

Chromatin-modifying and -remodeling complexes

Roger D Kornberg and Yahli Lorch

Nucleosomes have long been known to inhibit DNA transactions on chromosomes and a remarkable abundance of multiprotein complexes that either enhance or relieve this inhibition have been described. Most is known about chromatin-remodeling complexes that perturb nucleosome structure.

Address

Department of Structural Biology, Stanford School of Medicine
Stanford, California 94305, USA

Current Opinion in Genetics & Development 1999, **9**:148–151

<http://biomednet.com/elecref/0959437X00900148>

© Elsevier Science Ltd ISSN 0959-437X

Abbreviations

CHD chromodomain–helicase–DNA-binding domain
HAT histone acetyltransferase
HDAC histone deacetylase

Introduction

Chromatin-modifying and -remodeling complexes have attracted much recent attention [1–8]. For the most part — as biochemical assays, genetic analyses, homology searches, and other approaches continually bring new complexes to light — the focus has been on discovery. Identification of subunits has served to classify the complexes in a few broad families and to indicate relationships between them. Information remains scant, however, regarding the biological roles of the complexes, the consequences of their actions for the structure of chromatin, and the biochemical mechanisms involved. Here, we provide a brief description of new complexes, identified since the reviews cited above were written and discuss in detail the mechanism by which one major family perturbs the structure of chromatin.

All known chromatin-modifying and -remodeling complexes target the nucleosome, either through the acetylation or deacetylation of histone ‘tails’, or by perturbation of histone–DNA interactions. Modifying complexes contain one or more histone acetyltransferase (HAT) or histone deacetylase (HDAC) subunit. Remodeling complexes contain a DNA-dependent ATPase subunit, either a Swi2/Snf2 homolog, the more distantly related ISWI protein, or the recently described CHD (chromodomain–helicase–DNA-binding domain) proteins [9]. Modifying and remodeling complexes are presumed to be involved in gene regulation and the initiation of transcription, amongst other processes, although strong evidence has been obtained in only two cases. The first concerns the chromatin-remodeling complex, SWI/SNF, which was identified by genetic analysis in yeast, supported by subsequent biochemical studies. Although the requirement for SWI/SNF *in vivo* is limited to a small number of genes [10], the isolation of suppressors of *swi/snf* mutants in histone genes, as well as effects of *swi/snf* mutations on chromatin structure, leave little doubt of its

role in chromatin remodeling *in vivo* [11]. The second case concerns the acetyltransferase Gcn5, which was similarly identified by genetic and biochemical studies in yeast. Recent studies have documented the role of Gcn5 in histone acetylation during transcriptional activation *in vivo* [12].

The requirement for nucleosome disruption for gene activation and the involvement of histone acetylation bear out notions concerning the inhibition of transcription by histones and its reversal by histone modification that were put forward long ago. Recent studies, however, have revealed greater subtlety than earlier imagined. For example, the deacetylase complex NRD (also known as NURD) involved in transcriptional repression includes a homolog of the Swi2/Snf2 protein instrumental in activation (see below); and acetylation of certain histone tail residues is important for repression as well as for activation [1].

Newly identified complexes

NRD, the most recent addition to the family of chromatin-modifying complexes, was isolated from human cells and contains six previously identified polypeptides [13–16]. The Swi2/Snf2-related ATPase component, CHD4, includes two chromodomain and two PHD (plant homeodomain) zinc finger motifs. Chromodomains are found in a variety of proteins, including the *Drosophila* heterochromatin-associated proteins HP1 and Polycomb. PHD zinc fingers are evidently involved in CHD4–HDAC1 interaction. The chromatin-remodeling activity of NRD stimulates the deacetylation activity, suggesting that nucleosome disruption facilitates access of HDAC to the histone tails. The interdependence is not mutual, as an HDAC inhibitor has no effect on chromatin-remodeling, indicating that the acetylation state of the histone tails has little impact on nucleosome stability.

NRD differs from SAP, a complex that contains many of the same subunits, but which further includes mSin3 and which lacks CHD4 or any other ATPase subunit. SAP may be recruited to promoters and repress transcription by a network of interactions, for example non-liganded retinoic acid receptor interacting with a corepressor which in turn interacts with mSin3 [1,17,18]. The basis for recruitment of NRD and its role in transcriptional repression have yet to be elucidated.

RSF, a newly identified member of the family of chromatin-remodeling complexes, possesses remodeling and nucleosome-spacing activities [19]. Nucleosome spacing refers to an improvement in clarity of a ladder of bands in a gel following micrococcal nuclease digestion of chromatin, presumably a result of near periodic distribution of nucleosomes along the chromatin filament. RSF exhibits the simplest subunit composition of the chromatin-remodeling complexes described to date: a 135 kDa polypeptide

with 75% identity to *Drosophila* ISWI and a 325 kDa polypeptide of unknown identity or significance.

Activity of such a minimal complex in chromatin-remodeling can be understood in terms of the sufficiency of ISWI and of the Swi2/Snf2 ATPase component of human SWI/SNF complex, hBrg, for the remodeling process [20,21]. It was found that recombinant ISWI alone would support ATP-dependent remodeling and also ATP-dependent nucleosome spacing. Moreover, ATPase activity of the recombinant protein, like that of the larger remodeling complexes, was stimulated by nucleosomes but not by DNA or histones alone. Recombinant hBrg was similarly shown to support chromatin-remodeling and to mimic the behavior of intact hSWI/WNF complex in assays available *in vitro*.

Mechanism of remodeling: reaction intermediates

Progress has been made in defining the reaction pathway of chromatin remodeling by SWI/SNF and its close relative RSC, and in characterizing the intermediates involved. Complexes of the remodeling proteins with nucleosomes and naked DNA were resolved in minimally cross-linked polyacrylamide gels [22–24]. The complexes were shown to form with similar dissociation constants (~10 nM). Addition of ATP increased the affinity for nucleosomes but not for naked DNA. ATP also shifted the mobility of a portion of RSC–nucleosome complexes, apparently as a result of conformational changes in the nucleosome, RSC, or both. The shifted complexes were sensitive to digestion by all nucleases tested, whereas the unshifted complexes were resistant. Both the mobility shift and nuclease sensitivity persisted following the removal of ATP. These observations identify an ‘activated’ complex, in which the nucleosome is markedly perturbed, with a much-diminished histone–DNA interaction.

Perturbation of nucleosome structure also persisted following the removal of SWI/SNF or RSC from activated complexes. The resultant ‘altered’ nucleosome could be isolated by gel filtration or gradient centrifugation and characterized in more detail. Studies with yeast SWI/SNF, human SWI/SNF, and RSC showed somewhat different patterns of DNase I sensitivity of the altered nucleosome but were otherwise consistent with one another. Finally, it was shown that treatment of the altered nucleosome with human SWI/SNF or RSC and ATP restored the original state of the nucleosome.

Three states of the nucleosome are thus defined: activated, altered, and original. Two possibilities have been considered for the relationship between these states. On the one hand, it has been suggested that chromatin-remodeling complexes catalyze a rapid equilibrium between altered and original states. Implicit in this proposal is the idea that the altered and activated states are the same. On the other hand, the three states have been suggested to form a cycle, with a progression catalyzed by chromatin-

remodeling complexes from original to activated to altered and back to the original state again. Further work is needed to distinguish between these possibilities.

The precise nature of the perturbation of nucleosome structure in activated and altered states is also an important object of ongoing studies. The first evidence of such perturbation came from DNase I digestion of nucleosomes in defined locations on DNA. The digestion pattern was transformed by remodeling complexes from cleavage with a 10-base pair periodicity, characteristic of DNA bound on a surface, to the near uniform cutting pattern of free DNA. This change could be explained by randomization of the locations of nucleosomes rather than disruption of their structure. The subsequent demonstration of exposure of restriction enzyme cleavage sites, however, requires a structural change of the nucleosome. The situation is paradoxical: DNA is exposed along its entire length and yet remains associated with histones in the activated/altered nucleosome.

Mechanism of remodeling: unraveling from the ends

The tight binding of DNA in the nucleosome, wrapped nearly twice around the histone octamer, poses a problem for remodeling. It would seem unlikely that the DNA–histone interaction could be perturbed by attack on the central 100 base pairs or so of the nucleosome; any central segment of DNA is constrained by the tight binding of the DNA segments flanking it on both sides. Attack might occur, however, at the ends of DNA on the nucleosome, which are constrained by binding of a flanking segment on only one side. There is an ample precedent for a such a mechanism of nucleosome disruption: endonuclease III and RNA polymerases have been shown to invade a nucleosome from the ends [25,26]. These reactions might proceed by transient dissociation of the DNA–histone complex from the ends, a few base pairs at a time, followed by inward translocation of the enzymes along the nucleosomal DNA. It was suggested that such a mechanism of unraveling from the ends would be applicable to any enzyme acting processively on DNA [26].

Support for DNA–histone complex unraveling from the ends has come from two types of experiment. Analysis of the kinetics of restriction enzyme digestion of nucleosomal DNA provided evidence of an association–dissociation reaction of the ends, with the frequency of dissociation diminishing towards the center of the nucleosome [27,28]. Analysis of bacteriophage RNA polymerase transcription through a nucleosome indicated the release of a DNA end and its rebinding at a different location on the histone octamer isolated from human cells [29]. Transcription was thus thought to create a loop of DNA displaced from the octamer surface. Translocation of the loop with the transcribing polymerase would transfer the octamer to an adjacent site on the DNA.

In the case of a remodeling complex, the unraveling of nucleosomal DNA would be driven by translocation of the

ATPase/helicase subunit of the complex [30,31]. Several observations are consistent with remodeling in this way. First, in the absence of ATP, RSC interferes with restriction enzyme digestion of nucleosomal DNA near the ends, suggesting that it binds the ends [24•]. Second, the Swi2/Snf2 and ISWI ATPase/helicases are alone sufficient, in the absence of any other subunit of the remodeling complexes, for chromatin-remodeling [20•,21•]. Third, RSC catalyzes the transfer of a histone octamer from a nucleosome to naked DNA [32•]. This octamer transfer might occur by means of a duplex displacement loop in a manner analogous to that described for bacteriophage RNA polymerase transcription of nucleosomal DNA (pictured in [31]).

Despite the appeal of the unraveling mechanism, two observations are not immediately compatible: an activated RSC–nucleosome complex remains sensitive to nuclease digestion following the removal of ATP, and altered nucleosomes resulting from yeast SWI/SNF or human SWI/SNF action remain nuclease-sensitive following the removal of the remodeling complex. Either a duplex-displacement loop, once formed, can persist in the absence of remodeling complex, or an altogether different mechanism may be entailed.

Consequence of remodeling: octamer transfer *in cis*?

The recent demonstration of histone octamer transfer from a nucleosome to naked DNA by RSC raises the possibility of octamer transfer *in cis*, resulting in the translocation of a nucleosome on DNA. Although this possibility remains untested experimentally, it has the appeal of accounting for diverse activities of remodeling complexes. Catalysis of nucleosome spacing and of restriction site accessibility in nucleosome arrays — activities first found for the *Drosophila* ISWI remodeling complexes ACF and CHIRAC, respectively — could be explained by octamer transfer *in cis*. The question of how chromatin-remodeling complexes, shown to bind nucleosomes but not disrupt them entirely, might nonetheless expose a regulatory sequence or promoter DNA could also be answered by octamer transfer to adjacent DNA. One finding inconsistent with these ideas has come from the use of chromatin-remodeling complexes in chromatin-transcription assays. NURF and RSF have both been shown to potentiate transcription of nucleosomal templates [19,33], whereas other ISWI complexes and SWI/SNF were unable to do so [33].

Conclusions

The unifying hypothesis of nucleosomal DNA unraveled by ATP-driven translocases, leading to histone octamer transfer *in cis*, is tenable but far from established. Important contradictions must be reconciled or alternative hypotheses developed. Beyond these mechanistic issues lie such important unanswered questions as what directs remodeling complexes to promoters in chromatin, what controls their range of action, and what underlies their astonishing diversity.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Grunstein M: **Histone acetylation in chromatin structure and transcription.** *Nature* 1997, **389**:349-352.
 2. Wu C: **Chromatin remodeling and the control of gene expression.** *J Biol Chem* 1997, **272**:28171-28174.
 3. Hirose S: **Chromatin remodeling and transcription.** *J Biochem* 1998, **124**:1060-1064.
 4. Cairns BR: **Chromatin remodeling machines: similar motors, ulterior motives.** *Trends Biochem Sci* 1998, **23**:20-29.
 5. Varga-Weisz PD, Becker PB: **Chromatin-remodeling factors: machines that regulate?** *Curr Opin Cell Biol* 1998, **10**:346-353.
 6. Kadonaga JT: **Eukaryotic transcription: an interlaced network of transcription factors and chromatin-modifying machines.** *Cell* 1998, **92**:307-317.
 7. Gregory PD, Hörz W: **Life with nucleosomes: chromatin remodelling in gene regulation.** *Curr Opin Cell Biol* 1998, **10**:339-345.
 8. Travers A: **An engine for nucleosome remodeling.** *Cell* 1999, **96**:311-314.
 9. Delmas V, Stokes DG, Perry RP: **A mammalian DNA-binding protein that contains a chromodomain and an SNF2/SWI2-like helicase domain.** *Proc Natl Acad Sci USA* 1993, **90**:2414-2418.
 10. Holstege FCP, Jennings EG, Wyrick JJ, Lee TI, Hengartner CJ, Green MR, Golub TR, Lander ES, Young RA: **Dissecting the regulatory circuitry of a eukaryotic genome.** *Cell* 1998, **95**:717-728.
 11. Winston F, Carlson M: **Yeast SWI/SNF transcriptional activators and the SPT/SIN chromatin connection.** *Trends Genet* 1992, **8**:387-391.
 12. Kuo M-H, Zhou J, Jambeck P, Churchill MEA, Allis CD: **Histone acetyltransferase activity of yeast Gcn5p is required for the activation of target genes *in vivo*.** *Genes Dev* 1998, **12**:627-639.
 13. Tong JK, Hassig CA, Schnitzler GR, Kingston RE, Schreiber SL: **Chromatin deacetylation by an ATP-dependent nucleosome remodelling complex.** *Nature* 1998, **395**:917-921.
 14. Zhang Y, LeRoy G, Seelig H-P, Lane WS, Reinberg D: **The dermatomyositis-specific autoantigen Mi2 is a component of a complex containing histone deacetylase and nucleosome remodeling activities.** *Cell* 1998, **95**:279-289.
 15. Wade PA, Jones PL, Vermaak D, Wolffe AP: **A multiple subunit Mi-2 histone deacetylase from *Xenopus laevis* cofractionates with an associated Snf2 superfamily ATPase.** *Curr Biol* 1998, **8**:843-846.
 16. Xue Y, Wong J, Moreno GT, Young MK, Côté J, Wang W: **NURD, a novel complex with both ATP-dependent chromatin-remodeling and histone deacetylase activities.** *Mol Cell* 1998, **2**:851-861.
 17. Lin RJ, Nagy L, Inoue S, Shao W, Miller WH Jr, Evans RM: **Role of the histone deacetylase complex in acute promyelocytic leukaemia.** *Nature* 1998, **391**:811-814.
 18. Hassig CA, Tong JK, Fleischer TC, Owa T, Grable PG, Ayer DE, Schreiber SL: **A role for histone deacetylase activity in HDAC1-mediated transcriptional repression.** *Proc Natl Acad Sci USA* 1998, **95**:3519-3524.
 19. LeRoy G, Orphanides G, Lane WS, Reinberg D: **Requirement of RSF and FACT for transcription of chromatin templates *in vitro*.** *Science* 1998, **282**:1900-1904.
 20. Corona DFV, Längst G, Clapier CR, Bonte EJ, Ferrari S, Tamkun JW, Becker PB: **ISWI is an ATP-dependent nucleosome remodeling factor.** *Mol Cell* 1999, **3**:239-245.
- One of six recent papers concerning the mechanism of chromatin-remodeling that form the main subject of this review.
21. Phelan ML, Sif S, Narlikar GJ, Kingston RE: **Reconstitution of a core chromatin remodeling complex from SWI1/SNF subunits.** *Mol Cell* 1999, **3**:247-253.
- One of six recent papers concerning the mechanism of chromatin-remodeling that form the main subject of this review.

22. Côté J, Peterson CL, Workman JL: **Perturbation of nucleosome core structure by the SWI/SNF complex persists after its detachment, enhancing subsequent transcription factor binding.** *Proc Natl Acad Sci USA* 1998, **95**:4947-4952.
One of six recent papers concerning the mechanism of chromatin-remodeling that form the main subject of this review.
23. Schnitzler G, Sif S, Kingston R: **Human SWI/SNF interconverts a nucleosome between its base state and a stable remodeled state.** *Cell* 1998, **94**:17-27.
One of six recent papers concerning the mechanism of chromatin-remodeling that form the main subject of this review.
24. Lorch Y, Cairns BR, Zhang M, Kornberg RD: **Activated RSC nucleosome complex and persistently altered form of the nucleosome.** *Cell* 1998, **94**:29-34.
One of six recent papers concerning the mechanism of chromatin-remodeling that form the main subject of this review.
25. Prunell A, Kornberg RD: **Relation of nucleosomes to DNA sequences.** *Cold Spring Harbor Symp Quant Biol* 1978, **42**:103-108.
26. Lorch Y, LaPointe JW, Kornberg RD: **Nucleosomes inhibit the initiation of transcription but allow chain elongation with the displacement of histones.** *Cell* 1987, **49**:203-210.
27. Polach KJ, Widom J: **Mechanism of protein access to specific DNA sequences in chromatin: a dynamic equilibrium model for gene regulation.** *J Mol Biol* 1995, **254**:130-149.
28. Polach KJ, Widom J: **A model for the cooperative binding of eukaryotic regulatory proteins to nucleosomal target sites.** *J Mol Biol* 1996, **258**:800-812.
29. Studitsky VM, Clark DJ, Felsenfeld G: **A histone octamer can step around a transcribing polymerase without leaving the template.** *Cell* 1994, **76**:371-382.
30. Kornberg RD, Lorch Y: **Interplay between chromatin structure and transcription.** *Curr Opin Cell Biol* 1995, **7**:371-375.
31. Pazin MJ, Kadonaga JT: **SWI2/SNF2 and related proteins: ATP-driven motors that disrupt protein-DNA interactions?** *Cell* 1997, **88**:737-740.
32. Lorch Y, Zhang M, Kornberg RD: **Histone octamer transfer by a chromatin-remodeling complex.** *Cell* 1999, **96**:389-392.
One of six recent papers concerning the mechanism of chromatin-remodeling that form the main subject of this review.
33. Mizuguchi G, Tsukiyama T, Wisniewski J, Wu C: **Role of nucleosome remodeling factor NURF in transcriptional activation of chromatin.** *Mol Cell* 1997, **1**:141-150.