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Histone Octamer Transfer by a Chromatin-Remodeling Complex

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Summary

RSC, an abundant, essential chromatin-remodeling complex related to SWI/SNF complex, catalyzes the transfer of a histone octamer from a nucleosome core particle to naked DNA. The newly formed octamer–DNA complex is identical with a nucleosome in all respects. The reaction requires ATP and involves an activated RSC–nucleosome intermediate. The mechanism may entail formation of a duplex displacement loop on the nucleosome, facilitating the entry of exogeneous DNA and the release of the endogenous molecule.

Introduction

The first family of chromatin-remodeling complexes was identified through the evolutionary conservation of the Swi2/Snf2 DNA-dependent ATPase. Two yeast complexes, SWI/SNF and RSC, contain Swi2/Snf2 and the homologous protein Sth1, respectively (Winston and Carlson, 1992; Cairns et al., 1996). Two human complexes and a *Drosophila* complex containing related proteins are known as well (Imbalzano et al., 1994; Dingwall et al., 1995; Wang et al., 1996). The homology of SWI/SNF and RSC extends beyond the ATPase subunits: six proteins are either similar or identical between the two complexes (Cairns et al., 1998). Genetic evidence indicates a role for both SWI/SNF and RSC in transcription and implicates SWI/SNF in remodeling the chromatin structure of promoters in vivo (Winston and Carlson, 1992; Cairns et al., 1998). RSC is more abundant and, in contrast with SWI/SNF, is essential for cell growth.

Biochemical evidence for a direct role in chromatin remodeling has come from the perturbation of nucleosome structure by purified SWI/SNF and RSC complexes. It was first found that SWI/SNF alters the DNase I footprint of the nucleosome and facilitates binding of transcription factors to sites in nucleosomal DNA (Cote et al., 1994; Imbalzano et al., 1994). The DNase I pattern was transformed from periodic cutting, due to wrapping of the double helix on the histone core of the nucleosome, to more nearly uniform cutting, characteristic of naked DNA. Despite this action of SWI/SNF, and a similar action of RSC (Cairns et al., 1996), the nucleosome remained essentially intact, with no release of histones or DNA.

Further insight into the chromatin-remodeling process was gained by the resolution of RSC– and SWI/SNF–nucleosome complexes in polyacrylamide gels (Lorch et al., 1998; Schnitzler et al., 1998). RSC was shown to form complexes with naked DNA and nucleosomes with comparable affinities. Addition of ATP affected only the RSC–nucleosome complex, which was shifted to a more slowly migrating form. The structure of the nucleosome in this "activated complex" was grossly perturbed, as shown by nearly uniform accessibility to all nucleases tested, including restriction endonucleases, which do not ordinarily cut internal sites in nucleosomes. The activated complex was unaffected by the removal of ATP, but following removal of RSC, the nucleosome reverted to a stably altered state. The altered nucleosome was physically separable from the original, unaltered one, sedimenting more rapidly in a gradient and migrating more slowly in a gel. In these respects, the altered particle resembled a nucleosome dimer. The altered nucleosome did not exhibit the uniform sensitivity to nuclease digestion characteristic of the activated complex, but the strength of histone–DNA interaction was nonetheless diminished, as shown by marked instability at elevated ionic strength. Similar stable alteration of the nucleosome has been reported for yeast and human SWI/SNF complexes as well (Cote et al., 1998; Schnitzler et al., 1998).

Three states of the nucleosome are thus defined: the original, or basal state; an activated state, which exists only in a complex with RSC; and a persistently altered state. Conversion of the altered nucleosome back to the basal state by RSC and ATP completes a cycle of RSC action relating the three states (Figure 1). It may be asked whether either the activated or the altered state lies on a pathway to complete nucleosome disruption and the exposure of naked DNA. If so, then what is the natural acceptor for the histone octamer released in the process?

Related questions about the RSC and SWI/SNF mechanisms concern the disruption of histone–DNA contacts in the activated state. What length of DNA is affected? Are the contacts entirely broken, creating a region(s) of essentially naked DNA? Can the affected region propagate around the nucleosome? To address these questions, we investigated the interaction of exogeneous free DNA fragments with the activated RSC–nucleosome complex. The results revealed an unexpected functional activity of RSC, with implications for chromatin remodeling in vivo.

Results

RSC Catalyzes Free DNA–Core Particle Interaction

A 154 bp DNA fragment end-labeled with ^{32}P was added to a reaction mixture containing RSC, unlabeled nucleosome core particles, and ATP. After incubation, RSC was removed by competition with unlabeled DNA, and the products were analyzed in the native state by gel electrophoresis. The labeled DNA fragment appeared in a band with the mobility of a nucleosome (Figure 2). The rate of appearance of the band was far slower than

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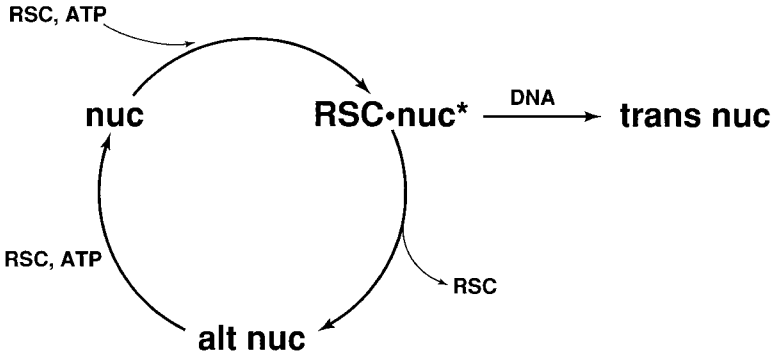

Figure 1. RSC Reaction Cycle and Pathway for Histone Octamer Transfer to Free DNA

It is not known whether an activated intermediate occurs in the conversion of an altered nucleosome ("alt nuc") to an unaltered nucleosome ("nuc"), but any intermediate is likely to differ from the activated RSC–nucleosome complex ("RSC-nuc*"), due to the difference in structure between altered and unaltered nucleosomes. "nuc," nucleosome; "RSC-nuc*," activated RSC–nucleosome complex; "alt nuc," stably altered nucleosome; "trans nuc," product of histone octamer transfer from the nucleosome to free DNA.

the rate of formation of an activated RSC–nucleosome complex (complete within a minute under the conditions used; data not shown). As the labeled DNA fragment was present in approximately 3-fold molar excess over RSC, the action of RSC was catalytic. ATP hydrolysis was required, as shown by a lack of detectable reaction in the presence of the nonhydrolyzable analog AMPPCP (data not shown).

The Product of RSC-Catalyzed DNA–Core Particle Interaction Is a Nucleosome

The labeled product of reaction of the free DNA fragment with core particles, RSC, and ATP was identified as a nucleosome on the basis of five criteria. First, as already mentioned, the reaction product migrated in a gel as a nucleosome. Second, the reaction product sedimented as a nucleosome. Following the addition of unlabeled competitor DNA, a reaction mixture was applied to a density gradient. Centrifugation yielded a major peak of radioactivity (Figure 3A) in the same position as a marker nucleosome run in a parallel gradient. Gel electrophoresis of the peak fraction revealed a band in the position of the reaction product and no free DNA or other labeled material.

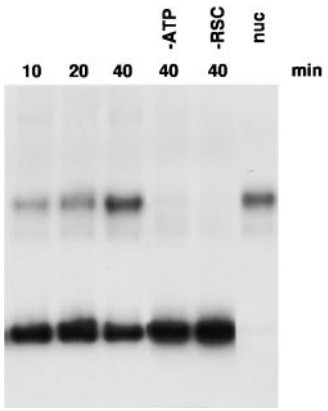

Figure 2. RSC Catalyzes Free DNA–Core Particle Interaction

A reaction mixture containing ^{32}P-labeled 154 bp DNA (10 ng), core particles (40 ng of DNA), and RSC (30 ng) was prepared as described, with the omission of RSC or ATP where indicated, and incubated for the times indicated at 30°C, followed by the addition of carrier DNA, gel electrophoresis, and autoradiography. A marker of a nucleosome assembled on the same DNA fragment is shown at the far right ("nuc").

Third, the reaction product displayed the DNase I footprint of a nucleosome. For this analysis, the peak fraction from the density gradient was subjected to brief DNase I digestion, followed by extraction of DNA and gel electrophoresis. An alternating pattern of cleavage and protection with the ten-base periodicity of the double helix, characteristic of a nucleosome and clearly distinct from the cutting pattern of naked DNA, was obtained (Figure 3B).

Fourth, the reaction product was resistant to digestion at an internal site by a restriction endonuclease. A DraI site about 15 bp from the center of the 154 bp DNA fragment used here was shown previously to be protected by assembly in a nucleosome. Addition of DraI to the reaction mixture containing both free DNA and reaction product resulted in complete cleavage of the former but no cutting of the latter (Figure 3C).

Finally, the reaction product was similar in stability at elevated ionic strength to a nucleosome (data not shown), indicating a similar strength of histone–DNA interaction. In this respect, as well as in gel electrophoretic and sedimentation behavior, the reaction product was clearly distinguishable from the altered nucleosome formed by the action of RSC.

Our conclusion that reaction of labeled DNA with core particles gives rise to labeled nucleosomes has the important corollary that the unlabeled DNA originally associated with the core particles is released in free form. A direct demonstration of this point may be found in the previous report of a stably altered nucleosome (Lorch et al., 1998). In that work, an activated complex of RSC with a labeled nucleosome was treated with an excess of unlabeled competitor DNA. In addition to the generation of the altered nucleosome, an appreciable amount of labeled DNA was released in free form (see Figure 7B, lanes 3 and 4, of Lorch et al., 1998).

Free DNA Interacts with an Activated RSC–Nucleosome Intermediate

Does the reaction of free DNA with RSC, core particles, and ATP proceed through the previously described, activated RSC–nucleosome complex? This possibility was raised by a comparison of reaction rates. The free DNA–core particle interaction catalyzed by RSC (Figure 2) was far slower than the formation of the activated complex (complete within a minute; data not shown). The activated complex was therefore kinetically competent to serve as an intermediate.

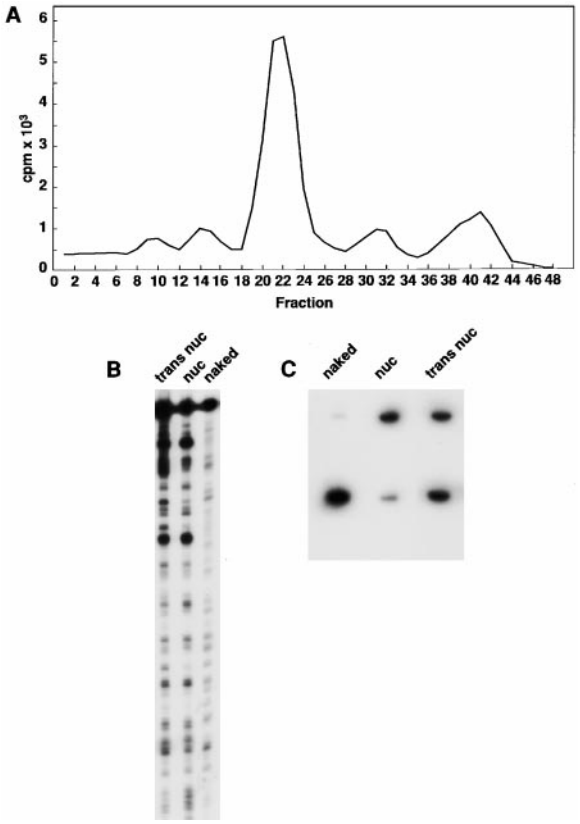

Figure 3. The Product of RSC-Catalyzed DNA–Core Particle Interaction Is a Nucleosome

(A) Sedimentation analysis of RSC reaction product. A reaction mixture (230 μl) containing ^{32}P-labeled 154 bp DNA (0.3 μg), core particles (4 μg of DNA), and RSC (1.5 μg) was prepared as described and incubated for 40 min at 30°C, followed by the addition of carrier DNA and sedimentation in a maltose gradient for 19 hr as described (Lorch et al., 1998). A marker nucleosome assembled on the same DNA fragment formed a peak in fraction 22 in a parallel gradient. The peak in fraction 31 was due to naked DNA.

(B) DNase I footprint analysis of RSC reaction product. The peak maltose gradient fraction from (A) ("trans nuc," 10 μl), the corresponding naked DNA ("naked," 3.5 ng), and the same DNA assembled in a nucleosome ("nuc," 3.5 ng) were treated with DNase I (50 ng) for 40 s at room temperature and analyzed by gel electrophoresis as described (Cairns et al., 1996). An autoradiograph of the gel is shown.

(C) Restriction endonuclease digestion of RSC reaction product. For the lane labeled "trans nuc," a reaction mixture (15 μl) containing ^{32}P-labeled 154 bp DNA (7.5 ng), core particles (40 ng of DNA), and RSC (30 ng) was prepared as described and incubated for 40 min at 30°C, followed by the addition of carrier DNA and further incubation for 5 min at 30°C. DraI (4 U) was added, followed by incubation for 30 min at 30°C, extraction of DNA, and electrophoresis in a 7% polyacrylamide gel. An autoradiograph of the gel is shown. For the lane labeled "naked," the same procedure was followed with the omission of core particles and RSC. For the lane labeled "nuc," the same procedure was followed with the omission of labeled DNA, core particles, and RSC and with the inclusion instead of the same labeled DNA assembled in a nucleosome. The partial protection of the RSC reaction product ("trans nuc") from DraI cutting reflects the partial conversion of the labeled DNA to nucleosomal form (Figure 1).

A more detailed kinetic analysis was strongly indicative of free DNA reaction with an RSC–nucleosome intermediate. If either DNA or core particles interacted first

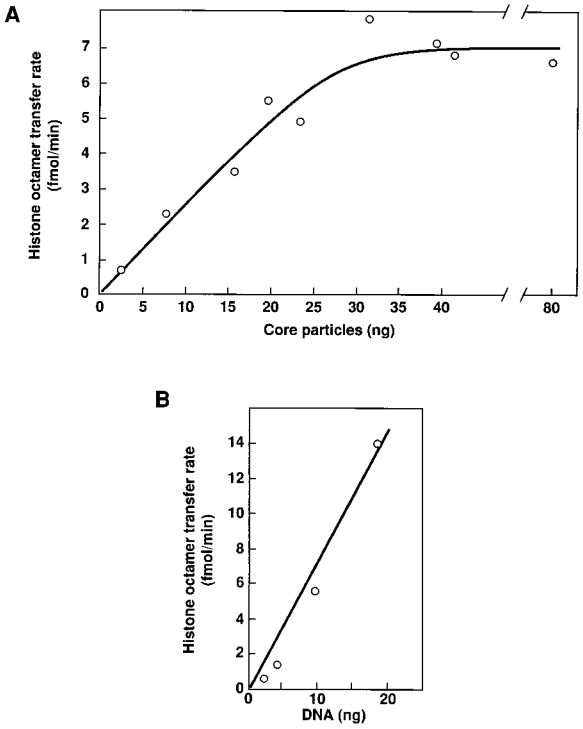

Figure 4. Kinetics of Histone Octamer Transfer Implicate an Activated RSC–Nucleosome Intermediate

(A) Dependence of histone octamer transfer rate on core particle concentration. Transfer reactions were preformed as described with ^{32}P-labeled 154 bp DNA (10 ng), RSC (30 ng), and core particles in the amounts indicated.

(B) Dependence of histone octamer transfer rate on free DNA concentration. Transfer reactions were preformed as described with core particles (40 ng of DNA), RSC (30 ng), and ^{32}P-labeled 154 bp DNA in the amounts indicated.

with RSC in a rate-limiting step, then the overall reaction rate should depend only on the concentration of that component. Rather, the observed rate at low DNA and core particle concentrations was proportional to both (Figure 4). Analysis over a more extended concentration range led to a K_M for the core particle of 0.8×10^{-8} M, essentially the same as the dissociation constant of about 10^{-8} M previously estimated for RSC–nucleosome interaction on the pathway to an activated complex (Lorch et al., 1998). We conclude that encounter of DNA with a RSC–core particle complex is rate determining, and that the RSC–core particle interaction is essentially the same as that involved in activated complex formation.

Discussion

Our findings show that RSC, and by inference, SWI/SNF, is able to transfer a histone octamer from a nucleosome to naked DNA. As one naked region becomes protected, another, previously covered by a nucleosome, is exposed. The capacity of a chromatin-remodeling complex to disrupt a nucleosome completely is thus established. All that is required beyond the remodeling complex, nucleosome, and ATP is an acceptor for the

histone octamer released. In our experiments, the acceptor was DNA. In vivo, DNA may also serve as an acceptor, for example a neighboring region of the chromosome or a region of an adjacent chromosome. It remains to be seen whether other nucleic acid or protein molecules function as octamer acceptors for chromatin remodeling as well.

Histone octamer transfer by RSC evidently proceeds through the activated RSC–nucleosome complex described previously. The involvement of this complex as an intermediate has implications for both the structure of the complex and the mechanism of the chromatin-remodeling process. It points to the occurrence in the complex of a region(s) of complete separation between histones and DNA. An invading DNA molecule may interact with histones in this region and then expand its contact to adjacent regions, displacing the resident DNA.

The possible involvement of the altered nucleosome in histone octamer transfer appears unlikely. The altered nucleosome is formed upon removal of RSC and differs in structure from the nucleosome in the activated complex. It shows less perturbation of the histone–DNA interaction and displays physical characteristics of a nucleosome dimer. It may be formed by DNA released from the histones in one activated RSC–nucleosome complex binding to the histones exposed in another activated complex.

Perhaps the closest equivalent to the mechanism envisaged here for histone octamer transfer is that demonstrated for transcription through a nucleosome by T7 RNA polymerase in vitro (Studitsky et al., 1994). DNA displaced from the octamer by the transcribing polymerase loops out and rebinds to the histone surface, preserving the structure of the nucleosome, but with retrograde transfer of the octamer along the DNA. Loop formation and movement are driven by the energy-dependent advance of the polymerase along the DNA. It remains to be seen whether the reaction catalyzed by RSC results from a similar ATP-dependent translocation on DNA.

Experimental Procedures

DNA Fragments

A 154 bp DNA fragment containing the nucleosome-positioning sequence of the *Xenopus laevis* 5S rRNA gene (Cote et al., 1994) and flanking DNA introduced to provide EcoRI and AvaI restriction sites was generated by PCR from pIC5x207(E,X) with the primers 5′-GCGCCGGAATTCCAACGAATAACTTCCAGGG-3′ and 5′-CAGCTG CCCGGGTATGCTGCTTGACTTCGGTGA-3′. This fragment was introduced between the EcoRI and AvaI restriction sites of pUC19 and excised and labeled by filling in the AvaI end with the use of α-^{32}P-dCTP. Alternatively, a 168 bp fragment was prepared by labeling the EcoRI primer at the 5′-end with γ-^{32}P-ATP and performing PCR with the use of the AvaI primer as well.

Nucleosomes

Rat liver core particles were prepared as described (Kornberg et al., 1989). Nucleosomes were assembled on labeled DNA fragments with the use of rat liver histone octamers by stepwise dilution from 2 M NaCl solution (Lorch et al., 1987), followed by centrifugation in a maltose gradient.

Histone Octamer Transfer Reaction

The transfer reaction was performed with DNA fragment, core particles, and RSC (purified as described [Cairns et al. 1996], followed by an additional step of chromatography on TSK-heparin) in the amounts indicated in a total volume of 5 μl containing 15 mM HEPES (pH 7.5), 3 mM $MgCl_2$, 10 mM potassium acetate, and 75 μg/ml BSA at 30°C for the times indicated. The reaction was terminated by the addition of 10 μg of a pUC19 plasmid DNA, followed by electrophoresis in a 3.2% polyacrylamide gel containing 10 mM TrisCl (pH 7.5) and 1 mM EDTA, except where indicated. Quantitation was performed with a PhosphorImager or equivalent device.

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