

Chromatin and transcription: where do we go from here?

Commentary

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Current Opinion in Genetics & Development 2002, **12**:249–251

0959-437X/02/\$ – see front matter

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Introduction

Chromosomal transactions related to transcription are under study in many laboratories and have been reviewed extensively [1–8]. The most recent information, extending from histone modification to cancer and therapeutics, is summarized in this issue's collection of reviews. Our purpose in writing a brief *Commentary* to finish this series is to call attention to what is *not* known, especially for those outside the field, for whom the avalanche of new data may tend to obscure the outstanding questions.

Not long ago, the very relevance of chromatin structure to transcription was in doubt. The structure of promoters and regulatory sequences could be manipulated and the consequences for transcription determined *in vivo* without regard to the packaging of the DNA in nucleosomes. Naked DNA could be employed in studies of transcription *in vitro*, reconstituting most aspects of transcription and its control, without the inclusion of histones or other chromatin proteins. It has since emerged that the role of chromatin is pervasive but could, nonetheless, be neglected in many studies on transcription for at least two reasons. First, numerous chromatin-remodeling complexes make DNA available *in vivo*, permitting DNA–protein interactions important for transcription despite packaging in nucleosomes and possibly without the complete disruption of chromatin structure. Second, there appear to be at least two levels of transcriptional regulation: one involving chromatin 'unfolding' and another involving the transcription machinery at RNA polymerase II promoters. The first level may be a prerequisite for the second, which can then proceed independently; that is, chromatin unfolding may expose promoters for assembly of the transcription machinery, explaining why transcription could be studied so successfully in the past with the use of naked DNA.

Chromatin structure and modification

Most research and thinking about the transcription of chromatin is based on the nucleosome, the conserved unit of chromatin organization. In reprising what is known, as a point of departure for discussing the outstanding issues in the field, it therefore seems most appropriate to begin with the nucleosome. X-ray structure determination of the histone octamer and nucleosome core particle has revealed a division between globular 'histone fold' and extended

'histone tail' regions [9,10]. The histone folds are responsible both for the interactions of the histones with one another to form the octamer and for the interactions with DNA that constrain it in a superhelical path around the octamer. The histone tails protrude, making no apparent contribution to either octamer or core particle structure. Inasmuch as the tails contain almost all sites of histone modification — including sites of acetylation, phosphorylation, methylation, and ubiquitination — it seems reasonable to conclude that these modifications do not affect the structure or stability of the core nucleosome directly. Effects on tail conformation, as well as indirect effects on core nucleosome structure, through the agency of specific tail-binding proteins, are possible but have not yet been demonstrated.

Histone modifications, featured in the reviews of this issue, are thought to impart functional diversity to an otherwise monotonous array of nucleosomes. Much of the work performed to date is descriptive, detailing the variety of modifications, the proteins and multiprotein complexes responsible, and the functional consequences. The results are impressive and exciting, showing direct connections with gene regulation, through histone-modifying enzymes that serve as transcriptional regulatory factors, and giving significant insight into the basis for epigenetic effects on gene expression. For the most part, however, the molecular mechanisms involved, and thus a full solution of the problem, remain elusive. Two types of mechanism have been considered: histone modifications as determinants of 'higher order' chromatin structure, and histone modifications as tags for recruiting further proteins to specific chromosomal regions. These mechanisms may be related, as in the case of histone acetylation and chromosome condensation.

It has long been thought that acetylation might affect the packing of nucleosomes in higher-order chromatin structures, and evidence has been presented for a decrease in compaction of chromatin fibers upon acetylation [11]. The difficulty is that chromatin structure beyond the nucleosome is still under investigation. Both a helical [12] and a zigzag [13] organization of chromatin fibers have been proposed, and although evidence favors the zigzag at present, decisive experiments must still be carried out. In the absence of an established structure, it is impossible to know for sure whether a modification such as acetylation perturbs the structure.

The clearest correlation between acetylation and chromatin structure is for heterochromatin in yeast: three sites of acetylation in the histone H4 tail are unmodified here [14]. This absence of acetylation is crucial and may alone be

sufficient for the establishment and maintenance of a heterochromatic state [15]. Inasmuch as heterochromatin in yeast exhibits many features in common with that in higher cells, including localization to the nuclear envelope and late replication, it may also be condensed as seen by cytological procedures in higher cells. The basis for this correlation of acetylation state with condensation state is thought to lie in interactions of the histone tails with nonhistone proteins, such as the SIR proteins in yeast heterochromatin [16].

Outstanding questions in regard to chromatin structure and modification therefore begin with the problem of chromatin organization beyond the nucleosome. Experimental criteria for higher-order chromatin structures are needed to assess the effects on such structures of histone modification, gene activation, and so forth. Detailed knowledge of higher-order structures is needed to understand the underlying mechanisms.

How might the problem of higher-order structures be addressed? One approach being pursued and likely to be important as an experimental criterion for higher-order structure in the future is cryoelectron microscopy. Evidence for the zigzag organization of chromatin fibers comes from this approach, and the results will become more detailed and informative as powerful new electron microscopes and allied technologies come into use. A second approach that might be taken is X-ray crystallography of heterochromatin. With some, if not all, of the protein components of yeast heterochromatin now defined, the way is at least open to relevant crystallization trials. Although the condensed state of yeast heterochromatin may differ in important regards from that of repressed genes or of heterochromatin elsewhere, some general principles of higher-order chromatin structure may emerge. Finally, biochemical studies of chromatin assembly may offer an avenue for elucidation of the components, intermediates, and range of end products of chromatin condensation. The development of a *Xenopus* oocyte extract that supports chromosome assembly and condensation can be further exploited for this purpose [17].

Promoter structure and remodeling

Both enhancers and promoters of transcriptionally active genes are generally exposed in so-called 'nuclease-hypersensitive' sites [18–20]. Fine mapping points to the occurrence of nucleosomes at these sites in the repressed state and disruption of the nucleosomes upon activation. A uniform accessibility to nuclease cutting upon activation, together with the excision of restriction fragments from hypersensitive sites in a protein-free state, led to the idea that histones are entirely dislodged from the DNA. Recently, two findings have called this idea into question. First, chromatin immunoprecipitation analysis has revealed hyperacetylated histones at the enhancers and promoters of transcriptionally active genes. Second, chromatin-remodeling complexes have been shown to

render nucleosomal DNA uniformly accessible to nuclease digestion *in vitro* without displacement of histones from the DNA [21]. The most fundamental attribute of transcriptionally active promoters, their nucleosomal state, is therefore an unresolved issue [3].

A definitive resolution for this issue can only come from the isolation and analysis of promoters in repressed and transcriptionally active states. A major limitation is the low abundance of natural promoter chromatin. This limitation can be overcome by the use of multicopy plasmids, whose isolation in the form of chromatin from yeast has been described [22]. Proper transcriptional regulation, however, requires a balance between gene and regulatory protein numbers, and the introduction of genes on multicopy plasmids will disturb this balance. The resulting chromatin preparation will be heterogeneous, containing both transcriptionally active and inactive genes. An alternative is the excision of single-copy genes from the yeast chromosome, which has been accomplished with the use of a site-specific recombinase [23]. The limitation of low abundance must then be overcome by large-scale isolation and sensitive methods of detection.

Beyond the problem of heterogeneity of promoter chromatin preparations lies the issue of whether different genes employ different transcriptional activation mechanisms. There are multiple chromatin-remodeling complexes in all cell types analyzed to date, and these complexes differ in aspects of their interactions with nucleosomes. The few promoter regions that have been analyzed in detail all differ in regard to arrangement of nucleosomes in the repressed state, requirement for acetylation, and chromatin-remodeling complex involved. It nonetheless stands to reason that there are common principles of the activation process, and the task before us is to discern those principles against the background of variation.

Conclusions: gene activation *in vitro*

Ultimately, the transcriptional activation process must be reconstituted *in vitro* with purified components. Not only the many proteins involved but also the chromatin template must be provided in a fully defined state. In studies done thus far, artificial chromatin templates, formed by the addition of purified histones to naked DNA templates, have been employed. This approach has yielded important information, but it has not dealt with more elaborate promoter chromatin structures involving nonhistone proteins and higher-order nucleosomal interactions. This concern can only be addressed by the use of natural chromatin templates, assembled *in vivo* and isolated without damage or loss of essential components. Cell extracts capable of transcribing such natural templates can then be fractionated to reveal the complete machinery and molecular mechanism of transcriptional activation.

Finally, biochemical studies of transcriptional activation must be complemented by genetic analyses to evaluate the

physiological relevance of the proteins involved. Crude cell extracts are rife with inhibitors of transcription, most of which are extraneous to the natural process — such as ribosomal and other highly basic proteins that bind tightly to DNA and RNA. Proteins that stimulate transcription but are also extraneous to the natural process, some serving as anti-inhibitors, are also commonly observed. Genetic analyses can test the relevance of both stimulatory and inhibitory proteins, and can reveal additional important factors, not immediately apparent from biochemical studies, as well. Only when such a combined biochemical and genetic investigation has been completed will the last word on transcriptional activation have been spoken.

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