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Chromatin Remodeling and Transcription[1]

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The chromatin structure is essential not only for the compact packaging of the eukaryotic genome but also for regulation of transcription. This article provides an overview of chromatin modification upon transcriptional activation or repression, and chromatin remodeling. Interestingly, recent data demonstrate that chromatin remodeling in the promoter region is necessary for transcription.

Key words: chromatin remodeling, histone acetylation, histone deacetylation, nucleosome, transcription.

The fundamental building unit of chromatin is the nucleosome, in which 146 base pairs of DNA wind around an octamer of core histones. The nucleosome arrays are further packed into a chromatin fiber to constrain the genome within the limited space in the cell nucleus. Studies on chromatin did not progress smoothly since R. Kornberg's discovery of the nucleosome (*1*) more than 20 years ago. However, many important findings have accumulated over the past decade and most people feel that chromatin studies are finally coming of age. In this review, I will focus on one aspect of these advances: chromatin remodeling and transcription. This article is written for a general audience; for more detailed information, please refer to other recent reviews in the field (Refs. *2-14*).

Yeast genetics opens up the avenue to success

In vivo evidence for a role of chromatin in the global repression of transcription has been obtained by placing a histone gene under the control of an inducible promoter in the yeast *Saccharomyces cerevisiae*. When expression of core histone H4 or H2B is repressed, certain genes including *PH05, CUP1*, and *HIS3*, that are normally inactive, become activated (*15*).

A link between chromatin and transcription has been also established by an independent approach. Insertion of a transposon Ty into the promoter region of the *HIS4* gene can disrupt expression of the gene in yeast by inhibiting normal transcription. Selection for suppressors of such insertion mutations has resulted in the identification of a set of genes (*SPT* for suppressor of Ty) required for normal transcription. Two of them (*SPT11* and *SPT12*) encode histones H2A and H2B, respectively (*16*). Many other *SPT* genes also appear to be involved in chromatin transcription (see the following section).

Still another line of studies has established a role of chromatin in the activation of a group of genes in yeast (*17*). Genetic analyses of transcriptional regulation in yeast have led to the identification of a number of *SWI* and *SNF* genes (*SWI* for mating type switching, *SNF* for sucrose non-fermenting). These genes are required for proper expression of certain inducible promoters including *PHO5*, *CUP1*, and *HIS3*. Among *SWI/SNF* gene products, SWI1, SWI2/SNF2, SWI3, SNF5, and SNF6 are functionally interdependent, suggesting that they exert their function as a complex. The link to chromatin structure has become apparent through the isolation of suppressor mutations that can induce transcription in yeast strains deficient for SWI/SNF function. These mutations identified a set of switch-independent (*SIN*) genes that include core histone genes.

Transcriptional activation through chromatin acetylation

Studies on transcriptional regulation in eukaryotes have revealed many regulatory proteins that bind to control elements on DNA. Typical regulators such as GAL4 and GCN4 consist of two domains (*18*), a DNA-binding domain that binds to the control element in a sequence-specific fashion and a transcriptional activation domain that somehow stimulates the basal transcription machinery. To elucidate the mechanisms of transcriptional activation, many researchers have concentrated on the identification of proteins termed co-activators or adaptors that would interact with the transactivation domain of a DNA-binding regulator. Again, the genetic approach has proven most powerful. Overexpression of a potent chimeric regulator GAL4-VP16, consisting of the DNA-binding domain of GAL4 and the transactivation domain of a helpes virus activator VP16, in yeast caused severe growth inhibition. This might be due to sequestration of a co-activator by GAL4-VP16. Selection for mutations that permit growth in the presence of GAL4-VP16 has led to the identification of a set of adaptor (*ADA*) genes (*19*). Further studies have demonstrated a functional interdependence among some of the *ADA* gene products, such as ADA2, ADA3, and GCN5, suggesting that these proteins form a complex.

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Abbreviations: CBP, CREB (cyclic AMP response element-binding protein)-binding protein; PCAF, p300/CBP-associated factor; TAF, TBP (TATA binding protein)-associated factor; SRC, steroid receptor co-activator; ACTR, nuclear receptor co-activator; N-CoR, nuclear receptor co-repressor; SMRT, silencing mediator for retinoid and thyroid hormone receptors; MCP, methy-CpG-binding protein.

Since the discovery of histone acetylation in the mid 1960s (*20*), it has been believed that hyperacetylation is correlated with transcriptional activation. Investigations on co-activators and histone acetylation have finally met in the remarkable finding of Allis and colleagues (*21*): purification and cloning of the catalytic subunit of *Tetrahymena* histone acetyltransferase (HAT) have revealed sequence homology with the yeast co-activator GCN5. Subsequent studies have demonstrated HAT activities in many co-activators including mammalian p300/CBP (*22*), PCAF (*23*), TAFII250 (*24*), SRC-1 (*25*), and ACTR (*26*). Yeast GCN5 can acetylate the free histone H3 but not nucleosomal histones. For acetylation of chromatin, a multiprotein complex termed ADA, containing ADA2, ADA3 and GCN5, or SAGA (SPT-ADA-GCN5-acetyltransferase) complex containing SPT3, SPT7, SPT8, and SPT20/ADA5 in addition to ADA2, ADA3, and GCN5, is required (*27*).

Transcriptional repression through chromatin deacetylation

Treatment of cells with inhibitors of histone deacetylase (HDAC) such as trichostatin A or trapoxin A results in repression of transcription of various genes. Human HDAC1 has been purified as a trapoxin-binding protein (*28*). Cloning of a cDNA encoding the catalytic subunit of HDAC1 has revealed a sequence homology with the yeast regulator RPD3 that is required for full repression of a subset of genes. An emerging picture from these findings is that chromatin deacetylation is targeted by transcriptional repressors. For example, yeast regulator UME6 bound to its recognition site upstream of the *INO1* gene represses the gene through chromatin deacetylation by recruiting a HDAC RPD3 through a co-repressor SIN3 (*29*). Similarly, the Mad:Max heterodimer represses its target genes by interacting with a complex of HDAC1 and mSIN3 (mammalian counterpart of yeast SIN3) (*30*). As shown in Fig. 1, a nuclear receptor represses transcription in the absence of its ligand by interacting with a co-repressor N-CoR or SMRT which recruits HDAC1 through mSIN3 (*31, 32*). Upon binding of the ligand, the nuclear receptor undergoes a conformational change. It no longer binds to the co-repressor but associates with co-activators such as p300/CBP, PCAF, and SRC-1 or ACTR, and activates transcription through chromatin acetylation (*22, 23, 25, 26*).

In vertebrate DNA, a small percentage of cytosines are methylated. Inactive genes tend to be hypermethylated while active genes are hypomethylated. The reason for this is now becoming clear. MeCP2 binds to 5-methylcytosine in DNA and recruits HDAC1 through mSIN3, which results in the deacetylation of chromatin and transcriptional silencing (*33*). These observations have led to a consensus that transcriptional activation and repression are achieved through chromatin acetylation and deacetylation, respectively.

Chromatin remodeling factors

Yeast SWI/SNF complex has been purified and found to facilitate site-specific binding of GAL4 to a reconstituted nucleosome in an ATP-dependent manner (*34*). It also changes the DNase I digestion pattern of a nucleosome. Among SWI/SNF proteins, SWI2/SNF2 contains a motif seen in ATP-dependent DNA helicases and has a DNA-dependent ATPase but no helicase activity. Components homologous to yeast SWI2/SNF2 are now known from *Drosophila* (BRAHMA) and human (BRG1 and HBRM). A chromatin remodeling activity similar to that displayed by the yeast SWI/SNF complex has been observed with human SWI/SNF complex containing BRG1 and HBRM (*35*). Another chromatin remodeling factor RSC (remodeling the structure of chromatin) has been isolated from yeast on the basis of homology to the SWI/SNF complex (*36*). It consists of 15 components and is approximately 10 times more abundant than the SWI/SNF complex in the yeast cell.

In *Drosophila*, three distinct chromatin remodeling factors, NURF, ACF, and CHRAC, have been identified in addition to the SWI/SNF (BRAHMA) complex. They all contain ISWI (imitation switch) as a common subunit, while their other subunits differ (*37-39*). ISWI shares homology with SWI2/SNF2 only in its ATP-dependent helicase domain. NURF (nucleosome remodeling factor) has been isolated on the basis of an ATP-dependent nucleosome disruption activity targeted by the DNA-binding transcription factor GAGA as depicted in Fig. 2 (*40, 41*). ACF (ATP-utilizing chromatin assembly and remodeling factor) can assemble periodic nucleosome arrays in the presence of core histone chaperons such as CAF-1 or NAP-1 (*38*). CHRAC (chromatin accessibility complex) has been purified as a factor that enhances accessibility of chromatin to restriction enzymes in an ATP-dependent manner (*39*). Both ACF and CHRAC have ATP-dependent activities that mobilize nucleosomes to form regularly spaced nucleosome arrays (nucleosome spacing activity), while NURF only perturbs the nucleosome structure. All of these chromatin remodeling complexes contain a SWI2/SNF2-related subunit harboring an ATP-dependent DNA helicase domain. It has been postulated that the SWI2/SNF2-related proteins may achieve ATP-driven DNA translocation to disrupt histone-DNA interactions (*13*).

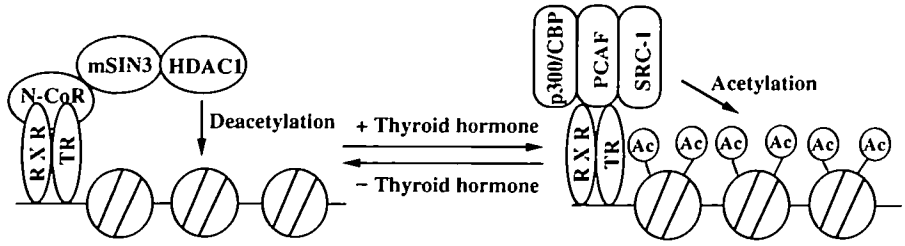

Fig. 1. **Ligand-dependent targeting of HAT or HDAC by thyroid hormone receptor.** In the absence of thyroid hormone, thyroid hormone receptor (heterodimer of RXR and TR) interacts with co-repressor N-CoR that recruits HDAC1 through mSIN3. HDAC1 deacetylates chromatin and represses transcription (*31, 32*). Upon binding of thyroid hormone, the receptor undergoes a conformational change to form a complex with co-activators p300/CBP, PCAF, and SRC-1. These co-activators acetylate chromatin, that leads to transcriptional activation (*22, 23, 25, 26*).

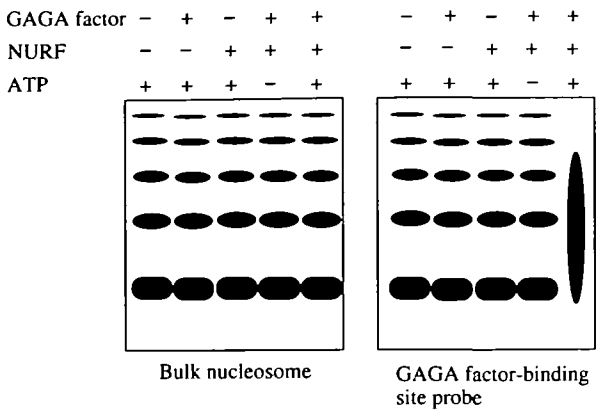

Fig. 2. **Assay for GAGA factor-dependent chromatin remodeling activity of NURF.** When chromatin DNA carrying a GAGA factor-binding site is incubated with GAGA factor and NURF in the presence of ATP and then partially digested with micrococcal nuclease, bulk DNA gives a typical nucleosome ladder. By contrast, Southern blot analysis using the GAGA factor binding site probe shows a smearing of the nucleosome ladder around the binding site (*40, 41*).

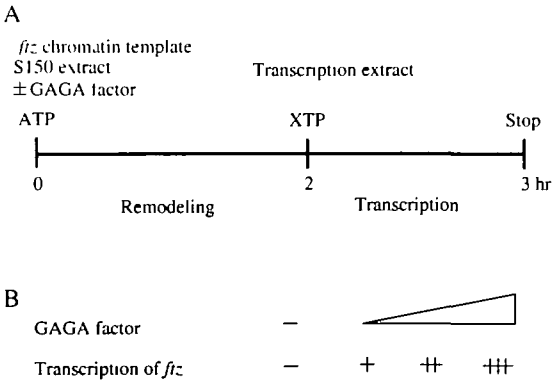

Fig. 3. **Transcriptional activation of *ftz* chromatin template by preincubation with GAGA factor and S150 extract.** Shown are schematic diagrams of a transcription experiment (A) and the results (B) (*42*).

Chromatin remodeling as a prelude to transcription

As described above, GAGA factor can target the chromatin remodeling with NURF in the *Drosophila* embryonic S150 extract. The promoter region of the *Drosophila fushi tarazu* (*ftz*) gene carries several binding sites for GAGA factor. Both the GAGA factor-binding sites and GAGA factor *per se* are necessary for the proper expression of *ftz in vivo*. We observed transcriptional activation of *ftz* when a pre-assembled chromatin template was incubated with GAGA factor and the S150 extract as schematically shown in Fig. 3 (*42*). The chromatin structure within the *ftz* promoter was specifically disrupted by incubation of the pre-assembled chromatin with GAGA factor and the S150 extract. Both the transcriptional activation and the chromatin disruption were blocked by an antiserum against ISWI or by base substitutions in the GAGA factor-binding sites in the *ftz* promoter region. These results indicate that GAGA factor- and ISWI-mediated disruption of the chromatin structure within the promoter region of *ftz* activates

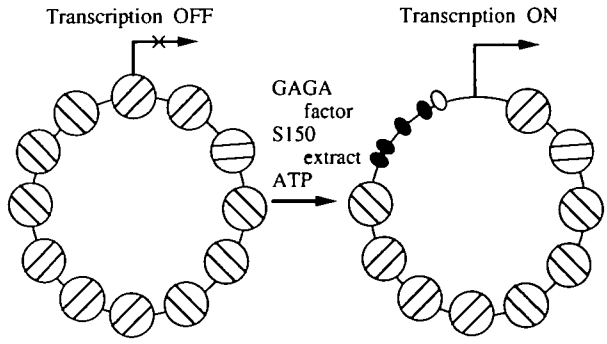

Fig. 4. **Transcriptional activation of *ftz* by GAGA factor-dependent chromatin remodeling (*42*).** The open and filled ovals represent the TATA element and four GAGA factor-binding sites on the *ftz* promoter, respectively.

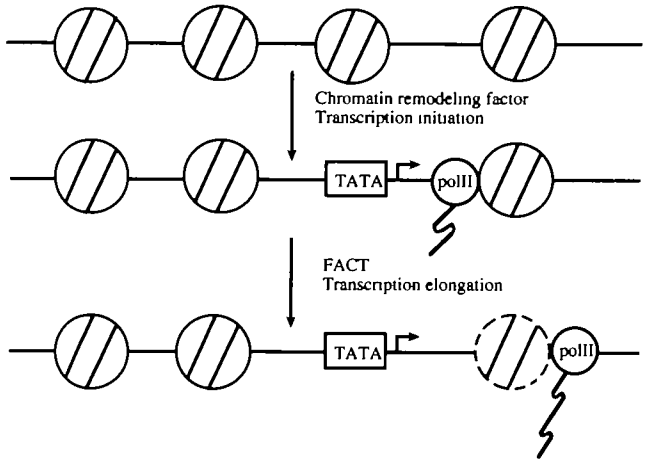

Fig. 5. **FACT is required for transcription elongation on a chromatin template.** polII and the arrows on DNA represent RNA polymerase II and the transcription initiation site, respectively. Modified from Ref. *44*.

transcription of the chromatin template (Fig. 4). This is the first demonstration of ISWI role in the transcriptional activation on chromatin template and has established a link between chromatin remodeling and transcriptional activation. Because GAGA factor does not directly interact with NURF, we are now searching for proteins that bridge between GAGA factor and NURF.

Mizuguchi *et al.* have shown that NURF is essential for GAL4-VP16-dependent transcriptional activation on chromatin templates (*43*). Interestingly, this transcription was stimulated by prior histone acetylation. Orphanides *et al.* have shown that hNURF-L (human NURF like) can remodel nucleosomes at a promoter to allow transcription initiation in an ATP and GAL4-VP16-dependent manner. They have also reported a new activity termed FACT (facilitates chromatin transcription) that allows RNA polymerase II to traverse through nucleosomes during elongation (Fig. 5) (*44*).

Although only a limited number of laboratories have succeeded in transcriptional activation from pre-assembled chromatin templates, these pioneer studies have shown interesting phenomena such as the FACT activity. In the

near future, many important findings will emerge from studies of transcription on chromatin templates.

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