COMMENTARY

Structure and dynamics of transcriptionally active chromatin

JUAN AUSIO

Department of Biochemistry & Microbiology, University of Victoria, Victoria, British Columbia, Canada V8W 3P6

Introduction

Chromatin is a highly dynamic macromolecular complex that undergoes continuous structural modification during the various stages of genetic activity. Such dynamic behavior is the result of a complex and perfectly balanced network of interactions involving histones, DNA and ions in an aqueous environment. In the past eighteen years since the discovery of the nucleosome particle, a great deal of information has been obtained on interactions responsible for maintaining the static three-dimensional structure of inactive chromatin (see Van Holde, 1988, for a recent review on chromatin). More recently, structural studies of chromatin have focused on the analysis of the molecular events involved in chromatin activation. A variety of models have been proposed, primarily to explain the structural transitions undergone by chromatin during transcription. In the following sections, I will briefly review what is known about the structure of transcriptionally active chromatin and propose a model for "activation" that would account for the chemical and ionic interactions that are possibly involved. I hope that this model will stimulate design of new experimental approaches with which to understand better the structure-function relationship in chromatin.

Active chromatin

Early studies carried out by Weintraub and Groudine (1976) demonstrated that active genes exhibited an altered chromatin structure with enhanced sensitivity to DNase I. However, the relationship if any, between these two phenomena remained unclear for many years. In the interim, the compositional characterization of active chromatin was further elucidated. Non-histone proteins HMG-14 and HMG-17 were found to be preferentially associated with active genes and initially held responsible for the DNase hypersensitivity (Weisbrod and Weintraub, 1979). Acetylated histones were also shown to be preferentially associated with the active chromatin template (Davie and Candido, 1978). However, it was subsequently shown that selective removal of the linker histones (histones of the H1 family), as well as HMG-14 and HMG-17 from active chromatin regions, failed to abolish DNase I sensitivity (Goodwin et al., 1985). A radical turn in this direction was taken with the demonstration that torsional stress, due to topoisomerase activity, was ultimately responsible for the nuclease hypersensitivity of transcriptionally active chromatin (Villapontea et al., 1984). Following this discovery, it was clear that gene activation involved dynamic structural changes in chromatin with distinctive compositional differences between the active and inactive state. Unfortunately this finding neither explained nor defined the nature of the altered chromatin structure initially observed by Weintraub and Groudine.

Weintraub and coworkers (Weintraub et al., 1976) have suggested that the structure of active chromatin involves an altered "unfolded" nucleosomal organization with changes primarily in the histone-histone interaction patterns. More recently, an unfolded nucleosome particle "lexosome" has been proposed (Prior et al., 1983) with an altered "more accessible" disposition of the SH groups of histone H3 (Chen and Allfrey, 1987). U-shaped particles possessing 20% less protein mass than normal globular nucleosomes (most likely as a result of the loss of an H2A-H2B dimer, see below) have also been recently observed in transcriptionally active chromatin (Locklear et al., 1990).

At the supranucleosomal level, reduced compaction of the active chromatin seems to be associated with a partial depletion of linker histones (Kamakaka and Thomas, 1990). This transient release of the linker histones as well as the histone octamer (Lorch et al., 1987; Pfafffle et al., 1990; Clark and Felsenfeld, 1991) and/or one histone H2A-H2B dimer (Baer and Rhodes, 1983) as a result of the RNA polymerase activity may also contribute to the altered conformation of chromatin within actively transcribed regions. Such RNA polymerase-mediated changes in chromatin structure could involve displacement of histones from their "native" positions and possibly binding to adjacent or neighboring secondary sites. Indeed, the association of nucleosome core particles with additional core histones at intermediate ionic strengths (Voordow and Eisenberg, 1978) and under physiological conditions (Stein et al., 1985) has been well documented. The extent of these compositional changes and thus the level of chromatin unfolding would ultimately depend on the

Key words: chromatin, transcription, histones.
particular kind of gene being transcribed (Van Holde, 1988).

**Histone acetylation: the unsolved puzzle**

Without a doubt, the structural motif most strongly correlated with chromatin activity is acetylation of the core histones (see Csordas, 1990, for a recent review). The biological relevance of this post-translational modification of histones, which involves charge neutralization of some of the lysine residues within the N-terminal regions (tails) of the histones, was already envisaged from the early days of its discovery. Because of the chemical nature of this phenomenon such modifications have been generally thought to participate in the loosening of the chromatin structure. In spite of the extensive experimental work carried out to support this hypothesis, most of this effort has failed to provide evidence of any significant structural changes. In the absence of any other factors, a histone acetylation-dependent unfolding of either nucleosomes or higher-order chromatin structure has not been observed. At the first level of chromatin organization, highly hyperacetylated nucleosomes remain essentially folded in solution at physiological ionic strength (Ausio and Van Holde, 1986). This finding is not surprising, however, since complete removal of the N-terminal regions of the histones by immobilized trypsin also fails to affect the overall structure of the chromatin subunit (Ausio et al., 1989). However, these observations do not preclude a partial loss of stability of the particle in response to mechanical forces, resulting from the weakening or disappearance of some of the histone-DNA interactions in these regions. Indeed, a selective unfolding of highly hyperacetylated nucleosome core particles has been observed in the electron microscope as a result of the additional shearing mechanical stress introduced by this technique (Oliva et al., 1990). In vivo, such stress may be introduced into the chromatin template by topoisomerase II activity or RNA polymerase passage (Pfaffle et al., 1990; Clark and Felsenfeld, 1991). At the next level of chromatin organization, histone hyperacetylation seems to have very little effect on the condensation state of the 30 nm fiber (McGhee et al., 1983). This observation is in contrast with the apparent complete absence of folding exhibited by trypsinized chromatin that had been further reconstituted with native histone H1 (Allan et al., 1982). It is important to emphasize, however, that the chromatin samples used in the acetylation experiments were obtained from HeLa cells grown in the presence of sodium butyrate in order to inhibit histone deacetylase activity (Riggs et al., 1977). Indeed butyrate treatment also enhances the expression of histone H1a (Chabanas et al., 1985). The presence of high levels of H1a could account for the condensed chromatin structure observed in these chemically perturbed cells. Therefore, results obtained under these conditions must be interpreted with caution.

A solution to the apparent lack of correlation between acetylation and chromatin unfolding may be ascertained from a careful analysis of the structural data already available. We observed that, during the preparation of nucleosome particles, the linker regions of hyperacetylated chromatin, which had been stripped of histone H1, were digested more readily by micrococcal nuclease than in the non-acetylated control (Liber-tini et al., 1988). In addition, only minor acetylation-induced structural changes were detected at the level of the nucleosome core particle (Ausio and Van Holde, 1986), while more extensive differences have been reported on nucleosome monomers containing undigested linker DNA (Bode et al., 1983). The conclusion from these studies is that in the absence of histone H1 the major structural effect of acetylation originates at the linker DNA component of chromatin. The finding that in H1-stripped chromatin the linker DNA is more accessible to nuclease attack in the hyperacetylated fractions clearly indicates that this DNA region has a more accessible conformation. Indeed it has been shown that histone acetylation reduces the nucleosome core-particle linking number change (Norton et al., 1989) and that it alters the capacity of exogenously added histones H1 to precipitate transcriptionally active/competent chromatin (Ridsdale et al., 1990). It is thus possible that upon neutralization (or removal by trypsinization) of the charges of the histone tails, and displacement of histone H1 by non-histone proteins, or other unknown effects, the exit and entry trajectories of the flanking DNA regions of the nucleosomes become substantially altered. This could in turn temporarily prevent histone H1 from binding to its native site and in combination with torsional stress may be responsible for maintaining the extended (partially depleted of histone H1) chromatin conformation necessary for the passage of RNA polymerase.

**A structural model for chromatin activation**

From what has been described above, it is clear that acetylation by itself cannot directly account for the structural changes observed in active/competent chromatin. Rather, I think that the role of acetylation is to poise chromatin for activation. This may be carried out in two ways (see Fig. 1). First of all, the finding that histone acetylation occurs sequentially throughout most of the chromatin (Perry and Chalkley, 1982) suggests a role in partial decondensation, unshielding some of the DNA sequences that may become available for protein recognition by regulatory proteins such as transcription factors and/or RNA polymerase itself (see Fig. 1A). Alternatively, acetylation may be required for further unfolding of the otherwise condensed 30 nm chromatin fiber. Such unfolding could be induced by the introduction of torsional stress mediated by the presence of topoisomerases or by polymerase advance during transcription (Pfaffle et al., 1990) or both (see Fig. 1B). Interestingly, topoisomerase II is found associated with nuclear matrix attachment regions (MARS) at the base of chromosomal loops (Cockerill and Garrard, 1986).
Fig. 1. A model for chromatin activation. (A) Acetylation of a chromatin domain (shaded region) relaxes slightly the higher-order structure within this region and unshields the DNA sequences for protein recognition by regulatory proteins. Chromatin is thus "poised" for activation. (B) Upon induction of torsional stress, the "poised" domain may partially unfold, releasing histone H1. The flanking DNA regions and the linker DNA of the nucleosomes involved become more extended, thus temporarily preventing H1 from binding to its primary nucleosomal site. The passage of RNA polymerase may induce a further unfolding of the nucleosome structure (shown here) with the displacement of an H2A-H2B dimer (see Fig. 2) or complete dissociation of the histone octamer depending on the class of gene being transcribed (van Holde, 1988). (C) Upon removal of the acetyl groups by deacetylases, and relaxation of the torsional stress (in the wake of RNA polymerase), histone H1 is able to recognize and bind again to its native binding sites and fold the nucleosome fiber back into its 30 nm higher-order inactive structure.

The activity of topoisomerase II at these sites could act in targeting specific DNA loci for expression. Regulation of the level of supercoiling of entire loops by topoisomerase II and other trans-acting factors (Gross and Garrard, 1987) in conjunction with acetylation would thus provide a mechanism for recognition of the DNA sequences to be transcribed. The resulting extended structure of this active chromatin conformation may promote local release or displacement of histone H1 molecules from their native binding sites. Non-histone proteins such as HMG 14 or HMG 17 may play an important role in stabilizing this transiently unfolded structure. In transcribed regions, the negative supercoiling of DNA in the wake of RNA polymerase (Pfaffle et al., 1990) and deacetylation of histones would restore the initial inactive chromatin structure (Fig. 1C).

The finding that histone residues targeted for acetylation occur non-randomly and to a different extent in response to physiological situations (i.e. in transcription compared to replication; Csordas, 1990) adds an extra level of complexity in the model proposed. However, beyond a critical number of about 12 acetyl groups per nucleosome particle, the structural changes observed at this level seem to be independent of the extent of acetylation (Ausio and Van Holde, 1986). Thus the function-dependent specific pattern of acetylation may play an additional regulatory role in the transitions involved (Loidl, 1988). Such distinctive patterns of chromatin acetylation may be important for recognition by deacetylases or in controlling the rate of removal of the acetyl groups by these enzymes.

At the nucleosome level, the combination of torsional stress and histone acetylation could also be responsible for the transient unfolding of the nucleosome particle (see Fig. 2), inducing U-shaped "lexo-
some"-like structures such as those observed in transcriptionally active chromatin (Locklear et al., 1990). The requirement for torsional stress could explain the elusive nature of this structure, which would be highly unstable (i.e. upon removal of torsional constraints by micrococcal nuclease cleavage at the adjacent DNA linker regions). It is noteworthy that the only "lexosomes" characterized thus far contained high levels of non-histone protein (Prior et al., 1983) and acetylated histones (Boffa et al., 1990).

Even if the model presented here is not entirely accurate, it seems clear that, the "tails" of the histone octamer play a more dynamic role in the modulation of chromatin structure than was initially envisaged. Through modification of the net charge distribution in these regions it is possible to modulate the functional state of chromatin (see also Earnshaw, 1987).

In their native fully charged conformation, the role of the tails may be to enhance the stability of the nucleosome core particle and provide the proper DNA conformation at the exit and entry points of the core particle, allowing for the proper binding of the linker histones. At the same time they may provide the necessary charge shielding on the DNA required for the folding and compaction of the chromatin fiber from its extended 10 nm configuration to its 30 nm higher-order structure. By simply altering this ionic balance it is possible to reverse these functions and thus poise chromatin for transcriptional activation.

I am very grateful to Nik Veldhoen for carefully reading the manuscript and also for his suggestions. I also thank Ms. Cheryl Gonnason for typing the manuscript.

References


Fig. 2. Possible unfolding of the nucleosome core particle as a result of chromatin activation. Upon acetylation, the nucleosome core particle remains essentially in its folded native (unshaded particles) conformation. Yet, it adopts a slightly looser conformation (shaded particles) probably arising from the release of the N-terminal regions (tails) of the histone octamer. It is possible that during this process some rearrangement of the core histones takes place.


