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Activation at a distance: involvement of nucleoprotein complexes that remodel chromatin

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Summary

Large-scale sequencing of the human genome has confirmed that genes are often spread over several thousand base pairs on a chromosome. The cis-acting regulatory elements that control gene transcription can be located at a considerable distance from the respective gene. Significant advances in understanding the mechanism of eukaryotic gene transcription have revealed a great deal about how the RNA polymerase initiation complex assembles on the promoter region of genes. However, the fundamental problem of how distant genetic regulatory elements, such as enhancers and locus control regions, communicate with proximal elements to confer cell- and tissue-specific patterns of transcription remains largely unsolved. The principle focus of this chapter will be on the role of chromatin structure in transcriptional regulation by RNA polymerase II, with an emphasis on long-range activation.

I. Introduction to the problem

The levels of cellular proteins are regulated by changes in the concentration of the respective mRNAs. Changes in steady-state concentrations of mRNA result from altered synthesis or stability of the mRNA, with alterations in transcription being common. Eukaryotic gene transcription requires a core promoter element, which is the site of assembly of the preinitiation complex, consisting of "basal transcription factors" and RNA polymerase II (Orphanides et al., 1996). Multiple layers of regulation converge to modulate initiation complex assembly and thus alter gene activity.

Initial studies on the mechanism of transcription initiation suggested that preinitiation complex assembly occurred by binding of the basal factor TFIID to DNA, followed by the sequential binding of other basal factors (Buratowski et al., 1989). However, more recent evidence supports a model in which a preassembled RNA polymerase II holoenzyme is recruited to the DNA as a unit (Greenblatt, 1997). The holoenzyme is a multiprotein complex consisting of the catalytic subunit of RNA polymerase and basal factors including TFIID, TFIIB and others. Macromolecular interactions between basal factors and RNA polymerase provide an important level of regulation of preinitiation complex assembly. A second level of regulation is conferred by transcription factors, distinct from the basal factors, which bind to recognition sites on the promoter. These sequence-specific DNA binding proteins engage in protein-protein interactions with components of the holoenzyme, resulting in enhanced transcription initiation. Furthermore, preinitiation complex assembly can be regulated by transcription factors bound at distant regulatory sites such as enhancers and locus control regions (LCRs). Thus, communication between distal regulatory elements and promoters or "action at a distance" provides a third way to modulate preinitiation complex assembly. The factors functioning through distal sites can be identical to factors that bind directly to promoters.

A wealth of data support models of long-range activation involving disruption of chromatin structure, which increases access of the promoter to the transcription machinery. The process by which trans-acting factors induce chromatin transitions, such as the localized disruption of a nucleosome or the unfolding of a broad region of condensed chromatin, is poorly understood. In this chapter, the major emphasis will be on the functional role of chromatin transitions in transcription initiation. A simple model will be developed, which incorporates both looping and chromatin-disruption, to explain "action at a distance". We will begin to address the influences of chromatin on long-range activation by reviewing fundamental aspects of chromatin structure.

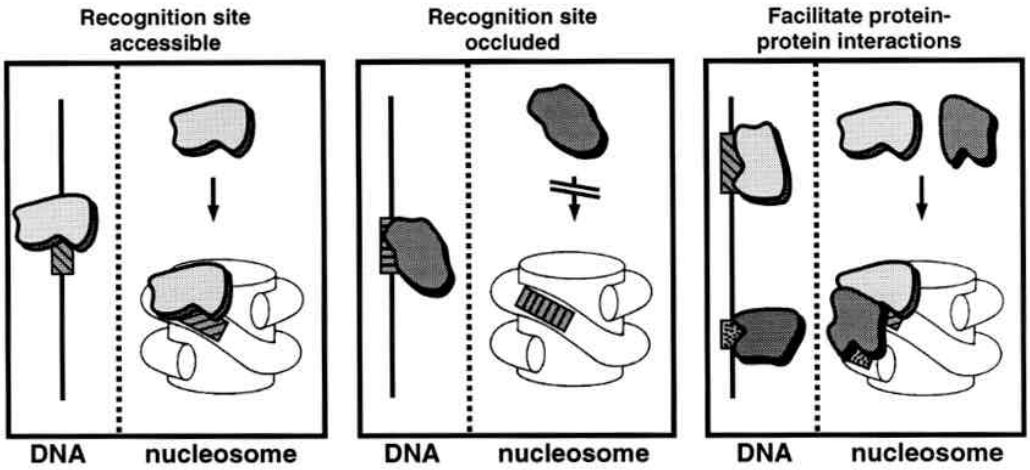

Figure 1. Functional consequences of nucleosomal organization of factor binding sites.

II. Influence of chromatin structure on transcription

At the simplest level, 146 base pairs of DNA duplex are wrapped around an octamer of histones (two H2A-H2B dimers and one H3-H4 tetramer) to form the *nucleosome*, the basic repeating unit of chromatin (Wolffe, 1995). The DNA between nucleosomes, the *linker DNA*, averages approximately fifty base pairs. The *linker histones* H1 and H5 interact asymmetrically with the nucleosome near the entry and exit points of the DNA (Hayes, 1996; Pruss et al., 1996). One role of the linker histones is to facilitate higher-order packaging of the 10 nm "beads on a string" nucleosomal filament to form the condensed 30 nm chromatin fiber. An additional level of packaging is believed to lead to the formation of loop domains, which, in turn are condensed into a chromosome.

The histone octamer can occlude recognition sites on DNA for certain sequence-specific DNA binding proteins that control DNA replication, transcription, and recombination (Hager et al., 1993) (**Fig. 1**). Consequently, the pattern of accessible sites on naked DNA and chromatin templates can differ considerably. However, the nucleosomal organization of DNA does not always negatively affect protein-DNA recognition, as certain proteins can form stable complexes with sites on the surface of a nucleosome (Adams and Workman, 1995; Archer et al., 1991; Li et al., 1994; Pina et al., 1990; Steger and Workman, 1997; Taylor et al., 1991) (**Fig. 1**). Thus, chromatin organization can also be permissive for transcription. The wrapping of DNA around a histone octamer can bring together distant regions of DNA, facilitating protein-protein interactions between bound regulatory factors (**Fig. 1**). In this scenario, the nucleosome would serve as an architectural element, enhancing the efficiency of transcription initiation (Quivy and Becker, 1996; Schild et al., 1993; Thomas and Elgin, 1988). By either allowing or precluding protein-DNA interactions, chromatin structure plays an active role in transcription initiation.

The specific positioning of the histone octamer on the DNA can be a critical determinant of recognition site accessibility (Wolffe, 1994). Two distinct types of positioning need to be considered. First, *rotational positioning* refers to the orientation of the DNA helix on the octamer core of the nucleosome. Rotational positioning refers to which face of the DNA is oriented toward or away from the octamer core. The prevalence of rotationally positioned nucleosomes in intact cells is unclear. Second, *translational positioning* refers to whether sequences reside within the core or the linker DNA. Preferred translational positioning has been observed in several systems and has important regulatory implications. However, examination of nucleosome positioning at base-pair resolution on the mouse mammary tumor virus (MMTV) promoter has revealed microheterogeneity of translational positions (Fragoso et al., 1995), despite the appearance of rigid translational constraints from lower resolution analysis. Further studies are required to determine whether microheterogeneity is common and functionally important. Nucleosome positioning can be modulated by both DNA binding proteins (Pazin et al., 1997; Roth et al., 1992) and the physicochemical properties of DNA (Muyldermans and Travers, 1994; Satchwell et al., 1986). Beyond the level of regulation conferred by nucleosome positioning, the association of linker histones with chromatin also has significant consequences for transcription (Shen and Gorovsky, 1996). As transcription factor binding sites are often positioned in linker regions (Bresnick et al., 1992; Thomas and Elgin, 1988), the linker histone association can modulate factor access. This could occur through a direct competitive mechanism or by facilitation of chromatin condensation (Graziano et al., 1988; Shen et al., 1995) and

occlusion of recognition sequences. Linker histones can also affect protein-DNA interactions by modulating mobility of the octamer core on DNA (Pennings et al., 1994; Ura et al., 1995). One consequence would be to ensure that certain recognition sites remain within the octamer core.

Condensation of the 10 nm nucleosomal filament into the 30 nm fiber may mask binding sites that remain accessible on nucleosomal DNA. It is difficult to envision a protein-DNA interaction, which requires precise contacts with the major or minor groove, occurring on the condensed 30 nm fiber. However, recent evidence suggests that the 30 nm fiber is a dynamic structure (Ericsson et al., 1990), which could allow a window of opportunity for a protein-DNA recognition event to occur. As assembly of the initiation complex on a promoter requires sequence-specific recognition of the TATA box or the initiator element on the DNA by basal transcription factors, it is not surprising that multiple levels of chromatin structure affect initiation. The principles of how chromatin influences transcription initiation are also likely to be relevant to elongation.

RNA polymerases can elongate through arrays of nucleosomes without completely displacing histones from the DNA (Ericsson et al., 1990). However, RNA polymerase pausing is enhanced on nucleosomal templates (Izban and Luse, 1991). Polymerase pausing is a functionally relevant step of the elongation process (Krumm et al., 1995; Rasmussen and Lis, 1995; Yankulov et al., 1994). Studies with a defined *in vitro* system using phage SP6 polymerase and reconstituted chromatin led to a detailed model for how RNA polymerase negotiates a nucleosomal template. As the polymerase approaches a nucleosome, the nucleosome is transferred to the DNA behind the polymerase via an intramolecular reaction (Clark and Felsenfeld, 1992; Studitsky et al., 1994). One can envision a variety of ways to modulate this transfer reaction to generate a highly regulated process. Additional studies are required to determine whether this mechanism is applicable to eukaryotic RNA polymerases transcribing chromosomal templates.

III. Regulatory elements that function over long distances on chromosomes

A. Enhancers

DNA regulatory elements that increase the transcriptional activity of promoters in a distance-and orientation-independent manner are called *enhancers* (Muller et al., 1988). The defining criterion of distance-independence is not entirely accurate, as the activity of certain enhancers can be significantly reduced when moved several thousand base pairs away from a promoter. In addition, the intervening DNA between an enhancer and promoter is not inert, and therefore, different DNA sequences differ in permissiveness for activation (Schreiber and Schaffner, 1989). Nevertheless, there is usually flexibility in the proximity of an enhancer to a promoter as well as enhancer location. Enhancers are normally located upstream or downstream of genes and even in introns. Several classes of enhancers exist, depending on the nature of the binding proteins.

A common feature of enhancers is the presence of clustered cis-acting elements that bind transcription factors. Differential distribution or modifications of these factors give rise to distinct classes of enhancers. Enhancers can have cell-specific, tissue-specific, or ubiquitous activity depending on the distribution of the factors. Furthermore, posttranslational modifications can modulate the activity of the enhancer binding proteins (Goldman et al., 1997; Hill and Treisman, 1995; Karin et al., 1997). Thus, enhancers can be constitutively active or induced by environmental cues such as hormones and nutrients.

B. Locus control regions

A second class of positive-acting genetic elements exists that shares certain features with enhancers, but appears to have a distinct activity. These *locus control regions* (LCRs) confer copy number-dependent and position-independent expression of a linked gene when integrated into chromosomal DNA (Bresnick, 1997). When an exogenous gene is stably integrated into a chromosome, the activity of the gene is often activated or repressed, depending on the DNA sequences flanking the integration site (Wilson et al., 1990). Integration of the gene next to an enhancer can elevate promoter activity. By contrast, integration into condensed chromatin or near a silencer, a genetic element that represses transcription (Hanna-Rose and Hansen, 1996), can reduce promoter activity. By overcoming the integration site position effects, LCRs generate an autonomously regulated gene or gene cluster (Forrester et al., 1987; Grosveld et al., 1987). Copy-number dependence simply refers to the fact that the expression level of a gene bears a linear relationship with the number of integrated gene copies.

Analogous to enhancers, LCRs have a strong activating function (Tuan et al., 1989) and function over long distances on chromosomes. A distinction between LCRs and enhancers is that certain enhancers are incapable of supporting copy number-dependent and position-independent expression of integrated genes (Trudel and Costantini, 1987). It is unknown whether this reflects a distinct mechanism for LCRs and enhancers or if simply a maximal quantum of enhancer activity is required to overcome position effects, and the mechanism is identical.

C. Activation mechanisms of enhancers and LCRs

Three classes of mechanisms are commonly invoked to explain "action at a distance" by enhancers and LCRs - *looping*, *tracking*, and *chromatin disruption*. First, analogous to well-characterized prokaryotic systems, enhancer-bound transcription factors physically interact with factors that bind to promoters. As the intervening DNA between distal and proximal sites forms a loop (Mastrangelo et al., 1991; Su et al., 1991), this type of model is called the

looping or protein-protein interaction model (Schleif, 1992). Physical interactions between distal- and proximal-bound transcription factors increase the local concentration of activators at the promoter, resulting in recruitment of the holoenzyme to the promoter or, potentially, the stabilization of a preexisting complex. Both actions would enhance transcription initiation. Parameters that affect the efficiency of activation include DNA binding affinity, the affinity of the protein-protein interaction, and potentially factors that modulate DNA bending. It seems intuitive that the fundamental mechanism of transcription would be conserved, and therefore a looping mechanism would also be used in eukaryotes. However, the chromatin organization of the eukaryotic nucleus provides a formidable impediment to DNA looping, demanding additional regulatory mechanisms to contend with chromatin structure.

Tracking mechanisms assume that regulatory factors bound at distal sites relative to a promoter processively move or "track" toward the promoter (Ouhammouch et al., 1997). The upstream factors could recruit RNA polymerase at the distal site, and migrate toward the promoter in conjunction with the polymerase. On the other hand, the upstream factors could track toward the promoter and then recruit polymerase at the promoter.

Similar consequences of chromatin organization would be predicted for looping and tracking models, i.e., DNA recognition by regulatory factors would be modulated. Chromatin would likely affect tracking in an analogous manner to its effects on transcriptional elongation. As discussed in detail below, the remodeling of chromatin by multiprotein complexes is a critical step in eukaryotic transcriptional regulation. It is easy to envision how regulatory factors could alter local histone-DNA contacts, leading to nucleosome disruption. However, it is more difficult to conceptualize how the chromatin structure of an entire domain could be modulated by LCRs.

IV. Long-range activation by locus control regions - a role for multiprotein complexes that remodel chromatin?

Since the initial description of the β-globin LCR (Forrester et al., 1987; Grosveld et al., 1987), a variety of genes have been reported to have LCRs (Carson and Wiles, 1993; Chauveau and Cogne, 1996; Dang and Taylor, 1996; Ess et al., 1995; Jones et al., 1995; LadekjaerMikkelsen et al., 1996; Lang et al., 1991; Madisen and Groudine, 1994; Montoliu et al., 1996; Talbot et al., 1994). Thus, it appears that inclusion of an LCR within a chromosomal domain may be a common means of establishing and/or maintaining an active chromatin structure. We expect that many more LCRs will be identified as the expression of more genes is analyzed *in vivo*. The transient transfection assay, which is often used to characterize promoter and enhancer activity, would not be expected to reveal an LCR requirement. The activation mechanism of LCRs involves decondensation of chromatin structure, and transiently transfected DNA is neither replicating nor assembled into organized chromatin. Although several LCRs have been described, much of what is known about the activation mechanism of LCRs is derived from the β-globin system.

A. Structure and function of the β-globin LCR

The human β-globin genes ε, Gγ, Aγ, δ, and β exist in a cluster on chromosome 11 (**Fig. 2**) and are differentially expressed in an erythroid-specific manner during development (Baron, 1997).

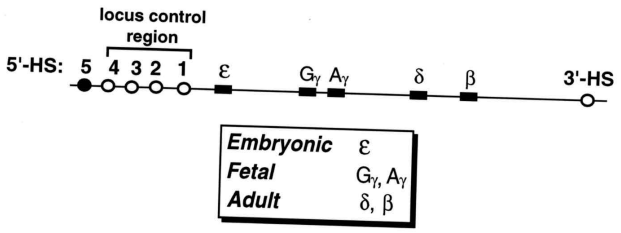

Figure 2. Organization of the human β-globin gene domain.

All of the genes share a common regulatory element, the β-globin LCR, which consists of four erythroid-specific DNaseI hypersensitive sites (HS1 - HS4), at the 5' end of the locus (Forrester et al., 1986; Tuan et al., 1985). HSs are regions of chromatin, typically encompassing about 200 base pairs of DNA, which are characterized by strong susceptibility to nuclease cleavage. This structural discontinuity of the chromatin fiber is a hallmark of a nucleoprotein complex (Becker, 1994).

The β-globin LCR is crucial for establishing an erythroid-specific chromosomal domain. Thus, the activation property of the LCR can be shared among multiple genes on a chromosome (Bresnick and Felsenfeld, 1994; Furukawa et al., 1994; Milot et al., 1996). However, the LCR does not appear to be involved in determining which globin gene is active at a particular stage of development. Evidence for lack of involvement of the LCR in globin gene switching comes from studies in transgenic mice whereby normal switching occurs with transgenes lacking the LCR (Starck et al., 1994). Despite this compelling observation, the neutral role of the LCR in globin gene switching remains controversial (Engel, 1993), as most transgenes lacking an LCR are subject to position effects. There is no question, however, that the LCR induces a decondensation of the chromatin structure of the β-globin locus, which is required for generation of a transcriptionally-competent β-globin domain.

The physiological significance of the β-globin LCR is illustrated by a human genetic disease, Hispanic thalassemia. This disease is characterized by deletion of a portion of the LCR, resulting in formation of condensed chromatin throughout the locus and silencing of the globin genes

(Forrester et al., 1990). The analysis of the global activity of the LCR to decondense chromatin has been complicated by the fact that no single factor mediates this activity (Caterina et al., 1994).

B. Protein components of the β-globin LCR

Each of the HSs contains multiple recognition sequences for both ubiquitous and erythroid-specific transcription factors (Bresnick and Felsenfeld, 1993; Caterina et al., 1991; Elnitski et al., 1997; Lam and Bresnick, 1996; Philipsen et al., 1993; Talbot et al., 1990; Yant et al., 1995). Considering that the HSs only exist in erythroid cells, one would expect the erythroid-specific factors to be crucial for formation and/or maintenance of the HSs. In this regard, the requirements for formation of HS4 have been studied (Stamatoyannopoulos et al., 1995). The recognition sites for two erythroid-specific factors, GATA1 and NF-E2, were important for formation of HS4. By contrast, a site that binds several ubiquitous factors (CACCC/Sp1) was not important. However, this does not exclude a role for ubiquitous transcription factors in LCR function, as these proteins could modulate the activity of the LCR, rather than being necessary for formation of a stable complex with chromatin. A common thread among the tissue-specific binding proteins of the LCR (GATA1, NF-E2, and TAL1) is that they are critical for hematopoiesis (Orkin, 1996).

In vitro protein-DNA interaction studies reveal whether a factor binds with specificity and high affinity to a recognition site. However, not all high-affinity interactions are physiologically relevant. In certain cases, without requisite posttranslational modifications or accessory factors, a factor may bind to a site with low affinity *in vitro*, in contrast to the intact cell. Of course, consideration of whether a site is conserved throughout evolution is suggestive of functional significance (Gumucio et al., 1992). The HSs of the LCR are each characterized by multiple conserved recognition sequences. *In vivo* footprinting studies support the notion that numerous binding sites within a single HS are occupied simultaneously by factors (Ikuta and Kan, 1991; Reddy et al., 1994; Strauss et al., 1992), suggesting that they function as an integrated complex.

The identification of proteins that function through cis-acting elements is often more complex than deduction based on the DNA sequence. More often than not, multiple factors interact with an identical or highly-related recognition sequence. Even if one can conclude that a site is important based on evolutionary considerations, the functional factor remains unknown. The NF-E2 binding site within HS2 is a good example of this scenario, as multiple proteins bind tightly to this sequence [NF-E2 (Andrews et al., 1993), AP1 (Lee et al., 1987), NRF1 (Caterina et al., 1994; Chan et al., 1993), and NRF2 (Moi et al., 1994)]. Complex methodologies based on immunoprecipitating nucleoprotein complexes with specific factor antibodies have the potential to determine which factor is bound to a site in intact cells (Orlando, 1997; Boyd and Farnham, 1997; Bresnick et al., 1992).

Another enigmatic issue is why the LCR consists of four distinct HSs, rather than a single cluster of cis-acting elements. Multiple HSs are required for the long-range activation property of the LCR (Bresnick and Tze, 1997; Bungert et al., 1995). Individual HSs, such as HS2 and HS3, can strongly activate transcription when positioned near a promoter but are incompetent for long-range activation (Bresnick and Tze, 1997). In addition, the ability of the LCR to confer position-independent gene expression appears to require multiple HSs. We postulated that multiple HSs may be required to form a stable nucleoprotein structure that reassembles with high fidelity after each round of DNA replication (Bresnick, 1997; Bresnick and Tze, 1997). An alternative possibility is that different sites recruit distinct coactivators, which could function synergistically.

We also postulated that chromatin modifying enzymes could be recruited through protein-protein interactions with LCR-bound factors (**Fig. 3**). These enzymes could mediate the long-range effects of the LCR on chromatin structure and transcription (Bresnick, 1997; Bresnick and Tze, 1997). It was recently shown that the p45 subunit of the heterodimeric factor NF-E2 physically interacts with the transcriptional coactivator, CBP (Cheng et al., 1997) (**Fig. 4**), which is a histone acetyltransferase (HAT) (Ogryzko et al., 1996). CBP, in turn, physically interacts with another HAT, PCAF (Yang et al., 1996). PCAF is present in a large macromolecular complex in human K562 erythroleukemia cells (Forsberg et al., 1997), in which the LCR is active. An understanding of long-range activation by the LCR requires knowledge of the nuclear machinery that mediate chromatin structure transitions.

V. Regulatory complexes that unfold chromatin

A. Nuclear signaling enzymes that acetylate histones

A common posttranslational modification of core histones is the acetylation of conserved lysine residues (Wade et al., 1997). The amino terminal "tails" of the core histones are believed to physically interact via electrostatic forces with the negatively charged phosphodiester backbone of DNA (Lee and Hayes, 1997). Several conserved lysine residues within the tails are subject to acetylation on their epsilon amino group (Gershey et al., 1968). Neutralization of the positive charge of the lysine would be expected to reduce the affinity of the tail for the DNA backbone, thus increasing the accessibility of DNA sequences within the nucleosome. Consistent with this idea, histone acetylation can enhance the binding of transcription factors to nucleosomal recognition sites (Lee et al., 1993; Vettese-Dadey et al., 1996). Genetic studies have verified the

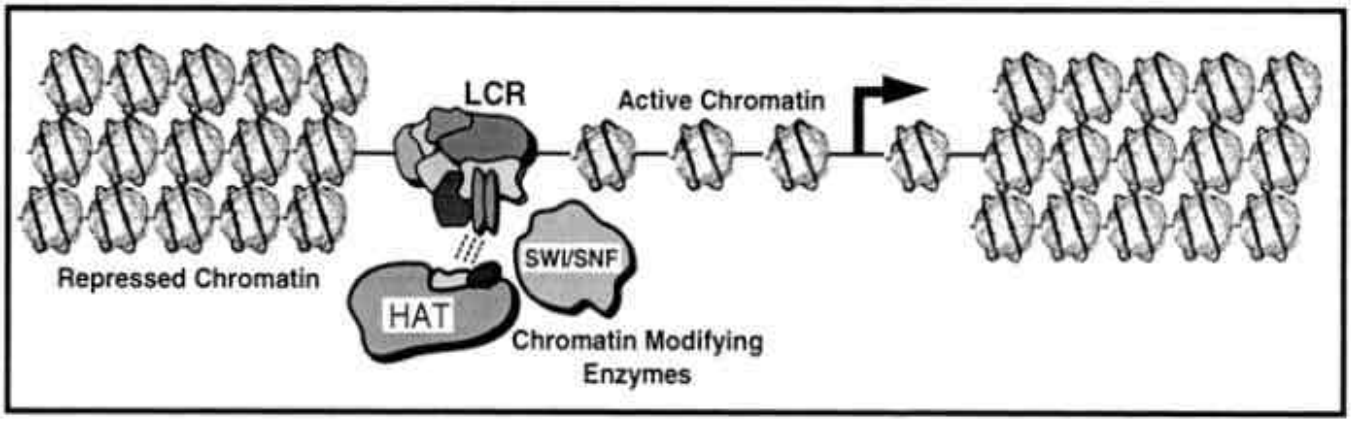

Figure 3. Model of chromatin modifying enzyme involvement in domain opening by the β-globin locus control region.

importance of the lysine residues that are acetylated (Durrin et al., 1991). The acetylation reaction is carried out by a class of nuclear enzymes termed HATs, which mediate chromatin remodeling (Wolffe, 1995).

At least one HAT, GCN5, is conserved from yeast to man (Candau et al., 1996), emphasizing the universal importance of histone acetylation. GCN5 exists in a large macromolecular complex in yeast (Grant et al., 1997; Marcus et al., 1994; Saleh et al., 1997) and human (Forsberg et al., 1997) cells. A paradoxical question is how do HATs regulate specific gene expression when they can acetylate histones globally? The answer is to employ a targeting mechanism to recruit HATs to specific genetic loci.

A seminal discovery that shed light on the specificity of histone acetylation was recently made by David Allis and colleagues. A *Tetrahymena* HAT was cloned, revealing that it was homologous to a yeast transcriptional coactivator, GCN5 (Brownell et al., 1996). Once it was determined that GCN5 had HAT activity, the picture rapidly unfolded. It was known that GCN5 was necessary for transcriptional activation by the transcription factor GCN4 (Georgakopoulos and Thireos, 1992). As GCN4 physically interacts with GCN5, a simple model emerged in which GCN4 binds to DNA and recruits GCN5 through a specific protein-protein interaction. Thus, a solution to the problem of how HATs are targeted is that protein-protein interactions between the HAT and DNA binding proteins mediate gene-specific recruitment. Many unsolved issues remain, such as once the HAT is recruited, does it modify chromatin by processively moving on the chromosomal template? What are the mechanisms that regulate the extent of the chromatin modification and terminate the activating signal? How can a limited number of HATs engage in protein-protein interactions with diverse DNA binding proteins? Does a common protein domain mediate interactions with HATs, or alternatively, do HATs have a moldable domain, analogous to chaperonins, which can interact with structurally diverse proteins? While the answers to these questions are unknown, it is clear that several HATs exist, with potentially distinct functions.

A human GCN5-related HAT, PCAF, was cloned, which does not appear to have a yeast homolog (Yang et al., 1996). PCAF shares a common carboxy-terminal domain with GCN5, but has a unique amino terminus. Analogous to GCN5, PCAF also exists in a large macromolecular complex (Forsberg et al., 1997; Yang et al., 1996; Grant et al., 1997). Two other proteins, CBP (Yang et al., 1996) and ACTR (Chen et al., 1997), directly interact with PCAF. Surprisingly, both CBP and ACTR have intrinsic HAT activity. This raises yet another question, i.e., why would multiple HATs be present in the same heteromeric complex? This could be explained by a model in which individual HATs have unique specificities and coordinately function to generate a specific pattern of acetylated histones in chromatin. Another possibility is that HATs might have substrates other than the core histones. In this regard, acetylation of the DNA binding tumor suppressor protein p53 recently was shown to stimulate sequence-specific DNA binding by p53 (Gu and Roeder, 1997). Further studies on HAT structure and function should reveal principles for how posttranslation modifications of chromosomal proteins regulate transcription. The identification of protein-protein interactions between HATs and nuclear proteins will likely provide important clues to their functional roles. Indeed, as indicated above, CBP physically interacts with the p45 subunit of NF-E2, which is a key regulator of the β-globin LCR.

B. Involvement of histone acetyltransferases in LCR function.

Considering that NF-E2 can interact with CBP, and CBP is present in a complex with PCAF (**Fig. 4**), it is reasonable to propose that the CBP-PCAF HAT complex is recruited to the β-globin locus by the β-globin LCR. The implications of this are significant, as HATs could mediate the long-range chromatin decondensing activity of the LCR.

A relevant observation is that analysis of the acetylated state of histones of the chicken β-globin locus revealed acetylated histones distributed throughout the locus (Hebbes et al., 1994). Thus, sites of acetylation were not restricted to regulatory sequences, such as promoters or enhancers, or coding regions of the globin genes. This observation is consistent with a role for acetylation in establishment and/or maintenance of active domains, rather than exclusive functions to determine promoter accessibility or to regulate transcriptional elongation. Besides HATs, other multiprotein chromatin remodeling complexes could also be important in long-range activation.

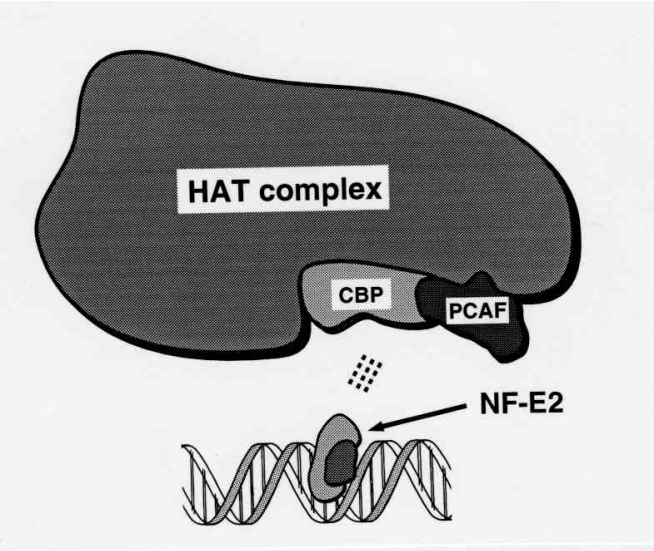

Figure 4. Recruitement of CBP- and PCAF-containing HAT complex by NF-E2.

C. Other chromatin remodeling complexes - SWI/SNF, NURF, and RSC

Genetic studies in yeast revealed a series of proteins (SWI - yeast mating type switching)/SNF - sucrose nonfermenting) that were critical for transcription of various genes. The SWI/SNF proteins (Winston and Carlson, 1992) were distinct from transcription factors and components of RNA polymerase. The proteins form a multimeric complex (Cairns et al., 1994; Peterson et al., 1994) and appear to function by modulating chromatin structure (Kruger et al., 1995) to increase the accessibility of cis-acting elements. The SWI/SNF complex isolated from yeast and human cells consists of at least twelve stably associated proteins and is estimated to be approximately two megadaltons (Cairns et al., 1994; Peterson et al., 1994; Wang et al., 1996). SWI/SNF may be physically associated with the RNA polymerase II holoenzyme (Wilson et al., 1996), although this remains debatable (Cairns et al., 1996). *In vitro* protein-DNA interaction studies have shown that SWI/SNF facilitates factor binding to nucleosomal templates (Cote et al., 1994; Imbalzano et al., 1994; Kwon et al., 1994; Owen-Hughes et al., 1996), suggesting that SWI/SNF disrupts the association of histones with DNA; the fate of the histones is unclear.

At least one component of SWI/SNF, SWI2/SNF2, has homology with DNA helicases (Khavari et al., 1993), which unravel double-stranded DNA (Lohman and Bjornson, 1996). The helicase homology may be an important clue to how SWI/SNF modulates chromatin structure and transcription. DNA helicases move processively on DNA in a way that is fueled by ATP hydrolysis (Lohman and Bjornson, 1996). SWI2/SNF2 hydrolyzes ATP similar to helicases. It is unknown, however, whether nucleotide hydrolysis confers upon SWI/SNF the ability to track on DNA. The nucleotide hydrolyzing activity is necessary to facilitate factor binding, but the permissive chromatin transition induced by SWI/SNF is stable without further nucleotide hydrolysis (Imbalzano et al., 1996). The current data are consistent with the hypothesis that SWI/SNF can track on DNA and disrupt nucleosomes, thus promoting factor binding.

It seems reasonable to propose that SWI/SNF may play a role in decondensation of higher-order chromatin structure. A combination of histone modification by HATs and chromatin disruption by SWI/SNF would ensure that a domain resides in an uncondensed state and is thus transcriptionally-competent (**Fig. 3**). Despite major advances in understanding how HATs are targeted to a chromosomal template, studies on the targeting of SWI/SNF are in their infancy.

The recruitment of SWI/SNF to chromatin may occur through a protein-protein interaction mechanism analogous to HATs. It was recently shown that binding of the glucocorticoid receptor to DNA stimulates the nucleosome disruption activity of SWI/SNF (Ostlund Farrants et al., 1997). Another DNA-bound transactivator, NF1, did not affect SWI/SNF activity. Thus, SWI/SNF may mediate the well-characterized activity of the glucocorticoid receptor to disrupt chromatin (Hager et al., 1993) by a protein-protein interaction, recruitment mechanism.

In addition to targeting mechanisms based on protein-protein interactions, HATs and SWI/SNF may function coordinately, and SWI/SNF may recognize features of active chromatin induced by HATs. A related chromatin-remodeling complex in *Drosophila*, NURF (nucleosome remodeling factor) (Tsukiyama and Wu, 1995), was recently shown to interact with amino terminal tails of core histones (Georgel et al., 1997). NURF shares certain features with SWI/SNF, such as a polypeptide related to SWI2/SNF2 containing helicase homology and ATPase activity (Tsukiyama et al., 1995), the ability to disrupt chromatin structure and facilitation of factor binding. Thus, analogous to NURF, SWI/SNF may interact with histone tails, and this interaction could be modulated by acetylation.

As multiple HATs are present within a cell, it is of interest to ask whether multiple chromatin disrupting complexes like SWI/SNF exist. Yet another chromatin remodeling enzyme complex, RSC (remodels structure of chromatin), was recently isolated from yeast (Cairns et al.,

1996). RSC shares certain features with SWI/SNF, such as a component similar to SNF2/SWI2. However, differences between RSC and SWI/SNF include an approximately ten-fold greater abundance of RSC and a requirement of RSC for mitotic growth.

VI. Concluding remarks

A great deal of knowledge is emerging rapidly on how multiprotein complexes mediate chromatin transitions. Intuitively, it is easy to conceptualize how factors binding to a promoter lead to nucleosome disruption and enhance transcription by facilitating preinitiation complex assembly. A more challenging intellectual puzzle, however, is to unravel the determinants of how distant regulatory elements function. Is long-range activity mediated by the same chromatin-remodeling enzymes necessary for nucleosome disruption on promoters? As identical transcription factors can function through upstream elements and promoters, the chromatin remodeling machinery may be identical. In addition, the requirement for multiple polypeptides within the regulatory enzyme complexes needs to be explained. These components could enhance the functionality of the complexes by modulating enzymatic activity, targeting the complex to genes, or regulating the subcellular localization or stability of the active component.

Based on evolutionary conservation of the transcription machinery, it is likely that looping is a fundamental step in eukaryotic transcripional activation. To incorporate the additional level of regulation demanded by chromatin, one can propose a bimodal activation mechanism. Disruption of chromatin structure would establish a transcriptionally-competent domain or local chromosomal region, in which promoters are accessible to the transcription machinery. Protein-protein interactions and DNA looping would stabilize and/or facilitate assembly of a bona fide preinitiation complex. If the promoter is rendered accessible by chromatin disruption, the initiation complex could assemble without protein-protein interactions with upstream activators, albeit at a low frequency. The assumption is that assembly of the preinitiation complex occurs in a stochastic or all-or-none fashion. The level of gene transcription in a cell population would therefore depend on the number of engaged initiation complexes. Analysis of gene expression in single cells has yielded data consistent with a stochastic activation mechanism (Fiering et al., 1990; Ko et al., 1990; Walters et al., 1995; Weintraub, 1988).

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