Chromatin-modifying enzymes in transcription and cancer

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Abstract

In recent years, our view of how gene expression is controlled has changed dramatically. The discovery of enzymes that modify histones has revealed that transcription is an enzymically driven process. Such modifications can recruit specific proteins and mediate chromatin changes that affect transcription either positively or negatively. Important biological pathways leading to cell proliferation are under the control of these enzymes, and several of them are found deregulated in cancer. The hope is that chromatin-modifying enzymes will be a rich source of targets for drug discovery.

Modifications in chromatin were known to exist for many years, but it was not until 1996 that enzymes which mediate them were discovered. Two pioneering discoveries led the way. Dave Allis and colleagues [1] showed that a yeast transcription factor GCN5 was a histone acetyltransferase and Stuart Schreiber and colleagues [2] identified a histone deacetylase (HDAC1) as the target for trichostatin A, a drug known to regulate of cell differentiation. The identification of these new enzymes catalysed experiments that showed that two well-studied human co-activators {CREB (cAMP-response-element-binding protein)-binding protein (CBP) and p300 [3,4]} as well as a basal transcription factor (TAF250) [5] possess histone deacetylase activity. These findings established that activation of transcription is not only a process mediated by protein–protein interactions, but that enzymes that acetylate chromatin also play a key role [6].

Now, many more modifications have been shown to take place on histones and to regulate transcription, including phosphorylation, methylation (of arginine and lysine residues) and ubiquitination [7]. All these have been recognized primarily by the sequencing of bulk histones. The truth is that there are likely to be many more modifications yet to be identified, as the technique to detect them is insensitive. For example, some modifications might well be below detection levels if they are specifically induced by a signalling pathway. Nevertheless, there are now 28 residues which are known to be modified on histones. Even with only this number of modifications, the potential exists for over 100 million differentially modified nucleosomes in the cell, if each modification can exist independently of the other. The fact is that some modifications are interdependent, so the number is likely to be smaller, but the calculation gives an indication of the magnitude of the task facing us in trying to decipher the mechanism by which modifications function.

Large areas of the genome are likely to have chromatin, which is modified in a particular way. Some modifications dictate that a region is 'permissive' towards gene expression, whereas others dictate that no transcription can take place. Experiments in the mating-type locus in yeast and the β-globin locus in chickens has shown that inactive heterochromatin is methylated at lysine-9 (K9) of histone H3, whereas active euchromatin is methylated at K4 of H3 and also acetylated at other lysine residues. There appear to be boundary elements between these different kinds of chromatin that keep these states in their right context. How these modification states are maintained is unclear, but most likely there are specific enzymes recruited by the boundary element, which modify the adjacent chromatin in a specific way.

Within active euchromatin regions, expression of a particular gene is dependent on specific transcription factors that associate with the promoter. These are the factors that are known to bring in the basal machinery and the RNA polymerase complex, and are also the factors which recruit additional chromatin-modifying enzymes needed to open up further the chromatin and allow RNA polymerase to progress through the gene to be transcribed. There are many examples of such enzyme-recruiting proteins, and some of these are known to regulate important pathways

Key words: acetylation, cancer, chromatin, chromatin-modifying enzyme, histone, transcription.
Abbreviations used: CARM1, co-activator-associated arginine methyltransferase 1; HDAC, histone deacetylase; K9, etc., lysine-9, etc.
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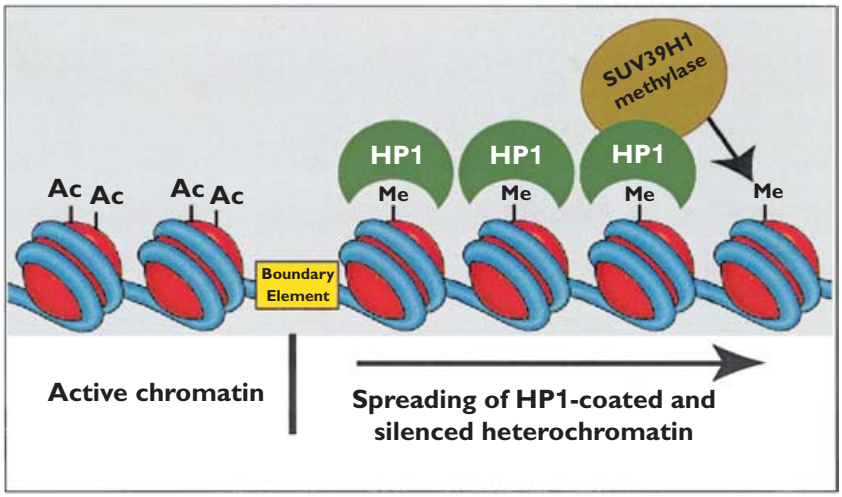
Figure 1 | Silencing of genes within heterochromatin is mediated by targeting of HP1 to heterochromatin sites



controlling cell growth. For example, the Retinoblastoma tumour suppressor protein regulates cell-cycle genes by recruiting to the promoter both histone deacetylases and methyltransferases [8,9]. Once on the promoter, the targets of these modifying enzymes may not only be histones, but also other proteins associated with the gene, such as transcription factors.

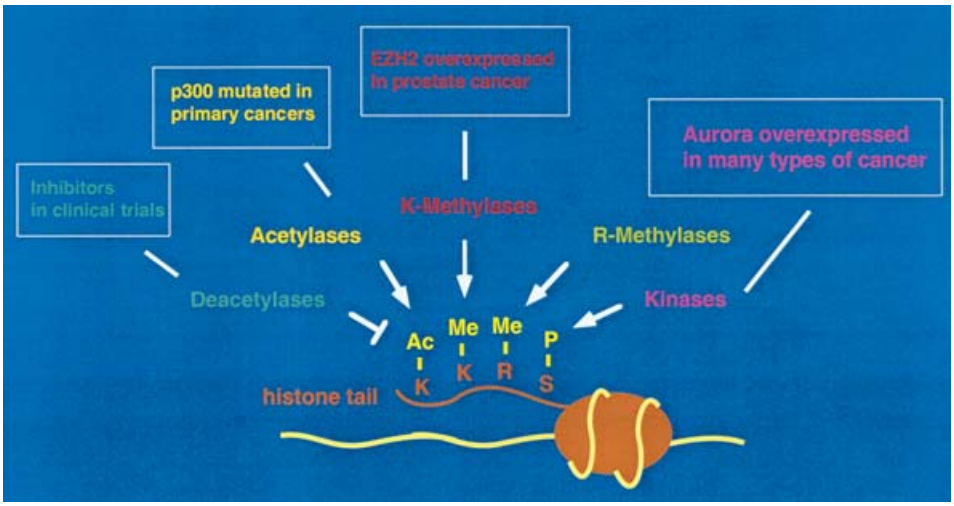
The delivery of chromatin-modifying enzymes to promoters leads to a complex pattern of modifications on histones. Some modifications (acetylation, phosphorylation, ubiquitination and arginine methylation) have almost exclusively been linked to activation of transcription, whereas lysine methylation can be either activatory or repressive, dependent on the particular lysine modified. In the course of transcriptional activation, a number of distinct types of modifications have been shown to communicate with one another. There are examples of 'cross-talk' between acetylation/phosphorylation, acetylation/arginine methylation and ubiquitination/lysine methylation. In these cases, a given modification may be a prerequisite for another to take place on the same chromatin, and in the cases that we know of so far, the dependence is only in one direction. For example, in the oestrogen-regulated pS2 gene acetylation of histone H3, K18 and K23 CBP is necessary for the methylation of R17 by CARM1 (co-activator-associated arginine methyltransferase 1), and not the other way round [10]. This is an instance where there is a proposed mechanism for the co-operation, namely that acetylation of histone H3 provides a binding platform for the CARM1 methyltransferase. This mechanism explains the one-sidedness of the communication.

There is also interference between different modifications. One example comes from the SUV39 methyltransferase, which methylates histone H3 K9. This enzyme is unable to methylate H3 K9 if this residue is first acetylated [11]. This provides the potential for a very nice on–off switch, since methylation at K9 is repressive for transcription, whereas acetylation of the same residues leads to activation. The existence of communication between modifications raises the question of whether there is a customized set of modifications that are needed for a particular form of gene expression. For example, are all oestrogen-regulated genes modified in the same way? Do different signalling pathways converging on a single gene mediate a distinct set of modifications ? These are fundamental issues which have yet to be resolved, at least partly because we don't yet understand the role that each modification plays in the control of chromatin structure.

Clues as to the mechanism by which modifications function has come from the finding that proteins exist that can discriminate and bind specifically to the modified versions of histones. Certain acetylated residues can be recognized via the Bromo-domain which is found in a number of acetylases. The Chromo-domain, present in the repressor protein HP1, can recognize the methylated version of H3 K9 [12,13]. The targeting of HP1 to heterochromatin sites, via H3 K9 methylation, mediates the silencing of the genes within heterochromatin (Figure 1). How the recruitment of proteins to modified sites regulates transcription is still unclear. One possibility is that additional activities are brought in, such as deacetylases or ATP-remodelling complexes, which then further compact chromatin. Alternatively, there may be compaction generated by the linking up of HP1 molecules present on different nucleosomes.

A number of lines of evidence now point to a role for chromatin-modifying enzymes in cancer [6,14]. First, circumstantial evidence has existed for many years, in the sense that viral-transforming proteins, such as the E1A protein from adenovirus and the E7 protein from papillomavirus, regulate cell proliferation by binding to and regulating acetylases or deacetylases. More tangible evidence comes from the observation that acetylases are found translocated in leukaemias and are mutated in primary cancers, the Aurora kinase (which phosphorylates H3 at Ser^{10} and Ser^{27}) is overexpressed in many types of cancer, and the lysine methyltransferase EZH2 is overexpressed in prostatic cancer

Figure 2 | Relationship between chromatin-modifying enzymes and cancer



(Figure 2). The most convincing evidence that these enzymes are valid and appropriate targets for rational drug discovery comes from the fact that inhibitors against deacetylases are now in clinical trials. Inhibitors against deacetylases are ahead of any other, since they were used initially for the discovery of HDAC1, and therefore have been around the longest. Suitable inhibitors against other enzymic families are now under way.

The effectiveness and specificity of deacetylase inhibitors for cancer cells is not easy to explain, especially given that these inhibitors affect all known HDACs. The argument goes that acetylation/deacetylation takes place on the chromatin of most if not all genes, so how can a broad-range inhibitor against most deacetylases behave so specifically against cancer cells? As scientists, what we would like to believe is that we know a lot about the pathways that lead to cancer, and we would therefore like inhibitors to be specific against only one of these. We would also prefer that this particular pathway was overexpressed only in the particular cancer against which the inhibitor is effective. However, this does not appear to be the case with these inhibitors. The likely explanation for the effectiveness of deacetylase inhibitors is that cancer cells may already have other critical pathways missing, which makes cancer cells more dependent an deacetylases compared with normal cells. Whatever the precise reason, the fact remains that deacetylase inhibitors appear to be effective in early clinical trials. This gives us hope that the new era of enzymically driven transcription is likely to uncover many more targets for rational drug design, not only for cancer, but for many other diseases.

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