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Review Article

Chromatin Assembly and Remodeling

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Chromatin is highly dynamic structure that plays essential role in regulating nuclear processes that utilize the DNA template including DNA repair,replication, transcription and recombination.Thus,the mechanisms by which chromatin structures are assembled and modified are questions of broad interest. Chromatin structure is linked to transcriptional regulation, and recent studies show how chromatin is altered so as to facilitate transcription. In addition, modification of chromatin structure is an important regulatory mechanism. Here I review the mechanism of chromatin assembly in vitro and the changes of chromatin structure related to transcriptional activation.

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Abbreviations: NAP-1, nucleosome assembly protein-1; ACF, for ATP-utilizing chromatin assembly and remodeling factor; CAF-1, chromatin assembly factor 1; HAT, histone acetyltransferase; NLP, nucleoplasmin-like protein; pol II, polymerase II;

Introduction to chromatin

The eukaryotic genome is packaged into nucleoprotein structure known as chromatin. When chromatin is digested with micrococcal nuclease in vitro, the resultant DNA has a characteristic ladder of fragments that derive from multiple units of the nucleoprotein complex. If the nuclease digestion proceeds further, the core particle, the basic structural unit of chromatin, is generated. The nucleosome core particle consists of 145 base pairs (bp) of DNA wrapped around an octamer of the core histones H2A, H2B, H3, and H4[1-7]. Crystallographic studies have shown that an H3-H4 tetramer occupies the central region of the nucleosome, and the H2A-H2B dimers bind to the peripheral region[8, 9]. In solution of physiological ionic strength, H2A and H2B form stable dimers, whereas H3 and H4 form tetramers. The core histone octamer, made of a central H3-H4 tetramer and two lateral H2A-H2B dimers, is stable only when in a complex with DNA in the nucleosome or at high ionic concentration[10, 11]. The instability of the histone octamer at physiological salt concentrations in the absence of DNA and the basic charge of all four core histones suggest two things. One is the necessity of the acidic molecules that stabilize core histones. The other is multistep histone octamer assembly on the DNA results in the nucleosome. Between these nucleosomes there is a variable length of linker DNA that is bound by the linker histones such as H1[12, 13]. The repeating nucleosome cores, namely the "beads on a string" structure[14, 15], further assemble into higher-order structures that are stabilized by the linker histone H1.

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Histone transfer

Previously biochemical studies have led to the identification of proteins that mediate the reconstitution of core histones into nucleosomes. Most of these factors are core histone-binding proteins called core histone chaperones. Some of these histone chaperones, such as nucleoplasmin[16] and nucleosome assembly protein-1 (NAP-1)[17, 18], exhibit a preference for binding to histones H2A and H2B relative to histones H3 and H4. In addition, NAP-1 is bound to H2A and H2B in whole-cell extracts[18, 19] and moves from the nucleus in early S phase, when much nucleosome assembly is taking place, to the cytoplasm in G2[18]. NAP-1's action has not yet been shown to depend on DNA replication or histone acetylation, although some nucleosome assembly probably occurs in vivo even without DNA replication. To varying extents, these core histone chaperones can mediate the deposition of histones onto DNA via an ATP-independent process that yields

chromatin consisting of randomly distributed nucleosomes. This ATP-independent histone deposition process can also be carried out with salts, such as 2 M NaCl, and polyanions, such as polyglutamate or RNA, which appear to interact with the histones and prevent their nonspecific aggregation with DNA[10, 11, 20-22]. This random deposition process, however, is generally inefficient and does not yield periodic arrays of nucleosomes, as is generally seen in native chromatin. The biochemical analysis of DNA replication and chromatin assembly led to the identification and characterization of the protein chromatin assembly factor 1 (CAF-1) from human cells[23, 24] and *Drosophila melanogaster* embryos[25]. hCAF-1 is a complex of three subunits, p150, p60, and p48[24], and in cells they can form a larger complex (CAC) that contains specific acetylated forms of histones H3 and H4[24]. In addition to CAF-1, the biochemical analysis of DNA replication-dependent chromatin assembly has led to identification of replication coupling assembly factor (RCAF) that facilitates the assembly of nucleosomes onto newly replicated DNA in vitro. RCAF comprises the *Drosophila* homologue of anti-silencing function 1 protein and histones H3 and H4[26].

Multistep histones transfer onto DNA

It has been proven that chromatin assembly is a staged process. Newly synthesized DNA is enriched with newly synthesized histones H3 and H4, whereas newly synthesized H2A and H2B associate with chromatin that has properties similar to those of bulk nonreplicating chromatin. It was concluded that newly synthesized histones associate with newly synthesized DNA in a sequential order: histones H3 and H4 are deposited first and then histones H2A and H2B. From crystallographic studies of the structure of the nucleosome, it is clear that histones H3 and H4 need to be deposited prior to the incorporation of H2A and H2B[8, 9]. The histone octamer is unstable under physiological ionic conditions in the absence of DNA, dissociating into the H3-H4 tetramer and two H2A-H2B dimmers[10].

An important issue is how chromatin assembly machinery ensures this ordered deposition of core histones onto DNA. Pfaffle and Jackson[27] showed that if the plasmid is negatively supercoiled, nucleosome transfer occurs very rapidly, even when using polyglutamic acid as a histone chaperone. The manner by which core histones bind to NAP-1 and DNA demonstrates how stepwise transfer occurs using the supercoiled plasmid, which facilitated core histone transfer only via a histone chaperone[28]. NAP-1 exhibits a greater affinity for histones H2A-H2B than does naked DNA, but in the presence of H3-H4, H2A-H2B dimers are transferred from NAP-1 to the plasmid templates. Because the H2A-H2B dimer was not transferred from NAP-1 to the DNA in the absence of H3-H4, these observations underscore the importance of a high affinity between H2A-H2B and NAP-1 for ordered transfer of core histones onto DNA. Thus, for core histones H2A-H2B, the order of the affinity is: (1) H2A-H2B and a subnucleosome consisting of DNA and H3-H4; (2) H2A-H2B and NAP-1; and (3) H2A-H2B and DNA. For H3-H4, the order of the affinity is: (1) H3-H4 and DNA; and (2) H3-H4 and NAP-1. These affinities explain why H3-H4 transfer occurs prior to H2A-H2B transfer. In contrast, *Xenopus* nucleoplasmin transfers H2A-H2B to DNA even in the absence of H3-H4[29]. In Drosophila dNAP-1, which has a higher affinity to core histones than dNLP (nucleoplasmin-like protein), works better than dNLP in the assembly reaction[30]. These reports strengthen the importance of NAP-1 as a H2A and H2B chaperone in chromatin assembly.

Because CAF-1 and RCAF are shown to bind newly

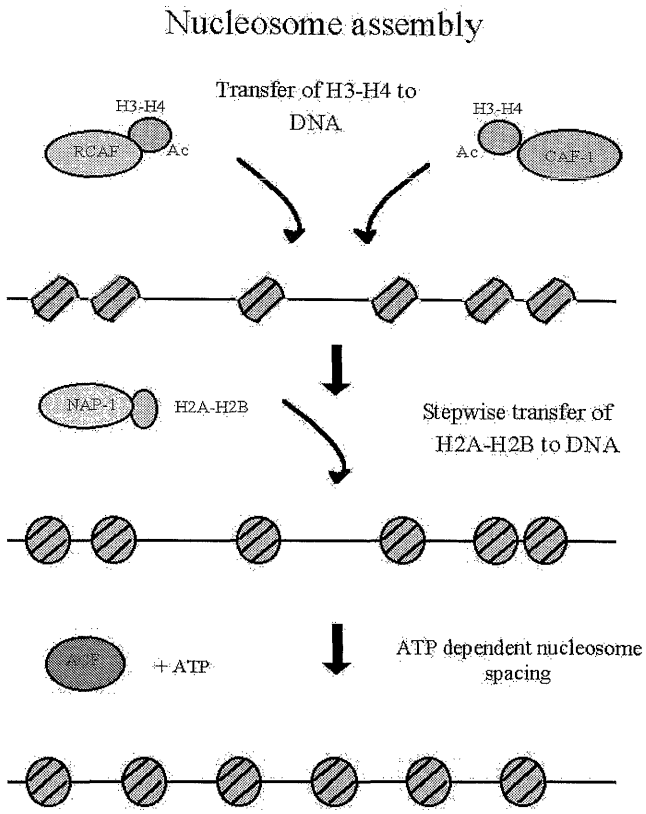

Figure 1. A model for nucleosome assembly. Chromatin assembly consists of an initial stepwise core histone transfer by a core histone chaperone (NAP-1) and subsequent chromatin maturation by ACF.

synthesized H3 and H4 and mediate replication-dependent chromatin assembly, it is reasonable to think that after DNA replication histones H3 and H4 are deposited first by chaperon such as CAF-1 or RCAF and then histones H2A and H2B by NAP-1 (Fig. 1).

ATP is required to generate periodic nucleosome arrays in vitro

Histone chaperones are not sufficient to generate regular arrays of nucleosomes with 180 -200 bp spacing in vitro. Iteansd, histone chaperones lead to the assembly of irregularly spaced or closely spaced nucleosome arrays in vitro. Presumably, factors in addition to histone chaperones are required in order to generate the physiologically spaced arrays of nucleosomes that are seen in the cell. Indeed, it has long been known that chromatin assembly in crude extracts requires ATP hydrolysis in order to generate regular arrays of physiologically spaced nucleosomes. After partial digestion of bulk genomic chromatin by micrococcal nuclease, which exhibits a preference for cutting in the linker DNA regions, a stepwise ladder results, indicating that genomic DNA is packaged into a regularly spaced nucleosomes[13]. Nucleosomal periodicity is likely a consequence of histone-DNA and internucleosomal histone-histone interactions[31]. However, these interactions alone do not seem to suffice for formation of regularly spaced nucleosomal arrays. In vitro study using salt dialysis to deposit a sufficient amount of nucleosomes onto plasmid DNA results in a close packing arrangement of nucleosomes with a periodicity of 145 bp with no linker DNA. In contrast, in the genome nucleosomal repeat length is typically 180-200 bp in the presence of linker histones[32].

The assembly of regularly spaced nucleosome arrays is an ATP-dependent process, which was initially observed in an extract derived from *Xenopus* oocytes[33]. In studies of chromatin assembly factors from *Drosophila* embryos, the ATP-utilizing component of the chromatin assembly machinery (termed ATP-utilizing chromatin assembly and remodeling factor, ACF) was identified and purified[34, 35]. Purified ACF acts catalytically (at approximately one ACF per 90 core histone octamers) in the spacing of the nucleosome to yield periodic nucleosome arrays in an ATP-dependent process. This chromatin assembly reaction, which can be carried out in a purified reconstituted system, requires ACF, core histones, DNA, ATP, and a histone chaperone (NAP-1 and hCAF-1 were each found to function as histone chaperones in conjunction with ACF). The most purified preparations of ACF were observed to contain ISWI protei n[35]. ISWI protein was identified as the SWI2/SNF2 superfamily[36]. Further purification of native ACF from *Drosophila* embryos led to the isolation of ACF consisting of Acf1 (both p170 and p185 forms) and ISWI subunits[37]. Acf1 did not, however, copurify with components of NURF[38], another chromatin remodeling complexes from *Drosophila* that similarly contain an ISWI subunit. Studies of purified recombinant ACF revealed that the Acf1 and ISWI subunits function synergistically in the ATP-dependent assembly of regularly spaced nucleosomal arrays[37]. CHRAC was also shown to have ATP-dependent activities related to the ordering of disordered nucleosomal arrays[39]. CHRAC was originally reported to contain topoisomerase II (topo II) as a subunit, but has since been found to be devoid of topo II; it does, however, contain Acf1 and two smaller (14- and 16-kDa) subunits[40]. Therefore, CHRAC is likely to be closely related to ACF. In addition to *Drosophila* ACF and CHRAC, human ACF, RSF (also an ISWI-containing factor), and yeast ISWI complexes are capable of converting randomly deposited chromatin into arrays of periodically spaced nucleosomes[41-43].

Nucleosome is mobilized in vitro

Native ACF and recombinant ACF comprised of ISWI and Acf1 can extend a closely packed nucleosomal repeat (~146 bp) made by salt dialysis to a more physiological repeat length of about 160 bp[28, 35]. In addition, recombinant ACF can mobilize nucleosomes and extend the repeat length from 148 bp to 160 bp gradually over time[28]. The other ISWI family of remodeling complexes, such as NURF and CHRAC, also exhibit the activity that stimulates nucleosome sliding, which is similar to the mobilization activity of ACF. In the reaction that mediates the ATP-facilitated assembly of chromatin, NAP-1 and ACF are essential for the formation of the regularly spaced nucleosomal arrays. Because NAP-1 is a core histone chaperone for nucleosome deposition, it is reasonable to think that the nucleosome mobilization activity of ACF plays a role in chromatin maturation. McQuibban et al.[44] reported that yNAP-1 can space nucleosomes without any ATP present. The major difference between chromatin made by yNAP-1 and that made by dNAP-1 and ACF is the repeat length of the nucleosomal array: 146 bp by yNAP-1 versus 160 bp by dNAP-1 and ACF. This difference supports the model that ACF plays a role in chromatin maturation by

mobilizing nucleosomes (Fig.1). In addition to the *Drosophila* ISWI-containing complex, the SWI/SNF ATP-dependent chromatin remodeling complex catalyses the redistribution of nucleosomes along DNA in cis, likely by sliding or tracking nucleosomes along DNA[45].

Chromatin remodeling

Chromatin remodeling factors are protein complexes consisting of between two and twelve diverse subunits. Chromatin remodeling is defined typically as a change in nucleosome structure as assessed by one of several different assays, usually involving digestion with nucleases. The assays include the loss of regularly repeating DNA ladders generated by micrococcal nuclease digestion and the loss of a characteristic 10-bp repeat of DNase I digestion in a rotationally positioned nucleosome[1, 2]. The packaging of DNA into chromatin has important consequences for the regulation of genes transcribed by RNA polymerase II (pol II). Nucleosomes act as general repressors of basal transcription, inhibiting transcriptional initiation and elongation by RNA pol II. The activation of transcriptionally repressed templates requires the actions of sequence-specific DNA-binding transcriptional activators, chromatin remodeling complexes, and histone acetyltransferases (HATs). As genes are transcribed by RNA pol II, the nucleosomal arrays positioned over promoters and transcribed regions are remodeled to counteract their repressive effect[46, 47]. Local changes in chromatin structure involve the disruption or alteration of nucleosomal arrays in the vicinity of the promoter and can be mediated by large multipolypeptide chromatin remodeling complexes[48-50]. Chromatin remodeling complexes use ATP to alter nucleosome positioning and structure[51]. All of the known chromatin remodeling complexes contain ATPase subunits that define three distinct families of complexes: SWI2/SNF2-like (e.g., SWI/SNF and RSC), ISWI-like (e.g., ACF, NURF, CHRAC, and RSF), and Mi-2-like (e.g., NURD). The SWI/SNF family of remodeling complexes catalyzes two distinct reactions: (1) nucleosome remodeling without the loss of nucleosomal components (i.e., histones); and (2) nucleosome transfe r[52, 53]. SWI/SNF and RSC have been shown to catalyze the stable alteration of nucleosome structures with mononucleosomal substrates. These remodeled nucleosomes retain all four core histones and the DNA, and in many cases can revert back slowly to the original state in the absence of continued remodeling activity. SWI/SNF also stimulates the transfer of histones from nucleosomes to nucleoplasmin in the presence of a transcriptional activator protein[52], whereas RSC stimulates the transfer of histones from chromatin to acceptor DNA[53]. The ISWI family of remodeling complexes (e.g., ACF, NURF, and CHRAC) exhibit a variety of assayable ATP-dependent activities related to nucleosome remodeling, such as the remodeling of local chromatin structure in the presence of a DNA-binding transcriptional activator protein[35, 38] and the induction of restriction endonuclease sensitivity on nucleosomal arrays[39]. In addition, ACF facilitates nuclear excision repair of ultraviolet-induced DNA damage[54]. Although the essential function of ATP-dependent remodeling factors still remain to be clarified, it interfaces with many biological phenomena including transcriptional activation and repair.

Chromatin fluidity and core histone acetylation

A most stimulating set of observations during the last two years documented the fact that nucleosome remodeling machines are able to induce the "sliding" of intact histone octamers to adjacent DNA segments in an ATP-consuming reaction. In addition diverse posttranslational modifications of the core histones occur, often on the tail domains. Among these, histone acetylation has been one of the most studied, in part due to the discovery of enzymes responsible for bringing about the steady-state balance of these modifications: HATs and histone deacetylase. The notion that histone acetylation facilitates transcription is strongly supported by the discovery that transcriptional regulatory proteins, such as GCN5, PCAF, p300, and CBP, possess intrinsic HAT activity[55-57]. The multiplicity of HATs identified suggests they may serve distinct functions. CBP and p300 are highly homologous coactivator proteins[58] that bind a number of sequence-specific transcriptional activators and have been suggested to be central integrators of transcriptional signals from various signal transduction pathways. Patterns of histone acetylation by the human coactivators p300 is well characterized within nucleosomal substrates. However, it is still yet to be clarified how these acetylases facilitate transcriptional activation. Recent studies showed that the binding of activator such as Gal4-VP16 to nucleosomal templates helps to increase the accessibility of nucleosomal histone tails to the p300HAT[59]. These results suggest that the VP16 activation domain of Gal4-VP16 is able to target p300 to nucleosomes via the Gal4 DNA-binding component. The binding of p53 to nucleosomal templates helps to increase the

accessibility of nucleosomal histone tails to the p300HAT, and thus stimulates transcription[60]. These observations are consistent with previous experiments showing that acidic activation domains can recruit HATs to promoters[61]; p300 functions as a coactivator of Gal4-VP16-mediated transcription[59]; and the HAT activity of p300 is required for full enhancement of Gal4-VP16-mediated transcription[59]. Prior activator-mediated, ATP-dependent chromatin remodeling is required for the efficient acetylation of nucleosomal core histones by p300.

The temporal relationship between activator-mediated chromatin remodeling and core histone acetylation by p300 is an interesting issue. It was clearly shown that activators and chromatin remodeling factors induce chromatin remodeling in the absence of histone acetylation; HATs such as p300 are recruited to the promoter by DNA-bound activators; and histone acetylation is important for a step subsequent to chromatin remodeling. These results are a good biochemical counterpart to the recent in vivo chromatin immunoprecipitation experiments of Cosma et al. and Krebs et al.[62, 63]. In these studies, analysis of the *Saccharomyces cerevisiae* HO promoter showed that the association of the SWI/SNF complex with the promoter is required for subsequent recruitment of histone acetylases (Gcn5p/SAGA complex) and increased acetylation of nucleosomal histones at the promoter[62, 63]. In other instances, however, histone acetylation can stabilize the binding of the SW1/SNF complex to promoter nucleosomes, suggesting that the function of HAT complexes may precede that of SW1/SNF during transcription activation[64]. Together, these studies raise the question of the role of histone acetylation in transcriptional regulation and chromatin remodeling.

Chromatin remodeling is considered to be any sort of detectable change in chromatin or mononucleosome structure, and chromatin remodeling assays are somewhat indirect and cannot discriminate changes such as nucleosome transfer, nucleosome sliding, or local changes in nucleosome structure. It is not well known what core histone acetylation does for chromatin remodeling. From biochemical assays, it is clear that ATP-dependent chromatin remodeling by chromatin remodeling complexes can occur in the absence of histone hyperacetylation[37, 42]. Thus, the role of core histone acetylation is an important issue. There was a report which suggests an answer to this question, namely that the role of histone acetylation is to alter the structure of nucleosomes (e.g., facilitate the loss of H2A-H2B dimers) that have been remodeled previously by the action of ATP-dependent chromatin remodeling complexes. This experiment indicated that NAP-1 acts as a histone acceptor after the activator-mediated acetylation of nucleosomal histones by p300. The presence of altered (i.e., H2A-H2B-depleted) nucleosomes at a transcriptionally active, chromatin-remodeled promoter may help to maintain an open chromatin structure conducive to multiple rounds of activated transcription (Fig. 2).

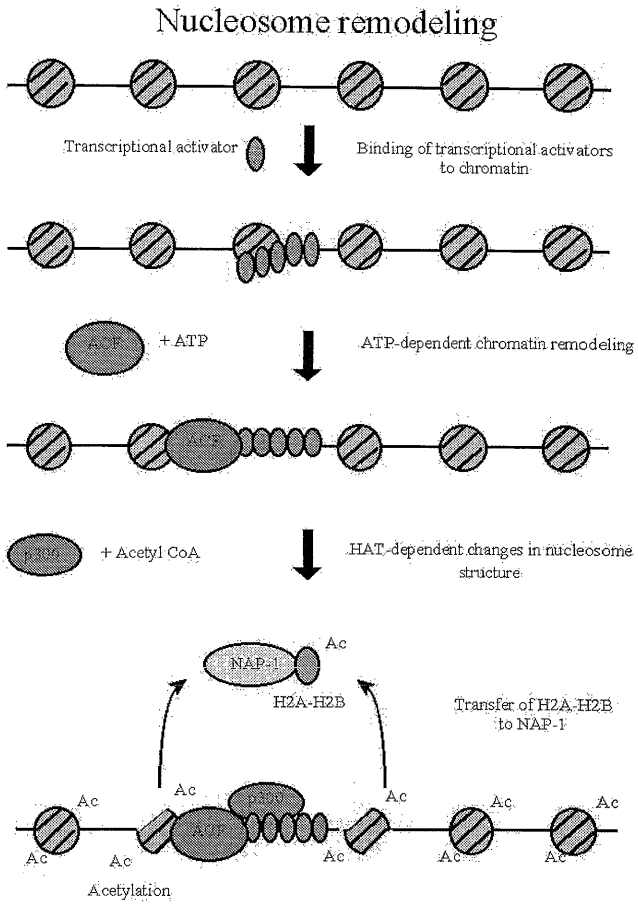

Figure 2. A model for histone transfer. ATP-dependent chromatin remodeling precedes histone acetylation by p300. Postremodeling histone acetylation facilitates the transfer of histone H2A-H2B dimers to a histone chaperone.

Conclusions and perspectives

It is clear that chromatin structure is dynamic and that structural changes in chromatin are highly regulated. Although H3 and H4 form part of the same nucleosomal structure as H2A and H2B, their stabilities in chromatin are clearly very different. Core histones H2A and H2B are exchanged more rapidly than H3 and H4, and some of this exchange appears to depend on continuing transcription[65]. It appears

that chromatin assembly and remodeling factors together with chromatin modifying enzymes such as HAT adjust chromatin fluidity.

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