Essay

On the Use of the Word ‘Epigenetic’

Mark Ptashne

Over the past few years we have seen an odd change, or extension, in the use of the word ‘epigenetic’ when describing matters of gene regulation in eukaryotes. Although it may generally be that it is not worth arguing over definitions, this is true only insofar as the participants in the discussion know what each other means. I believe the altered use of the term carries baggage from the standard definition that can have misleading implications. Here I wish to probe our use of language in this way, and to show how such a discussion leads to some more general considerations concerning gene regulation.

To begin, consider an example of an epigenetic change (I’ll formally define the term in a moment) which involves a feedback loop. In this case a transcriptional activator — perhaps in response to an extra-cellular signal — binds DNA and turns on transcription of a second transcriptional activator; if the latter activates expression of its own gene, the new state of gene expression will be maintained even in the absence of the original activator. Such feedback loops are found widely in nature from phage lambda, to bacteria, to *Drosophila* and higher eukaryotes. Because the eukaryotic transcriptional machinery is so complicated — with its co-activators and co-repressors, histone modifiers and so on — it is rather easy to lose sight of the logic of regulatory systems: genes are turned on and off, often in feedback and interacting loops, by specific DNA-binding proteins. And these specific binding proteins typically work by recruiting the various components of the machinery to specific genes. These specific DNA binding proteins are expressed according to internal programs and, sometimes, in response to extracellular signals [1].

It is easy to see how the kind of feedback loop described above fits the classical definition of an epigenetic change in gene expression: “..a change in the state of expression of a gene that does not involve a mutation, but that is nevertheless inherited in the absence of the signal (or event) that initiated the change”. As a glance at the literature will reveal, however, that histone modifications — acetylation, phosphorylation, methylation, and so on — are now often explicitly called ‘epigenetic modifications’. This despite the fact that, so far as I am aware, no histone modification has been shown to be heritable. (Eukaryotic DNA is of course wrapped in histones to form strings of nucleosomes, and in what follows I will refer interchangeably to modifications of histones and nucleosomes.)

Nucleosome modifications

Certain histone modifications — methylation and acetylation, for example — are often found at actively transcribed genes. The enzymes that effect these modifications have no inherent specificity for one group of nucleosomes — such as those at a gene to be activated — over the nucleosomes found throughout the genome. Rather, like the transcriptional machinery itself, these modifying enzymes must be recruited to specific genes by specific DNA- binding proteins — transcriptional activators, for example. And, where examined, such modifying enzymes dissociate from the gene when the activator stops working, and the modifications are (actively or passively) lost [2]; these enzymes, of course, then work non-specifically and at a lower rate as part of the background.

A similar picture holds for gene repression. For example, silent mating type loci in yeast are maintained ‘off’ by a group of Sir proteins, bound to the chromatin, one of which bears a required histone deacetylase (HDAC) activity that removes acetyl groups from nucleosomes. This complex does not form spontaneously, neither is it self-perpetuating: formation of the complex requires specific DNA-binding proteins that recruit the Sir proteins, and loss of those recruiters causes the complex to fall apart [3]. Another example is provided by those cases in which RNA interference (RNAi) triggers silencing of transcription with concomitant recruitment of nucleosome modifiers. In some of these cases, specific DNA-binding proteins are not required — the silencing machinery is specifically recruited by the RNAi machinery. Here the continual production of specific RNA (and hence of RNAi) is required for maintenance of silencing and of those nucleosome modifications [4].

The transient nature of nucleosome modifications should not be surprising, because there is no known mechanism for self-propagation of such modifications. That is, there are no known enzymes that recognize a specific modification on a nucleosome and ensure that that modification will be transmitted to the next generation. This situation might change, of course (a possibility I return to later), but in the meantime the use of the word epigenetic implies a fundamental property of the system — self-propagation — that is, so far as we now know, not true of nucleosome modifications.

Regulation

I wish now, partly in light of what I have said, to comment briefly on the possible roles of histone

modifications in regulating transcription of genes, and on the words sometimes used to describe the roles of these modifications.

It is often said that genes "are regulated by chromatin modifications". Isn't this rather misleading? The simple fact that the nucleosome modifiers have no inherent specificity — beyond that for one or another residue on any of the many nucleosomes in the cell — tells us that these modifying enzymes cannot in any informative way be said to 'regulate' gene expression. Genes are typically regulated, in the meaningful sense, by specific DNA binding proteins that recruit transcribing or repressing complexes — including nucleosome modifiers. As noted, it is true that certain nucleosome modifications are typically found associated with active genes. But so is RNA polymerase, an observation that speaks to the events that occur during activation, but not to the key regulatory step.

One way nucleosome modifiers are sometimes said to regulate genes is by "opening or closing the chromatin structure", thereby allowing (or preventing) access of the transcriptional machinery to the DNA. Such a view is problematical on the face of it: how would the nucleosome modifiers 'know' which genes to pack or unpack unless instructed by a specific DNA binding recruiter? Moreover, the example we discussed — the silent mating type loci in yeast — suggests that even in 'silenced' regions (called heterochromatic regions), the DNA must be accessible to specific DNA binding proteins. Recall that, in that case, continual recruitment by the specific DNA-binding regulator is required to maintain the silenced state. The straightforward conclusion would be that this heterochromatic region is silent, not because of lack of access to specific DNA-binding proteins, but rather because the specific proteins that bind to the relevant sites recruit proteins, such as Sirs, which somehow diminish the efficiency with which an activator, binding to the same general region, can recruit the transcriptional machinery. Indeed, strong activators have been shown to overcome silencing in yeast, flies and in mammalian cells [5–8], and more direct experiments show that regulatory protein binding sites are accessible in heterochromatic regions in yeast [9,10]. And, of course, regions silenced by RNAi must be accessible to the RNA polymerase that transcribes the RNA necessary to maintain repression.

Whatever nucleosome modifications do — 'loosen the chromatin' as mentioned, or, another idea, provide binding sites to help in the recruitment of other proteins — it is very unlikely that such modifications suffice for efficient gene activation. Rather, it seems that an activator must contact the transcriptional machinery to recruit its various parts (directly or indirectly), and these interactions cannot be dispensed with for efficient expression. Thus, for example, nucleosome-depletion experiments in yeast (admittedly a messy business) show that for most genes, depletion suffices for no more than a modest increase in basal transcription [11]. Moreover, in yeast, various fusion proteins bearing a DNA-binding domain covalently attached to one or another component of the mediator (a protein complex required for efficient transcription of many genes) can activate transcription to a very high level (by directly recruiting to the gene a component of the transcriptional machinery), but only much smaller effects have been seen with fusions of a DNA-binding domain to one or another component of a complex that modifies nucleosomes [12]. Mutations in histone residues subject to modification have small effects on gene activation in yeast (Mary Ann Osley, personal communication), and it is now suspected that at least some of these modifications are involved in transcriptional elongation [13].

The notion that removing inhibitory proteins from DNA does not, in general, suffice for efficient gene expression is not unprecedented. In bacteria, for example, removal of a specific repressor rarely suffices for full transcription of a gene. Most bacterial genes are controlled by activators as well as by repressors, and removal of the repressor, in the absence of the activator, elicits in general only rather low (basal) expression [1]. The surmise that histone modifications per se do not suffice to activate or repress a gene is consistent with the findings that purported modified histone–target protein interactions are weak. Interactions that are weak can nevertheless be important, of course, but in each case their significance would remain to be shown. For example, the interesting idea that the interaction between a *Drosophila* Polycomb group protein and a methylated histone suffices to direct the Polycomb group protein to specific sites has not survived critical tests [14].

It is perhaps worth emphasizing again that some nucleosome modification might turn out — contrary to what we now know — to be self-perpetuating. One part of a possible mechanism is suggested by the phenomenon of 'spreading' of silencing in yeast. That effect —– which can extend silenced regions over long stretches of DNA — involves the Sir proteins mentioned above, and in particular the HDAC activity (also referred to above) of one of these proteins. The idea is that a recruited Sir deacetylates an adjacent nucleosome and thereby increases the affinity of that nucleosome for an additional Sir complex, and so on [15]. The Sirs, as we noted, require a recruiter to bind and to maintain deacetylation of histones. But one might imagine some other modification that, in the absence of a recruiter, would be self-reinforcing; and, we could imagine, that that modification would be inherited as nucleosomes were distributed to daughter chromosomes upon division. This remains to be seen. But if some such scenario holds, our current understanding requires that any such modification would have to be triggered by specific DNA

binding proteins or by RNAi. It is interesting in this context to consider DNA methylation.

DNA methylation

In contrast to histone modifications, DNA methylation —
a modification found in higher eukaryotes and bacteria, but not in yeast and flies — can be self-sustaining: maintenance methylases recognize hemi-methylated DNA, the product of replication of fully methylated DNA, and add methyl groups to the unmethylated DNA strand. It is believed that tumor suppressor genes are often turned off by aberrant methylation and kept off by epigenetic inheritance of that state. Assuming this is so, the question then arises as to what triggered methylation of these genes in the first place. Did the methylase spontaneously hop on and begin to work at (many) specific place? Or was there a genetic event, that for example, placed a methylase recruiting site nearer the gene, or caused over-production of a methylase recruiter? Another possibility arises from the suggestion that a specific histone modification can trigger DNA methylation [16]. The idea might then be that the hypothetical mutational event caused aberrant histone modification that, in turn, triggered DNA methylation.

Experiments with 5-AzaC cast doubt on the 'spontaneous methylation' idea. Treatment with this methylation inhibitor typically results in de-methylation. But [17,18] when one removes the 5-AzaC the aberrant DNA methylation typically reappears. Thus whatever mechanism (presumably requiring a genetic change) that initiated the methylation remains in place to restore the methylation. In bacteria there are several cases in which DNA methylation of crucial sites occurs spontaneously unless blocked by specific DNA-binding proteins. A mutant lacking such a protein would display aberrant methylation, a pattern that would be — as for the hypothetical scenarios I outlined above for tumor suppression — caused by a genetic event [19].

Unfortunately, especially in dealing with eukaryotes, there can be a very high bar to actually proving that the event that initiated self-propagating aberrant methylation did not involve a genetic change. One needs, in effect, to demonstrate that no mutation that might have initiated the changes occurred, something very hard to do.

A further comment and summary

Why might one be pre-disposed to misuse the term epigenetic? The term is sometimes used in the context of "maintaining stable states of gene expression", as though some 'locked in' mechanism, involving histone and/or DNA modifications, were required for stable states of gene expression in eukaryotes. But, in the first instance, there would seem to be no such special requirement: lambda lysogens are essentially infinitely stable in the absence of the specific signal that inactivates the repressor, and this system —of course — involves no histones. Beyond that, there is evidence against the idea that special irreversible mechanisms are required in higher organisms. Thus for example, fusion of a human muscle cell with a rat fibroblast produces a 'cybrid' which expresses rat muscle genes, a kind of experiment that has been repeated with an array of different cell types [20]. The surmise is that these highly differentiated cells bear, in their cytoplasms, factors that turn on (or in other cases off) transcription of crucial genes, and there is no special 'lockdown' that prevents these specific DNA-binding proteins from working. And, as already noted, there are several examples of inactivated genes being reactivated by strong transcriptional activators in an array of eukaryotes.

Whatever elaborations might be discovered, we have one clear picture for how patterns of gene expression are usually maintained as cells divide. In eukaryotes, as in bacteria, regulatory proteins (typically specific DNA-binding proteins) are distributed to progeny cells, and in the absence of changed signals (or new transcriptional programs initiated by the inherited regulators) these patterns, reinforced by feedback loops, will be maintained. Patterns of histone modification are often strongly correlated with patterns of inherited gene expression, and it has often been assumed that these modifications must cause them —hence the questionable use of the term 'epigenetic' to describe all such modifications. However, there are no examples where these modifications have been shown to be self-propagating, and there are explicit examples where these modifications are shown not to be self-propagating. Histone modifiers can play roles in gene expression, but they (as well as enzymes that trigger DNA methylation, in some cases at least) must be recruited to genes by specific DNA binding proteins. This general description is of course not complete: in mammals, for example, the phenomena of X-chromosome inactivation and imprinting show that genes can be maintained in an off state even in the presence of activators sufficient to turn on genes in homologous chromosomes (see also [21,22] for examples of similar phenomena in yeast). How these special cases arise and are maintained is not yet understood.

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Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, 1275 York Av., Box 595, New York, New York 10021, USA. E-mail: m-ptashne@mskcc.org