Chromatin and transcription factors : who's on first?

Recent results suggest that nucleosomes and transcription factors interact dynamically to determine the transcriptional state of eukaryotic genes.

Chromatin is the physiologically relevant template for transcription: chromatin structure and transcriptional initiation must therefore be mechanistically linked. Nucleosome coating of the DNA severely restricts the access of transcription factors to promoters. Nucleosomal templates are transcribed much less efficiently than naked DNA templates *in vitro*, and gross disruption of chromatin structure *in vivo* by histone loss results in increased transcription. More generally, changes in chromatin structure are very likely to affect the access and/or the function of transcription factors, thereby altering patterns of gene regulation. Conversely, the binding of transcription factors to DNA must perturb chromatin structure by affecting nucleosome conformation, positioning, stability or removal. For these reasons, correlations between chromatin structure and transcription, though easy to find, are not very informative. Furthermore, activator proteins can stimulate transcription from naked DNA templates *in vitro*, leading some to view chromatin structure as simply a mechanism for compacting DNA, with only a passive, though generally inhibitory, role in transcription. However, work over the past few years has provided increasing evidence that chromatin structure plays a more active role in the transcription process.

The access of transcription factors to promoters does not inherently require a special chromatin state, such as might occur during DNA replication or mitosis. Although nucleosomes constitute a significant barrier, transcription factors can bind DNA at essentially any time in the cell cycle and rapidly cause changes in chromatin structure that can extend over several nucleosomes [1,2]. In the promoter of the rat *TAT* gene, hormone-activated binding of the glucocorticoid receptor alters chromatin structure to allow a distinct protein, HNF5, to bind to the same sequence [1]. As the glucocorticoid receptor and HNF5 cannot simultaneously occupy this sequence, the induced structural change must persist long enough to permit HNF5 access to DNA. Chromatin structural changes often precede transcriptional activation during the development of multicellular organisms.

Because chromatin structure *in vivo* is assayed by the indirect method of nuclease digestion, it is unclear whether nucleosome disruption by transcription factors reflects nucleosome removal. *In vitro*, it is clear that some transcription factors can bind to nucleosomal DNA. Of the proteins with binding sites in the promoter of the mouse mammary tumor virus (MMTV), the glucocorticoid receptor, but not NF1, can bind when a nucleosome is positioned on the promoter [3,4]. It is unclear whether this difference in binding reflects the location of the nucleosome with respect to the binding sites or distinct properties of the proteins. However, the transcription factors GAL4 and HSF (heat shock factor) appear to be mechanistically distinct, because only GAL4 can bind randomly positioned or rotationally phased nucleosomes [5]. The GAL4–nucleosome complex is metastable; it dissociates into nucleosomes and GAL4–naked DNA complexes upon addition of competitor DNA [6].

Histone acetylation can increase the access to nucleosomal DNA of at least one transcription factor, TFIIIA, without influencing the extent of histone binding or the DNA helical repeat [7]. TFIIIA also binds more efficiently to modified nucleosomes lacking the amino-terminal tails of the histones. Thus, the histone tails inhibit the access of transcription factors, possibly through the presence of basic residues that interact with the phosphodiester backbone of DNA in the nucleosome. Acetylation might prevent the histone tail from associating with DNA and/or alter the path of the DNA in the nucleosome. These *in vitro* results may be relevant *in vivo*, because acetylated nucleosomes are associated with transcriptionally active chromatin.

The binding of one protein to nucleosomal DNA can facilitate binding by others. GAL4 derivatives bind cooperatively to multiple sites in nucleosomal but not naked DNA, and HSF can bind nucleosomes in the presence, but not the absence, of TFIID [5]. In these examples, cooperative binding occurs only on nucleosomes, and hence is unlikely to involve the conventional mechanism of direct interactions between the relevant proteins. Instead, it is likely that binding by the first protein disrupts, but does not displace, nucleosomes so that the second protein can then bind more easily. Because this nucleosome-disruption mechanism of cooperative binding does not require specific protein–protein interactions, it may explain why transcriptional activation is often synergistic and promiscuous — promoters containing multiple binding sites for unrelated proteins are generally much more active than promoters with single sites. In this regard, transcriptional synergy depends on the number of proteins bound to the promoter, not the number of activation domains [8].

In vitro, nucleosomes severely repress basal TATA-dependent transcription, but only modestly affect transcription activated by GAL4–VP16 chimeric molecules, consisting

of the GAL4 DNA-binding domain linked to the potent VP16 activation domain [9]. A similar phenomenon is observed on DNA templates coated with histone H1 instead of nucleosome cores [10]. This relief of nucleosome repression requires the activation domain and is not due to local disruption caused by DNA binding. Alleviation of repression also may require RNA, perhaps to accept histones that otherwise would be present on the transcription template [10]. Other proteins, such as SP 1 and the GAGA-factor, relieve H1 and nucleosome repression, but the GAGA-factor is unable to activate transcription on naked DNA. These observations have led to the suggestion that activator proteins may stimulate transcription by relieving nucleosome repression, in addition to directly stimulating the transcription machinery. Clear interpretations are difficult, however, because the experimental conditions may not be physiologically relevant and because the contributions of the nucleosomal template and the activation domains are cleanly separated.

Transcriptional activation domains can disrupt nucleosome structure *in vivo*. Analysis of estrogen receptor derivatives in yeast indicates that the degree of chromatin disruption is related to the strength of the transcriptional activation domain [11]. This disruption of chromatin structure requires multiple estrogen receptor binding sites and correlates with transcriptional competence; hence it is difficult to distinguish the cause-and-effect relationship between transcriptional activity and chromatin structure. GAL4 disrupts a nucleosome in the *GAL* promoter region in a manner dependent on the activation domain [12]. Importantly, nucleosome disruption in the *GAL* promoter is caused by the activation domain and is not a consequence of transcriptional activity: it is observed even when transcription is blocked by mutational inactivation of the TATA element.

The yeast SNF and SWI proteins, which do not bind DNA but are important for transcriptional enhancement by a variety of DNA-bound activators, may activate transcription by affecting chromatin structure. Several lines of evidence suggest that these proteins function together, possibly as a large complex that possesses a transcription activation domain(s). SWI3 interacts directly with DNA-bound activators and is necessary for transcriptional activation *in vitro* at an early stage of the reaction [13]. SNF2 and SNF5 affect chromatin structure in a manner that is influenced by histone dosage but is independent of the transcriptional status of the promoter [14].

The observations above suggest that activation domains might stimulate transcription by altering chromatin structure. However, DNA-bound activator proteins and the putative SWI–SIN complex, which may associate with such activators, function *in vitro* on non-nucleosomal DNA templates. Thus, disruption of chromatin structure, though likely to be important for transcriptional activation, is clearly not sufficient to explain the full process. It is also likely that the relative contributions of chromatin disruption and stimulation of the transcription machinery will differ among activators and promoters. For example, the *DED1* upstream element can stimulate transcription by T7 RNA polymerase in yeast cells presumably by increasing access to the promoter, whereas the *GAL* enhancer cannot [15].

Perhaps the strongest argument for an active role of chromatin structure comes from histone mutations that cause specific transcriptional effects in yeast cells. Deletions of the histone H4 amino terminus and mutations that prevent H4 acetylation severely compromise transcriptional enhancement by several activator proteins [16]. This effect on activation is specific to H4: it is not observed in strains containing amino-terminal deletions of the other histones. In fact, amino-terminally deleted and non-acetylatable derivatives of H3 increase activation [17]. A sub-region of the histone H4 amino terminus, distinct from that involved in acetylation and activation, plays a specific role in transcriptional silencing of the yeast mating-type genes [18] and of genes near chromosomal telomeres [19]. Mutations in the H4 amino terminus that abolish silencing also disrupt nucleosomes positioned by the α2 repressor in minichromosomes [20]. These and other results suggest that silencing is likely to involve a repressed chromatin state that can be propagated through many cell division cycles.

For nearly two decades, opinions about chromatin structure have oscillated between it being the key determinant or a passive bystander in transcriptional regulation. It is now clear that activation and repression of specific genes results from the dynamic interactions between nucleosomes and transcription factors with DNA and with each other. The molecular mechanisms are just beginning to be understood.

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