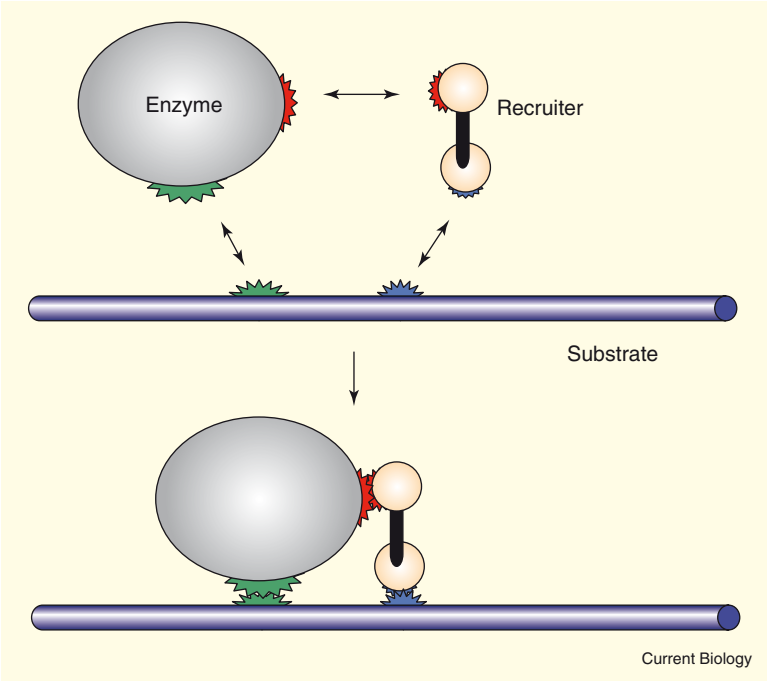

Figure 1. Activation. A recruiter and an enzyme are shown binding cooperatively to a common target, with three crucial pairs of interacting sites highlighted.

As discussed in the text, the recruiter might be an E3 ligase, a transcriptional activator, a splicing regulator, and so on. The recruiter might, as explained, be pre-bound, but the recruiting reaction remains essentially the same. Absent a recruiter, unless prevented from doing so, the enzyme will naturally work on its substrates at a lower level as dictated by its unaided affinity for its target..

one protein-protein interaction. Even a weak interaction between the proteins — say a kcal or two — can increase binding by a factor of 10–100, a large effect in physiological terms.

Recruitment

Alex Gann and I used 'recruitment' to describe how certain transcriptional activators, such as Gal4, work [1]. As I have mentioned, and will explore further in the next section, the word (and the mechanism) applies to an array of important regulatory processes [2]. 'Recruitment' has been criticized and sometimes misconstrued: it has a 'militaristic' aspect, we have heard; or it implies some 'molecular beacon' effect; or it specifies some particular interaction (for example recruiter–polymerase) that might not actually obtain for certain cases. We think the word apt, however, and we see no obvious alternative.

Figure 1 shows a protein recruiting an enzyme to a specific substrate. Note the three pairs of interacting surfaces: substrate–recruiter; recruiter–enzyme; and enzyme–substrate. Were the enzyme *Eschericia coli* RNA polymerase, the recruiter could be the bacterial protein lambda repressor (working as an activator); and the substrate would be DNA bearing two sites: an operator for binding repressor, and a promoter for the polymerase. The figure, with different names inserted, equally well describes recruitment of a specific protein to the ubiquitylating machinery; recruitment of the dosage compensation complex to X chromosomes in one sex of *Caenorhabditis elegans*; recruitment of the RNA splicing machinery to specific RNA sites, and so on.

All recruiters of a given class (transcriptional activators, for example) use one surface to bind to the enzymatic machinery and another to bind to a specific substrate. The kinds of surfaces required for recruitment are just like those required for cooperative binding –simple binding surfaces. Recruitment typically differs from cooperative binding only trivially: in the case of transcription, for example, the activator might be constitutively bound to DNA (as is the case for Gal4) and, only upon the appropriate signal would its 'activating region' (that which binds the transcriptional machinery) become exposed, and recruitment ensue. Recruitment is not synonymous with 'increasing local concentration': as the figure suggests there might be ways to bring the enzyme near its target (hence 'increasing the local concentration') in an inappropriate orientation.

And in the eukaryotic world especially, the recruiting reaction can be more elaborate than is suggested by the figure. For example, some 50 proteins, found in an array of complexes, must be recruited to a yeast gene to elicit transcription. Some of these proteins are recruited directly — are touched by the activator — and others then bind cooperatively with those directly recruited. Some proteins (including even an inert RNA polymerase) might be pre-bound to a gene, but in each of these cases the job of the activator is to recruit, by a simple binding reaction, some necessary component to trigger transcription initiation, to foster transcriptional elongation, and so on.

Regulation

Our discussion of recruitment suggests one appropriate way to use this word. As we have noted, the cell contains an array of active enzymes — polymerases, ubiquitylators, proteases, RNA splicers, histone modifiers – each of which has multiple possible targets. Each of these enzymes is *regulated*, then, by directing it to one or another of its possible substrates — a specific gene or protein, for example. I have emphasized the use of protein recruiters (such as transcriptional activators and E3 ligases) to effect this regulation, but other

kinds of molecules can also work as recruiters. Double-stranded RNA, for example, directs the RNA interference machinery to specific sequences in mRNA and in DNA, and thereby regulates a form of gene silencing.

Signals typically are conveyed to recruiters and, as implied by the discussion thus far, not to the enzymatic machineries themselves. For example, the sugar galactose (which we'll call a signal) causes the inhibitor bound to Gal 4 (called Gal80) to dissociate from Gal4, thereby triggering recruitment of the transcription machinery. This rule (that signals go to recruiters) is not ironclad: during formation of germ cells in *Drosophila*, for example, the transcriptional machinery is turned off entirely, and it would seem proper to call this a form of regulation.

Despite our lack of a precise definition, there are uses of the word 'regulation' that are inappropriate. For example, let us say that RNA polymerase, once recruited along with whatever else is required for transcription, undergoes some conformational change as it begins to work — surely it does. But to call such a conformational change 'regulatory', without any evidence that such a step is subject to modulation by changing signals in the cell, is to embark on a trail of endless regress in which every event on a biochemical pathway can be called 'regulatory'.

This discussion has centered on 'activation', the imposition of specificity of by recruitment. 'Repression', as we shall see in our next encounter, is often, especially in eukaryotes, another manifestation of recruitment.

References

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