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The Many Faces of Chromatin Remodeling: SWItching beyond Transcription

Minireview

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In the eukaryotic cell nucleus, hundreds of millions of base pairs of DNA are assembled into chromosomes. Chromosomes are dynamic entities that vary in structure throughout the cell cycle and serve as the substrate for many nuclear processes such as DNA replication, recombination, repair, and transcription. Chromatin, the central nucleoprotein filament of chromosomes, has many different forms, which range from interphase euchromatin and heterochromatin to condensed metaphase chromatin. The packaging of DNA into chromatin provides the cell with the means to compact and to store its nuclear DNA, but it also creates an impediment to the function of DNA binding factors. To counterbalance the repressive nature of chromatin, a variety of chromatin remodeling factors use the energy of ATP hydrolysis to facilitate the interaction of proteins with nucleosomal DNA. The ability of these factors to participate in transcriptional regulation has been extensively studied (for reviews, see Flaus and Owen-Hughes, 2001; and Fry and Peterson, 2001, and references therein). To provide another perspective, we will explore the functions of chromatin remodeling factors in processes other than transcription.

Diversity of Chromatin Remodeling Factors

ATP-dependent chromatin remodeling complexes are characterized by the presence of an ATPase subunit from the SNF2-like family of the DEAD/H (SF2) superfamily of DNA-stimulated ATPases (Table 1) (Gorbalenya and Koonin, 1993; Eisen et al., 1995). The ATPase subunits share homologous helicase motifs with the SWI2/SNF2 protein of the prototype chromatin remodeling complex, SWI/SNF. Despite their similarity to DNA helicases, chromatin remodeling factors (and their individual ATPase subunits) have not been observed to possess helicase activity (with the exception of the INO80 complex [Shen et al., 2000], which also contains two polypeptides that are related to the bacterial RuvB DNA helicase). The current evidence suggests that these factors use the energy of ATP hydrolysis to generate superhelical torsion in DNA, to alter local DNA topology, and to disrupt histone-DNA interactions, perhaps by a mechanism that involves ATP-driven translocation along the DNA.

Many chromatin remodeling complexes have been found to affect transcriptional regulation, but it also appears that chromatin remodeling is important for processes other than transcription. Moreover, proteins in the SNF2-like family of ATPases have been found to participate in diverse processes such as homologous recombination (RAD54), transcription-coupled DNA repair (ERCC6/CSB), mitotic sister chromatid segregation (lodestar; Hrp1), histone deacetylation (Mi-2/CHD3/CHD4), and maintenance of DNA methylation states (ATRX). It thus appears that there is a broad range of nontranscriptional functions of chromatin remodeling proteins (Figure 1).

In addition, the recent sequencing of eukaryotic genomes has led to the identification of many novel SWI2/SNF2-related putative ATPases. For example, there are at least seventeen SWI2/SNF2-related open reading frames in the *Drosophila* genome, and only six of the corresponding proteins have been analyzed. In their native state, SWI2/SNF2-related proteins have been generally found to exist as subunits of multiprotein complexes. Thus, the purification of the native forms of the novel SWI2/SNF2-like proteins would likely reveal many new chromatin remodeling complexes. It will be an interesting and important challenge to identify the functions of these new factors.

Chromatin Remodeling Factors in DNA Replication

Chromatin structure is an important component of eukaryotic DNA replication (reviewed in Demeret et al., 2001). Nucleosomes appear to be generally inhibitory to replication. For instance, the positioning of a nucleosome over a yeast autonomously replicating sequence (ARS) inhibits plasmid DNA replication in vivo, and the packaging of DNA into chromatin represses SV40 DNA replication in vitro. It has been found that sequence-specific DNA binding factors, such as the yeast origin recognition complex, can establish an arrangement of nucleosomes that allows the initiation of DNA replication (see, for example Lipford and Bell, 2001; and Demeret et al., 2001). Hence, in the cell, it is possible that replication-competent chromatin structures are generated by the coordinate action of the DNA replication machinery and ATP-dependent chromatin remodeling factors.

To examine the effect of chromatin remodeling upon DNA replication, T antigen-mediated SV40 DNA replication reactions were performed in the presence or the absence of a chromatin remodeling factor termed CHRAC (*chr*omatin *a*ccessibility *c*omplex) (Alexiadis et al., 1998). In these studies, CHRAC was found to stimulate the sequence-specific binding of SV40 large T antigen to the SV40 origin of replication, as well as to enhance the initiation of SV40 DNA replication in vitro with chromatin templates, but not with naked DNA templates. These results therefore suggest a function of CHRAC in the initiation of DNA replication. On the other hand, CHRAC did not appear to affect the elongation of replication with DNA or chromatin. There may be other activities that are involved in the progression of DNA polymerase through chromatin.

Other studies have revealed a connection between the SWI/SNF complex and DNA replication. First, a direct interaction was identified between the Ini1/hSNF5 subunit of human SWI/SNF complex and the human papillomavirus E1 replication protein, which is a sequence-specific DNA binding factor that functions in a manner similar to SV40 large T antigen (Lee et al., 1999). Transient transfection, antisense, and mutational analyses revealed that the interaction between Ini1/hSNF5 and E1 is essential for the efficient replication of papilloma-

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Table 1. Functions of SWI2/SNF2-like Proteins

Remodeling Complex	ATPase Subunit	Role in Transcription	Role in Other Processes
ySWI/SNF, dBRM, hSWI/SNF	ySNF2, dBRM, hBRG1, hBRM	+	DNA replication, recombination
yRSC, hSWI/SNF-B	ySTH1, hBRG1	+	Chromosome structure?
dACF, dCHRAC, yISW1,2, xACF, xISWI-A,-B,-D, hACF/WCRF, dNURF, hRSF	dISWI, yISW1, yISW2, xISWI, hSNF2h	+	Chromatin assembly, global remodeling of nuclei, chromosome structure, nucleotide excision repair
NuRD/NURD/NRD/Mi-2	Mi-2α/CHD3, Mi-2β/CHD4	+	Histone deacetylation
Not known	yRAD54, dOKR	?	Homologous recombination
Not known	hATRX	+	DNA methylation maintenance
Not known	yRAD26, hERCC6/CSB	?	Transcription-coupled DNA repair
yINO80.com	yINO80	+	DNA repair

virus DNA. It was not determined, however, whether Ini1/hSNF5 protein facilitates papillomavirus replication by itself or as a subunit of the SWI/SNF complex.

In a separate work, the function of yeast SWI/SNF complex in DNA replication was investigated by using a mitotic plasmid stability assay that reflects the replication efficiency of ARS-containing minichromosomes (Flanagan and Peterson, 1999). These experiments showed that mutations in subunits of the SWI/SNF complex cause a decrease in the maintenance of plasmids that contain one particular ARS but not three other ARSs that were tested. In addition, the recruitment of a LexA-SWI2 fusion protein was observed to increase the maintenance of an ARS-containing plasmid. These findings indicate that the SWI/SNF complex can, in some instances, increase the efficiency of DNA replication.

Chromatin Assembly and Chromosome Structure

Chromatin assembly is a fundamental biological process by which nuclear DNA is packaged into nucleosomes. ACF (*A*TP-utilizing *c*hromatin assembly and remodeling *f*actor) was identified and purified on the basis of its ability to mediate the ATP-dependent assembly of periodic nucleosome arrays, and it consists of two subunits: ISWI and a polypeptide termed Acf1 (Ito et al., 1999). The Acf1 subunit functions cooperatively with the ISWI subunit for the assembly of chromatin. ACF-mediated chromatin assembly can be carried out with purified recombinant ACF, purified recombinant NAP-1 (a core histone chaperone), purified core histones, DNA (either linear or circular), and ATP. ACF requires the hydrolysis of ATP for both the deposition of histones onto DNA as well as the establishment of periodic nucleosome arrays. In addition to its function in the assembly of chromatin, ACF can catalyze the ATP-dependent mobilization of nucleosomes, and is therefore a chromatin remodeling factor. Thus, ACF provides an example of a chromatin remodeling factor that has been purified and characterized based on its function in a nontranscriptional process.

The ISWI subunit of ACF is also present in the NURF (*nu*cleosome *r*emodeling *f*actor) and CHRAC chromatin remodeling factors. NURF was identified on the basis of its ability to disrupt the regularity of a periodic nucleosome array in the presence of the GAGA factor (a sequence-specific DNA binding factor), whereas CHRAC was identified as a factor that increases the accessibility of restriction enzymes to DNA packaged into chromatin. CHRAC was originally reported to contain topoisomerase II as a subunit, but has since been found to be devoid of topoisomerase II as well as to contain Acf1 and two smaller (14 and 16 kDa) subunits (Eberharter et al., 2001). It is therefore likely to be closely related to ACF. NURF, on the other hand, appears to be distinct from ACF, aside from the common ISWI subunit.

The analysis of ISWI in *Drosophila* revealed that ISWI is essential for viability and is localized to both euchromatic and heterochromatic sites in polytene and mitotic chromosomes (Deuring et al., 2000). The localization of ISWI and RNA polymerase II was found to be mostly nonoverlapping, and thus, ISWI is present mainly at sites that are not actively transcribed. In addition, mutations in ISWI caused a gross change in the structure of the male X chromosome. In *Xenopus*, ISWI was found to be required for the ATP-dependent global remodeling of nuclei (Kikyo et al., 2000). Thus, these results are consistent with a function of ISWI-containing complexes in the establishment and maintenance of chromatin structure.

The RSC (*r*emodel the structure of *c*hromatin) chromatin remodeling complex, which contains the STH1 ATPase, has the ability to catalyze the transfer of a

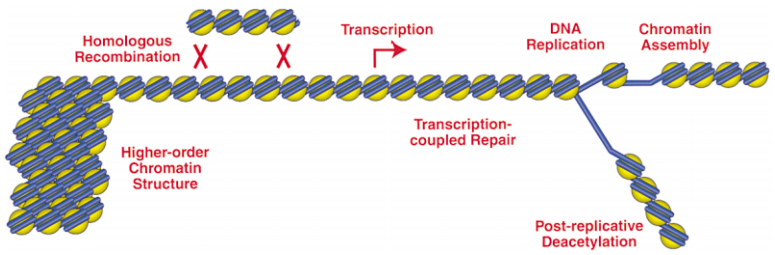

Figure 1. Diverse Functions of Chromatin Remodeling Factors

histone octamer from a nucleosome core particle to naked DNA (Lorch et al., 1999). This activity may reflect a specialized function of RSC in the establishment and/or maintainance of chromosome structure, such as the transfer of preexisting histones to newly synthesized DNA during replication or the nonreplicative exchange of histones. Mutations in subunits of RSC cause a G2/M cell cycle arrest (Angus-Hill et al., 2001, and references therein), which could be due to defects in chromatin assembly and chromosome structure.

DNA Repair and Recombination

Chromatin structure is an important component of DNA repair in eukaryotes (reviewed in Meijer and Smerdon, 1999). The DNA repair machinery must have access to the DNA lesions in chromatin, and the newly-repaired DNA must also be reassembled into chromatin. A recent biochemical study of chromatin structure and nucleotide excision repair revealed that ACF is able to facilitate the excision of pyrimidine (6-4) pyrimidone photoproducts in a dinucleosome (Ura et al., 2001). In that study, chromatin-mediated repression of nucleotide excision was only partially relieved by ACF, but it nevertheless appears that ACF can increase the efficiency of nucleotide excision in chromatin.

Analysis of the SWI2/SNF2-like ATPase, INO80, in *Saccharomyces cerevisiae* led to the purification of the INO80 chromatin remodeling complex (INO80.com), which contains INO80 along with about 11 additional polypeptides (Shen et al., 2000). Mutation of INO80, but not SWI2/SNF2, causes sensitivity of yeast to hydroxyurea, methyl methanesulfonate, ultraviolet radiation, and ionizing radiation. These findings suggest a function of INO80.com in DNA repair. INO80.com also contains two RuvB-like subunits termed RVB1 and RVB2. In prokaryotes, the RuvB DNA helicase is a component of the RuvAB complex, which binds to Holliday junctions and promotes branch migration. Thus, RVB1 and RVB2 may contribute to the function of INO80.com in DNA repair.

Another SWI2/SNF2-related protein with a function in DNA repair is Cockayne syndrome B protein (CSB). CSB is involved in the coupling of nucleotide excision repair to transcription. Purified recombinant CSB polypeptide is a DNA-dependent ATPase that exhibits ATP-dependent chromatin remodeling activity (Citterio et al., 2000, and references therein). These findings suggest that CSB, possibly as a component of a multisubunit complex, may function to remodel chromatin during transcription-coupled repair.

Chromatin remodeling is also likely to be important for DNA recombination. For instance, human SWI/SNF complex was observed to stimulate the cleavage and processing of DNA by the RAG1 and RAG2 proteins that are involved in V(D)J recombination (Kwon et al., 2000). In addition, RAD54 is a SWI2/SNF2-like protein that is involved in the recombinational repair of double-strand breaks and homologous recombination during meiosis. It is thus possible that RAD54 functions to remodel chromatin during homologous recombination.

Perspectives

Here, we have presented a handful of examples of chromatin remodeling in processes other than transcription. These studies are likely to be the proverbial "tip of the iceberg" of an exciting and important area of chromatin research. One of the key challenges for the future will be to devise chromatin remodeling assays that accurately reflect the specific functions of the factors in the cell. In this manner, the activities of different chromatin remodeling factors could be distinguished and characterized. It will also be a significant challenge to identify the biochemical and biological activities of yet-to-be-characterized proteins that are related to SWI2/SNF2. In this regard, genetic data will likely provide key insights. In addition, the purification of the native forms of the proteins will reveal whether or not they are present in a multisubunit complex (and the identification of other associated proteins, if they exist, would likely provide clues on the functions of the factors). From the mechanistic standpoint, it would be interesting to investigate whether chromatin remodeling factors function by the same basic mechanism in transcription, replication, repair, and recombination, or if different remodeling complexes possess unique activities that specifically facilitate each of the separate processes in the context of chromatin. Thus, in the future, these efforts should lead to the replacement of the somewhat vague activity that we currently call "chromatin remodeling" by more specific descriptive terms of the rich and diverse functions of these ATP-driven chromatin-reorganizing factors.

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