Nucleosome mobilization and positioning by ISWI-containing chromatin-remodeling factors

Gernot Längst and Peter B. Becker*

Adolf-Butenandt-Institut, Schillerstraße 44, München 80336, Germany

*Author for correspondence (e-mail: Peter.Becker@mol-bio.med.uni-muenchen.de)

Journal of Cell Science 114, 2561-2568 (2001) © The Company of Biologists Ltd

Summary

ATP-dependent chromatin-remodeling machines of the SWI/SNF family are involved in many cellular processes in eukaryotic nuclei, such as transcription, replication, repair and recombination. Remodeling factors driven by the ATPase ISWI make up a subgroup of this family that exhibits defined mechanistic and functional characteristics. ISWI-induced nucleosome mobility endows nucleosomal arrays with dynamic properties and recent results suggest that ISWI-type remodelers have diverse functions that range from transcriptional regulation to chromatin assembly and maintenance of chromosome structure.

Key words: ISWI, Chromatin remodeling, Nucleosome assembly

Introduction

DNA in eukaryotic cells is packaged into chromatin, a complex structure in which DNA is associated with histones and a multitude of other proteins. The basic, repetitive unit of chromatin is the nucleosome, formed by the wrapping of ~150 bp of DNA around an octamer of four different histones (see Fig. 1). Nucleosomes are separated by a short 'linker' DNA to which linker histones, such as H1, bind. Nucleosomal arrays are coiled up into higher order-structures, most notably the 30 nm fiber, which is the predominant structure of interphase chromatin. This structure serves as a substrate for all nuclear processes dealing with DNA, such as transcription, replication, repair and recombination (Muchardt and Yaniv, 1999). These processes involve reorganization of the chromatin structure at the level of the nucleosomal array, which facilitates the access of DNA-binding regulators. The functional state of chromatin can be regulated by at least three different strategies: differential association of non-histone proteins, covalent modification of the histones themselves and ATP-dependent mobilization of nucleosomes. The latter, energy-dependent alterations are brought about by so-called chromatin-remodeling factors, multiprotein complexes containing ATPases of the SWI2/SNF2 subfamily (Eisen et al., 1995; Workman and Kingston, 1998; Peterson, 2000; Vignali et al., 2000).

The first hint of a role for nucleosome-remodeling during gene activation came from genetic analyses in yeast. Different screens had identified the SWI/SNF proteins as global regulators of transcription and these were later shown to reside in one large, 2 MDa complex (Peterson, 2000; Vignali et al., 2000). The function of this complex was linked to chromatin when histones and other chromatin components were identified as suppressors of the swi/snf phenotype (Winston and Carlson, 1992). Further analyses showed that the SWI/SNF complex is necessary to relieve chromatin-mediated repression of a set of inducible genes and is particularly important for those genes that are transcribed in late anaphase, when the mitotic condensation of chromatin is still not fully reversed (Krebs et al., 2000). Biochemical analyses of purified yeast SWI/SNF complex and its human counterpart demonstrated that the machinery can modulate histone-DNA interactions such that the accessibility of nucleosomal DNA is much enhanced, facilitating the interaction of proteins with their binding sites on DNA. The SWI2/SNF2 subunit of the SWI/SNF complex, a protein that has similarity to DNA helicases, proved to be responsible for the ATP-dependent nucleosome disruption. Structural and functional homologs of this ATPase reside in related remodeling machineries in all higher eukaryotes (reviewed by Muchardt and Yaniv, 1999). The SWI/SNF complex and the related human BRM and BRG1 complexes have since been shown to be involved in many important processes involving chromatin substrates, such as transcription that leads to cell cycle progression (Muchardt et al., 1998), cellular differentiation (de La Serna et al., 2001), replication (Flanagan and Peterson, 1999), recombination (Kwon et al., 2000) and repair (Ura et al., 2001).

ATP-dependent nucleosome-remodeling was also discovered by an entirely independent and purely biochemical approach. Wu, Becker and colleagues screened crude (and enzymatically rich) *Drosophila* embryo extracts for activities that allow transcription factors to access nucleosomal binding sites in vitro (Tsukiyama et al., 1995; Varga-Weisz et al., 1997). Whereas the wrapping of DNA around the histone octamer frequently prevents the interaction of proteins with recognition sequences, access of a variety of proteins was virtually unhindered in the presence of embryo extract. When the transcription factors gained access to DNA, the nucleosome that had occupied the site before could no longer be detected by footprinting assays. The realization that this mysterious 'nucleosome remodeling' required ATP hydrolysis triggered a hunt for energy-dependent nucleosome-remodeling enzymes in the extract, which led to the identification of two novel complexes (Tsukiyama and Wu, 1995; Varga-Weisz et al., 1997). In a parallel effort, Kadonaga et al. fractionated the embryo extracts into components required for the assembly of regularly spaced nucleosomal arrays. The ATP-consuming 'spacing factor' (see below) was also able

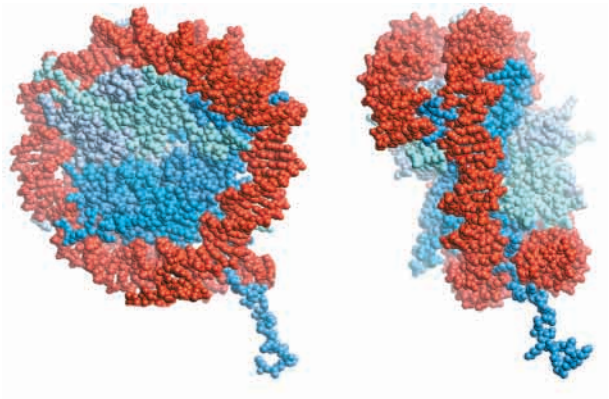

Fig. 1. The nucleosome modeled according to the 2.8 Å crystal structure (Luger et al., 1997).

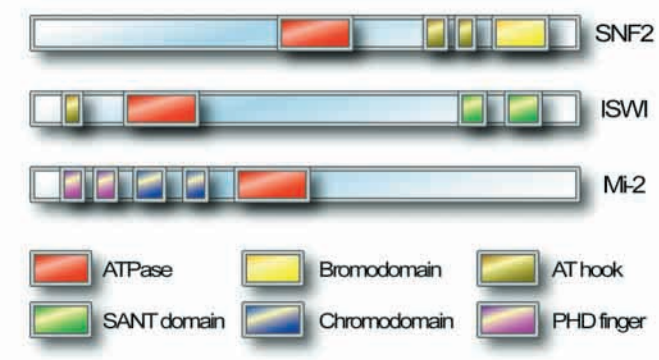

Fig. 2. Domain structure of the SWI2/SNF2-, ISWI- and Mi-2 types of nucleosome-remodeling ATPase.

to facilitate the interaction of DNA-binding proteins with nucleosomal DNA (Ito et al., 1997). Remarkably, all three factors isolated contained ISWI, an ATPase that had been identified earlier on the basis of sequence similarity to the SWI2/SNF2 homolog in *Drosophila*, Brahma (BRM) and was therefore called Imitation SWItch (Elfring et al., 1994).

SWI2/SNF2 belongs to the DEAD/H superfamily of nucleic-acid-stimulated ATPases

Enzymes that resemble SWI2/SNF2 in their ATPase domains form a distinct subfamily within the family of nucleic-acid-stimulated DEAD/H ATPases (Eisen et al., 1995; Peterson, 2000). The SWI2/SNF2 subfamily can be further divided into at least three groups of enzymes, according to their domain structures (see Fig. 2). ATPases of the SWI2 group contain a bromodomain, whereas ISWI-like ATPases feature a SANT domain. CHD-type enzymes, such as Mi-2, contain chromodomains and PHD fingers (Peterson, 2000). These three groups can also be distinguished by their biochemical properties and mechanisms of nucleosome remodeling (Boyer et al., 2000; Brehm et al., 2000; Guschin et al., 2000b).

Since SWI/SNF-type remodeling machines were the first to be identified, a wealth of biochemical and functional data has accumulated. Less is known about ISWI-containing remodeling factors. However, during the past three years a considerable body of data has accumulated, demonstrating that the two types of remodeling machines are functionally distinct. Here we summarize recent data on the ISWI group of remodelers and their function in chromatin dynamics and organization.

ISWI powers several nucleosome-remodeling machines

Three different ISWI-containing nucleosome-remodeling complexes were purified from *Drosophila* embryo extracts: nucleosome remodeling factor (dNURF), ATP-dependent chromatin-assembly and -remodeling factor (ACF) and chromatin accessibility complex (CHRAC). NURF was traced in the Wu laboratory following an assay that monitored the ATP-dependent interaction of the GAGA transcription factor with nucleosomal binding sites, which leads to DNaseI hypersensitivity at the *hsp*70 promoter in vitro (Tsukiyama et al., 1995; Tsukiyama and Wu, 1995). In NURF, ISWI is associated with three other subunits, which have molecular weights of 300 kDa, 55 kDa and 38 kDa (see Fig. 3). NURF-55 is a WD40 protein that was found earlier as a subunit of the histone chaperone CAF-1 and the highly related RbAp48 family of factors that associate with NURD, Mi-2 and Sin3 complexes (Martinez-Balbas et al., 1998; Wade et al., 1999; Zhang et al., 1999). NURF-38 is a pyrophosphatase whose function in nucleosome remodeling is not known (Gdula et al., 1998).

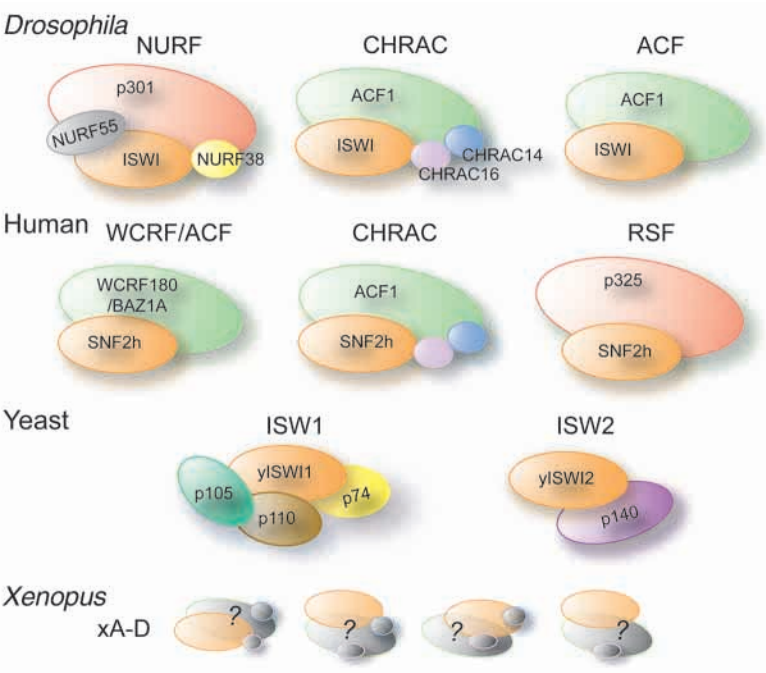

Fig. 3. Summary of the known ISWI-containing nucleosome-remodeling complexes. The known subunits are represented as spheres; protein families are indicated by colour coding. References: NURF (Tsukiyama et al., 1995); dCHRAC (Varga-Weisz et al., 1997); dACF (Ito et al., 1997); WCRF/hACF (Bochar et al., 1999; LeRoy et al., 2000); h CHRAC (Poot et al., 2000); RSF (LeRoy et al., 1998); ISW1 and ISW2 (Tsukiyama et al., 1999); *Xenopus* complexes (Guschin et al., 2000a).

ACF was discovered in the Kadonaga laboratory during a systematic fractionation of the embryo extracts for components required for the assembly of nucleosomal arrays that have a regular spacing. However, not only can ACF catalyze the establishment of regularity within an unordered succession of nucleosomes, but it can also mobilize nucleosomes to facilitate the interaction of DNA-binding proteins (Ito et al., 1997). In ACF, ISWI associates with Acf1, a 170 kDa factor featuring a bromodomain and PHD fingers (Ito et al., 1999).

Our group purified dCHRAC from an activity that promoted a global, energy-dependent increase in accessibility of chromatin (Varga-Weisz et al., 1997). The earlier hypothesis (reflected in the name) that such an activity should somehow 'crack chromatin open' was challenged by the observation that CHRAC can also function as a nucleosome-spacing factor and hence play a role during chromatin assembly. CHRAC is related to ACF, since it also contains Acf1 (Eberharter et al., 2001). In addition, however, CHRAC also contains the novel histone-fold proteins CHRAC-14 and CHRAC-16 (Corona et al., 2000; see Fig. 3). Because of its extensive copurification and co-immunoprecipitation we originally suggested that topoisomerase II (Topo II) is a subunit of dCHRAC (Varga-Weisz et al., 1997); however, TopoII can be separated from CHRAC without affecting CHRAC function or integrity (Eberharter et al., 2001).

Homology searches led to the identification of two ISWI homologs in yeast (yISW1 and yISW2) and mammals (SNF2h and SNF2L), as well as a *Xenopus* ISWI homolog. The yeast enzymes form two distinct complexes (Tsukiyama et al., 1999; see Fig. 3). In frogs, at least four different complexes exist, the simplest one being of the ACF type (Guschin et al., 2000a). Besides hCHRAC (Poot et al., 2000), several ACF-like complexes have been identified in human cells (Bochar et al., 2000; LeRoy et al., 2000). Acf1 is a member of a growing family of proteins that have similar domain architectures (Bochar et al., 2000; Jones et al., 2000; Poot et al., 2000), including WSTF, whose gene is invariantly deleted (among others) in the genome of William-Beuren syndrome patients (Peoples et al., 1998). Association of ISWI with a novel, 300 kDa protein produces the remodeling and spacing factor (hRSF; LeRoy et al., 2000).

The analysis of the activity of recombinant *Drosophila* ISWI expressed in bacteria and therefore removed from the context of other subunits demonstrated that the enzyme, in principle, can trigger a nucleosome-remodeling reaction. Its ATPase activity is stimulated maximally by the presence of nucleosomes and it is able to catalyze basic nucleosome-remodeling and -spacing reactions (see below; Clapier et al., 2001; Corona et al., 1999; Längst et al., 1999). However, the activity of ISWI is stimulated substantially (Ito et al., 1999; Hamiche et al., 1999; Längst et al., 1999; Eberharter et al., 2001) and modulated qualitatively (Eberharter et al., 2001) by other subunits within the remodeling complexes. ISWI has never been isolated on its own from a physiologically relevant source following a functional assay. Most, if not all, ISWI is therefore probably associated with other proteins in the cell.

Genetic analyses suggest complex functions for ISWI

The little we know about the biological roles of the ISWI-group remodelers comes from genetic analyses in fruit flies and yeast. Visualization of ISWI on *Drosophila* polytene chromosomes showed no overlap between ISWI and RNA polymerase, arguing against a major role for ISWI complexes in transcriptional activation (Deuring et al., 2000). Homozygous deletion and expression of dominant negative forms of ISWI in flies suggest that ISWI is involved in control of cell viability, developmentally regulated gene expression and chromosome structure (Deuring et al., 2000). The complex phenotype may be explained if the three ISWI remodeling complexes in flies (see above) have non-redundant functions and indirect effects.

Drosophila lacking ISWI die in the late larval or early pupal stage, presumably because maternal RNA and protein still supports development until then. This allows visualization of the polytene chromosomes of dying larvae. Remarkably, the X chromosome appears severely distorted in male mutant cells, whereas normal X chromosome morphology is observed in female cells. In flies the male X chromosome is targeted by the dosage-compensation machinery, which leads to hypertranscription of many X-linked genes throughout the chromosome. This involves the loosening of chromosome structure by site-specific histone acetylation. In the absence of ISWI, the structure of this 'sensitized' chromosome is no longer maintained, which points to a requirement for ISWI for organization of higher-order chromatin folding (Deuring et al., 2000).

In contrast to the lethal phenotype in flies, no significant phenotype is evident in yeast lacking either *ISW1* and *ISW2* under normal growth conditions, although transcription of a number of genes is altered (Hughes et al., 2000). *ISW1* and *ISW2* homozygous mutants exhibit defective early stages of sporulation (Trachtulcova et al., 2000). Tsukiyama and colleagues noted that in an *ISW2* mutant several meiosis-specific genes are derepressed under normal growth conditions (Goldmark et al., 2000). They found that repression of the meiotic *REC104* promoter involves the targeting of the ISW2 complex to the promoter through direct interaction with the sequence-specific Ume6p repressor. Ume6p requires the ISW2 complex to establish a repressive chromatin structure, which is further stabilized by deacetylation by the RPD3-SIN3 deacetylase complex. Targeting of the nucleosome remodeler correlates with altered nucleosomal positions. Kent et al recently showed that specific nucleosome positioning at several promoters depends on ISW1, ISW2 or both ATPases (Kent et al, 2001). The concept that emerges from these analyses of physiological chromatin structure is that ISWI-containing nucleosome-remodeling factors are involved in the (re)positioning of short arrays of nucleosomes at regulatory sites and is consistent with their biochemical identification as nucleosome mobilizers.

Chromatin remodeling increases factor access and repositions nucleosomes

Without exception, all ISWI-containing factors are able to increase the accessibility of nucleosomal DNA to incoming DNA-binding proteins. In vitro, they facilitate the interaction of these proteins with chromatin in a rather untargeted manner. Depending on the experimental conditions, native transcription factors (Tsukiyama and Wu, 1995; Längst et al., 1997; Di Croce et al., 1999; Dilworth et al., 2000), synthetic activators

(Ito et al., 1997; Mizuguchi et al., 1997; LeRoy et al., 1998), the replication initiator T-antigen (Alexiadis et al., 1998) and even prokaryotic restriction enzymes (Varga-Weisz et al., 1997; Boyer et al., 2000) can profit from the remodeling reaction. In appropriate experimental systems in which the regulatory DNA-binding protein is limiting a downstream reaction, remodeling can lead to complex read-outs, such as transcription (Di Croce et al., 1999; Ito et al., 1997; LeRoy et al., 1998; Mizuguchi et al., 1997; Okada and Hirose, 1998) or replication (Alexiadis et al., 1998) from chromatin templates. The few direct comparisons between remodelers containing ISWI in different contexts indicate that there might be preferred circumstances for each activity (Mizuguchi et al., 1997; Varga-Weisz et al., 1997). There appear to be clear and fundamental differences in the way in which different remodeling factors deal with nucleosomal arrays. Excess NURF will, for example, lead to a non-specific global perturbation of a regular nucleosomal array, if its stoichiometry approaches that of the nucleosomal substrate (Tsukiyama et al., 1995). ACF and CHRAC, in contrast, will not disturb a nucleosomal array, but instead catalyze the opposite reaction, the improvement of nucleosome 'spacing' within an array. This property correlates with the ability of these factors to function as 'spacing factors' in defined chromatin reconstitution systems (see below). These different features must be due to the additional subunits that associate with ISWI to form the different remodeling factors.

As DNA-binding proteins gain access to their binding sites, the nucleosomes that previously occupied the position are remodeled such that they can no longer be detected. However, neighboring nucleosomes are also affected: frequently, randomly positioned nucleosomes acquire an optimal distance from a DNA-bound protein (Längst et al., 1998; Längst et al., 1999; Pazin et al., 1997; Tsukiyama and Wu., 1995; Varga-Weisz et al., 1995; Wall et al., 1995). This phenomenon shows that nucleosomes that have been a substrate for ATP-dependent remodeling are not irreversibly disassembled, but instead repositioned.

Some ISWI remodelers can improve the regularity of nucleosomal arrays

The phenomenon of ATP-dependent nucleosome remodeling is compatible with the intuitive assumption that energy should be needed to unfold a nucleosome, which is held together by many histone-DNA interactions. The finding that several of the remodeling machines powered by ISWI stimulate the assembly of nucleosomal arrays came as a surprise. Chromatin assembly, associated with the replication fork or the repair machinery in non-proliferating cells, requires flexibility of chromatin structure. At the replication fork or lesion site, the regular nucleosomal array characteristic of the bulk of physiological chromatin must be re-established. The first steps of chromatin assembly lead to the wrapping of a DNA segment around a histone octamer, the formation of a nucleosome. In a subsequent ATP-dependent process, unordered successions of nucleosomes are converted into arrays that exhibit regular spacing between individual particles (reviewed by Varga-Weisz and Becker, 1998). Remarkably, *Drosophila* and human ACF and CHRAC complexes, human RSF (Ito et al., 1997; LeRoy et al., 1998; LeRoy et al., 2000; Poot et al., 2000; Varga-Weisz et al., 1997) and the yeast ISW1 complex (Gelbhart et al., 2001) are able to function as nucleosome-spacing factors. Under certain circumstances ACF can even increase the efficiency of nucleosome assembly (Ito et al., 1997; Ito et al., 1999).

The question of what 'spacing' of nucleosomes means in mechanistic terms still has not been conclusively answered (Varga-Weisz and Becker, 1998). Regularity might be established simply through relocation of nucleosomes to facilitate their setting in a regular array of presumed low energy. However, 'spacing factors' might have an additional positive role in the assembly of nucleosomes (Ito et al., 1999; see Fig.4). The observation that the higher-order folding of the acetylated male X chromosome in *Drosophila* is disrupted in the absence of ISWI (Deuring et al., 2000) lends further support to the Janus nature of ISWI remodelers: they may be involved in the assembly of folded chromatin but at the same time assure that the resulting structure remains flexible rather than static.

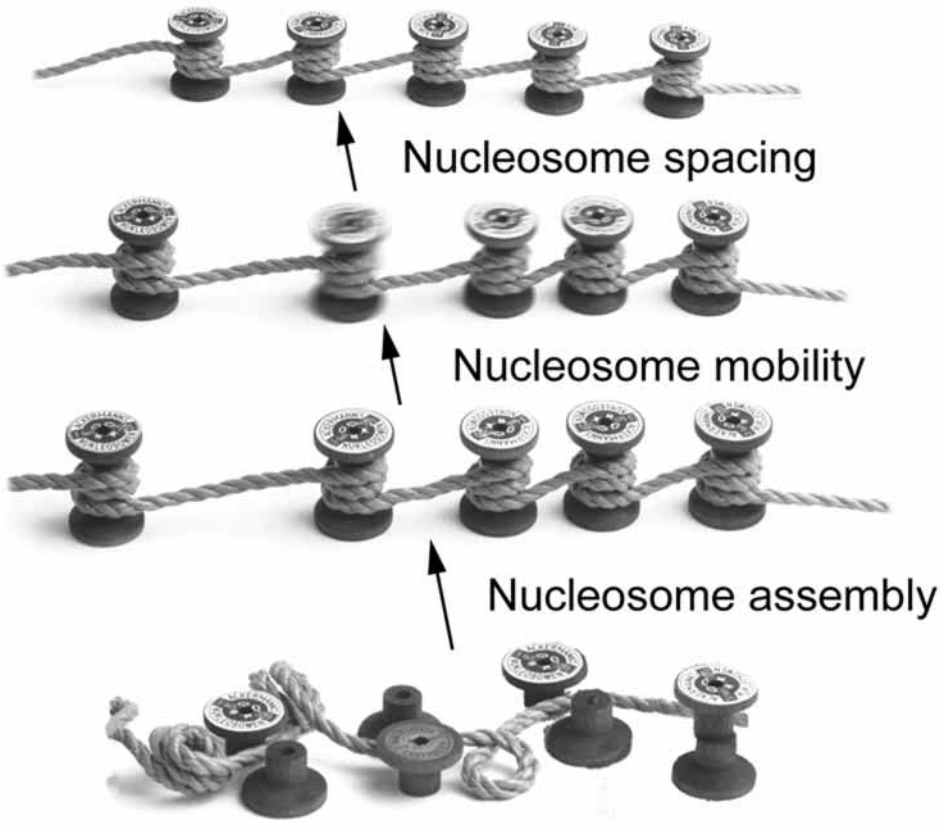

Fig. 4. The processes that ISWI-containing factors are known to be involved in. Graphics by Petra Riedinger/Volker Wiersdorf, EMBL.

ISWI complexes facilitate the sliding of histone octamers

The seemingly opposing activities of ACF, CHRAC and RSF - improving chromatin structure and rendering it transparent to DNA-binding factors - can be reconciled if one assumes that nucleosome-remodeling machines facilitate the translational movement of histone octamers relative to a given DNA segment. Obviously, many processes would profit from moving nucleosomes about. Theoretically, histone octamers could be moved by fast disassembly and reassembly on a neighboring DNA fragment. Alternatively DNA might be translocated relative to an intact octamer. This latter possibility is supported by an in vitro assay that monitors the relocation of a single histone octamer on a small DNA fragment. In this assay, NURF and CHRAC are able to trigger the ATP-dependent movement of intact histone octamers relative to segments of DNA in *cis*, without obvious transfer of histones to competing DNA fragments - a process that, in the absence of mechanistic insight, is termed nucleosome sliding (Hamiche et al., 1999; Längst et al., 1999). The new position of the nucleosome is probably a function of histone-DNA interactions and hence depends on sequence and curvature of the underlying DNA and the specifics of the interaction of the remodeling factor with the nucleosome (see below).

Other remodeling ATPases, such as the CHD-type ATPase Mi-2 and the SWI/SNF complex, also facilitate nucleosome sliding on linear DNA (Brehm et al., 2000; Guschin et al., 2000b; Whitehouse et al., 1999). Recombinant ISWI is also able to mobilize nucleosomes, but interestingly the outcome of such mobilization differs when ISWI and CHRAC are compared. Whereas CHRAC is able to move a nucleosome from one end to a more central position of a DNA fragment (but not back), ISWI triggers the converse reaction, the sliding of a nucleosome from the middle of a fragment to its end (Längst et al., 1999). Clearly, at least one other subunit modulates the outcome of ISWI-induced nucleosome remodeling. This factor has recently been identified as Acf-1, the largest subunit of CHRAC and ACF. Association of Acf-1 with ISWI not only stimulates the activity of ISWI by an order of magnitude but also reverses the directionality of nucleosome sliding to resemble the CHRAC-type mobility (Eberharter et al., 2001). Acf-1 therefore provides an example of regulation of a core remodeling machinery by protein-protein association.

Mechanistic considerations

The wrapping of DNA around a histone octamer involves multiple interactions between DNA and histones that render a nucleosome particularly stable. However, sliding of histone octamers on DNA can be induced under conditions in which histone-DNA interactions are weakened, such as extended incubation at elevated temperature and ionic strength (Beard, 1978; Pennings et al., 1991; Ura et al., 1995; for review see Guschin and Wolffe, 1999). Van Holde and Yager suggested early on that Brownian energy might suffice to induce nucleosome mobility by 'twist diffusion' (see below; Van Holde and Yager, 1985). ISWI and other remodeling ATPases may simply catalyse a reaction that would otherwise proceed rather inefficiently under physiological conditions. Spontaneous mobility of nucleosomes is inhibited by binding of the linker histone H1 to the DNA at its nucleosomal entry and exit sites (Pennings et al., 1994; Ura et al., 1995). There are hints that linker histones do not necessarily restrict ATP-dependent mobilization (Varga-Weisz et al., 1995), but the effect of H1 under defined conditions of catalyzed mobility has yet to be determined.

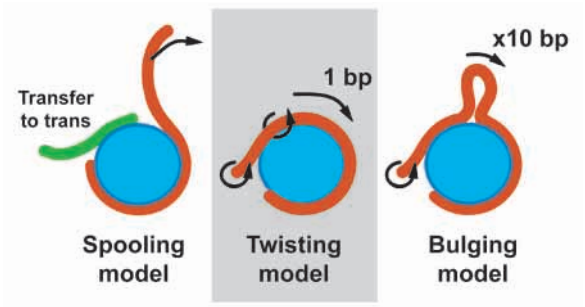

Fig. 5. The essential features of the three distinct models (spooling, twisting and bulging) for nucleosome mobility (see text).

How, then, could nucleosome mobility be facilitated? And why does mobilization lead to directional nucleosome movements? The observed stabilization of the histone octamer by ISWI and CHRAC (Varga-Weisz et al., 1997; Längst et al., 1999) and the fact that no histone transfer is detected argues against a disassembly model that invokes complete or partial disassembly of the histone octamer. The situation may be different for SWI/SNF-induced nucleosome remodeling, in which, particularly at high enzyme concentrations, transfer of histones to competitor DNA can be observed (Lorch et al., 1999; Phelan et al., 2000). SWI/SNF-type remodeling leads to prominent perturbation of histone-DNA interactions, as determined by DNaseI footprinting and an obvious reduction of constrained superhelicity - phenomena that so far have not been documented for ISWI-type remodelers.

Three model scenarios for nucleosome repositioning can be envisaged: spooling, twisting and bulging (Fig. 5). The 'spooling model' (Pazin and Kadonaga, 1997) was inspired by experiments that monitored the transcription of RNA polymerases through nucleosomes (Bednar et al., 1999; and references therein). In this scenario the movement of the enzyme on DNA leads to the peeling off the histone octamer surface of larger DNA segments. The patch of free histone is then available to capture a different DNA segment (see Fig. 5). The 'twisting model' argues that ISWI alters the topology of DNA and thereby changes histone-DNA interactions (Havas et al., 2000; Varga-Weisz and Becker, 1998). As discussed by van Holde and Yager, thermal energy could alter the twist of DNA (van Holde and Yager, 1985), effectively disrupting a set of DNA-histone interactions at the site of entry into the nucleosome and replacing them by analogous interactions involving the neighboring base-pair. Since small distortions of the helix geometry can be accommodated in the nucleosome (Luger and Richmond, 1998), it is possible that the locally altered twist is propagated over the surface of the nucleosome (twist diffusion). Once the helix distortion emerges on the other side of the nucleosome, the DNA will have been displaced by one base pair relative to the octamer surface (see Fig. 5). The 'bulging model' combines aspects from both spooling and twisting models. In analogy to the spooling model ISWI would disrupt histone-DNA interactions, but, as in the twisting model,

only the first contact of the DNA helix at the edge of the nucleosome would be affected. The free histone valency would then interact with DNA one helical turn 'outside' of the nucleosomal realm, effectively bulging out a short DNA segment on the surface of the histone octamer. Propagation of this 'bulge' over the nucleosome surface would lead to displacement of the DNA relative to the histones. Whereas the twisting model predicts that the 'unit length' of nucleosome mobility would be a single base pair as the DNA is screwed over the histone surface, a bulging model would be more consistent with a unit length of mobility of a DNA helix turn (~10.5 base-pairs), and the mobility could be broken down to steps of translational rather than rotational translocation of the DNA relative to the histone octamer.

Recently, Owen-Hughes and colleagues have shown that a variety of remodeling machines, including recombinant ISWI, are able introduce negative superhelicity into linear DNA fragments (Havas et al., 2000), suggesting that these machines alter the twist of DNA and at the same time constrain the resulting superhelical stress within a topological domain. Remarkably, recombinant ISWI was able to induce local DNA supercoils only in the presence of nucleosomes, which is consistent with the fact that its ATPase activity is largely stimulated by nucleosomes. This result could indicate that ISWI needs direct histone contact in order to twist DNA. Alternatively, the nucleosome could itself participate in the formation of a topological domain that allows the accumulation of superhelical stress. By contrast, the SWI/SNF complex is able to generate superhelicity even in the absence of nucleosomes, possibly through its ability to bind two DNA segments and constrain the intervening DNA into a tight loop (Bazett-Jones et al., 1999).

Enzyme-substrate interactions

Intuitively one might assume that an enzyme that alters the position of DNA relative to a histone octamer might have to contact both components and move them relative to each other. There is indeed evidence for both types of interaction. ISWI binds only poorly to nucleosomal cores but interacts well with particles that contain additional linker DNA (Brehm et al., 2000). A domain that might mediate DNA binding is its SANT domain, which resembles the DNA-binding domains of some transcription factors (Aasland et al., 1996). An interaction with histone can be inferred from the recent observation that deletion of the H4 N-terminal tail from nucleosomes prevents ISWI from recognizing the substrate (Clapier et al., 2001).

The role of ATP hydrolysis

How might ATP hydrolysis drive nucleosome mobility? Several scenarios can be envisioned. ISWI might hydrolyze ATP to power a molecular motor that promotes its translocation on DNA - by analogy with helicases and polymerases. If ISWI is immobile, as footprinting experiments using nucleosomal substrates suggest (G.L. and P.B.B., unpublished), it might hydrolyze ATP to twist DNA, thereby converting the chemical energy of ATP into superhelical stress, which could lead to dissociation of a segment of DNA from the histone surface. Theoretically, however, ISWI does not need to manipulate the nucleosome in any active manner. Since thermal energy suffices to untwist the loosely attached DNA at the point of entry into the nucleosome, ISWI might simply endow the natural 'twist diffusion' with directionality. In any case, the binding of ATP is likely to influence the way that ISWI interacts with the nucleosomal substrate. Nucleotide binding, subsequent hydrolysis and nucleotide exchange might constitute a cycle of enzyme conformations that in turn determine distinct interactions with the nucleosome.

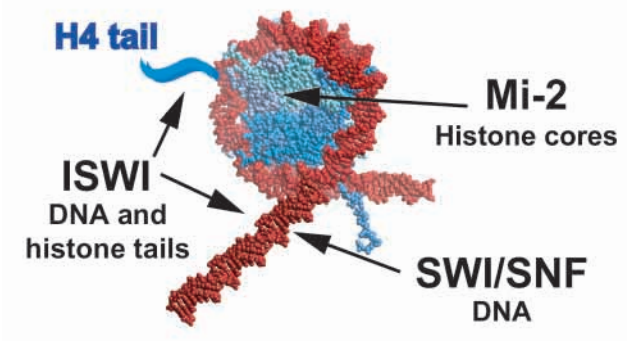

Fig. 6. Summary of contact points on the nucleosome for the nucleosome-remodeling ATPases listed in Fig. 2.

Directionality

The observation that Acf-1 is able to improve the efficiency and alter the directionality of ISWI-induced nucleosome sliding in vitro is intriguing (Eberharter et al., 2001). Despite the non-physiological nature of short chromatin fragments, the results still indicate profoundly different interaction of the remodeling factor with the nucleosome. Since bromodomains exhibit a preference for binding to an acetylated isoform of the H4 N-terminus (Owen et al., 2000), Acf-1 activity might be modulated by stable modification of the H4 N-terminus. Whether the PHD fingers and the WAC domain of Acf-1 contribute to the remodeling mechanism, or function in complex assembly and/or targeting of the enzyme to specific nuclear compartments, remains to be determined. It is already becoming clear that the result of nucleosome mobilization is a function of histone-DNA interactions (which are in turn determined by DNA sequence and curvature) and enzyme-DNA interactions.

Not all remodeling enzymes are equal

Although ATPases of the ISWI, CHD and SWI/SNF classes can all catalyze nucleosome sliding, the substrate requirements differ for each enzyme. The ATPase of the SWI/SNF complex is already maximally stimulated by free DNA (Boyer et al., 2000) and can induce superhelicity into DNA in the absence of histones (Havas et al., 2000). Nucleosome remodeling by SWI/SNF does not require histone N-termini (Boyer et al., 2000). Mi-2 represents a different case: its ATPase is stimulated by nucleosomal DNA but not at all by free DNA and yet histone N-termini are dispensable for remodeling (Brehm et al., 2000). The ATPase of ISWI is partially activated by DNA and further stimulated by nucleosomes. However, deletion of the H4 tail abolishes the recognition of the nucleosome substrate (Clapier et al., 2001; see Fig. 6). There are other indications that nucleosome remodeling by ISWI differs fundamentally from SWI/SNF-type remodeling. High concentrations of the latter type of factor eventually lead to eviction of nucleosomes from the DNA fragment and to the accumulation of nucleosomes that have stable structural

changes and resemble ‘dinucleosome particles’ in many respects (Lorch et al., 1998; Lorch et al., 1999; Phelan et al., 2000; Schnitzler et al., 1998). It is unclear as yet whether these structures correspond to trapped remodeling intermediates or non-productive ‘dead-end’ molecules. Neither octamer eviction nor stable structures representing the ‘remodeled’ state have so far been observed during remodeling by ISWI factors. Apparently, there is more than one mechanism by which a remodeling ATPase can mobilize a nucleosome.

Perspectives

Although only little is known about the physiological functions of the ISWI family of nucleosome-remodeling factors in higher eukaryotes, they have great potential in regulation of diverse nuclear functions. Mobilization of nucleosomes seems to be an elegant solution to the problem of rendering chromatin structure transparent while maintaining a high degree of charge neutralization of DNA. Clearly, there are still more open questions than have been answered. Are there levels of chromatin organization that restrict nucleosome mobility, or nuclear compartments that are ‘off limits’ for remodeling factors? Is the remodeling activity of ISWI regulated as a function of cell type, cell cycle or environmental signals? Are ISWI-containing nucleosome-remodeling factors targeted to specific gene loci, chromosomal domains or nuclear compartments by interaction with specific factors? A recent report from the Wolffe lab provokes an even more fundamental question: are there any other substrates for ISWI besides nucleosomes? Kikiyo et al. observed that ISWI was involved in active displacement of the general transcription factor TBP from the nuclear matrix of differentiated cells during the global remodeling that occurs if nuclei are incubated in *Xenopus* egg extract (Kikiyo et al., 2000). Our current bias towards nucleosomal substrates may thus simply reflect the lack of a comprehensive survey for alternative substrates.

Work in the laboratory of the authors was supported by Deutsche Forschungsgemeinschaft and TMR Network Grant ‘Gene Regulation by chromatin’ from the European Union.

References

Aasland, R., Stewart, A. F. and Gibson, T. (1996). The SANT domain: a putative DNA-binding domain in the SWI-SNF and ADA complexes, the transcriptional co-repressor N-CoR and TFIIIB. *Trends Biochem. Sci.* **21**, 87-88.

Alexiadis, V., Varga-Weisz, P. D., Bonte, E., Becker, P. B. and Gruss, C. (1998). In vitro chromatin remodelling by chromatin accessibility complex (CHRAC) at the SV40 origin of DNA replication. *EMBO J.* **17**, 3428-3438.

Bazett-Jones, D. P., Cote, J., Landel, C. C., Peterson, C. L. and Workman, J. L. (1999). The SWI/SNF complex creates loop domains in DNA and polynucleosome arrays and can disrupt DNA-histone contacts within these domains. *Mol. Cell. Biol.* **19**, 1470-1478.

Beard, P. (1978). Mobility of histones on the chromosome of Simian Virus 40. *Cell* **15**, 955-967.

Bednar, J., Studitsky, V. M., Grigoryev, S. A., Felsenfeld, G. and Woodcock, C. L. (1999). The nature of the nucleosomal barrier to transcription: direct observation of paused intermediates by electron cryomicroscopy. *Mol. Cell* **4**, 377-386.

Bochar, D. A., Savard, J., Wang, W., Lafleur, D. W., Moore, P., Cote, J. and Shiekhattar, R. (2000). A family of chromatin remodeling factors related to Williams syndrome transcription factor. *Proc. Natl. Acad. Sci. USA* **97**, 1038-1043.

Boyer, L. A., Logie, C., Bonte, E., Becker, P. B., Wade, P. A., Wolffe, A. P., Wu, C., Imbalzano, A. N. and Peterson, C. L. (2000). Functional delineation of three groups of the ATP-dependent family of chromatin remodeling enzymes. *J. Biol. Chem.* **275**, 18864-18870.

Brehm, A., Längst, G., Kehle, J., Clapier, C. R., Imhof, A., Eberharter, A., Muller, J. and Becker, P. B. (2000). dMi-2 and ISWI chromatin remodelling factors have distinct nucleosome binding and mobilization properties. *EMBO J.* **19**, 4332-4341.

Clapier, C. R., Längst, G., Corona, D. F., Becker, P. B. and Nightingale, K. P. (2001). Critical role for the histone H4 N-terminus in nucleosome remodeling by ISWI. *Mol. Cell. Biol.* **21**, 875-883.

Corona, D. F., Längst, G., Clapier, C. R., Bonte, E. J., Ferrari, S., Tamkun, J. W. and Becker, P. B. (1999). ISWI is an ATP-dependent nucleosome-remodeling factor. *Mol. Cell* **3**, 239-245.

Corona, D. F., Eberharter, A., Budde, A., Deuring, R., Ferrari, S., Varga-Weisz, P., Wilm, M., Tamkun, J. and Becker, P. B. (2000). Two histone fold proteins, CHRAC-14 and CHRAC-16, are developmentally regulated subunits of chromatin accessibility complex (CHRAC). *EMBO J.* **19**, 3049-3059.

de La Serna, I. L., Carlson, K. A. and Imbalzano, A. N. (2001). Mammalian SWI/SNF complexes promote MyoD-mediated muscle differentiation. *Nat. Genet.* **27**, 187-190.

Deuring, R., Fanti, L., Armstrong, J. A., Sarte, M., Papoulas, O., Prestel, M., Daubresse, G., Verardo, M., Moseley, S. L., Berloco, M. et al. (2000). The ISWI chromatin-remodeling protein is required for gene expression and the maintenance of higher order chromatin structure in vivo. *Mol. Cell* **5**, 355-365.

Di Croce, L., Koop, R., Venditti, P., Westphal, H. M., Nightingale, K. P., Corona, D. F., Becker, P. B. and Beato, M. (1999). Two-step synergism between the progesterone receptor and the DNA-binding domain of nuclear factor 1 on MMTV minichromosomes. *Mol. Cell* **4**, 45-54.

Dilworth, F. J., Fromental-Ramain, C., Yamamoto, K. and Chambon, P. (2000). ATP-driven chromatin remodeling activity and histone acetyltransferases act sequentially during transactivation by RAR/RXR in vitro. *Mol. Cell* **6**, 1049-1058.

Eberharter, A., Ferrari, S., Längst, G., Straub, T., Imhof, A., Varga-Weisz, P. D., Wilm, M. and Becker, P. B. (2001). Acf1, the largest subunit of CHRAC, regulates ISWI-induced nucleosome remodelling. *EMBO J.* (in press).

Eisen, J. A., Sweder, K. S. and Hanawalt, P. C. (1995). Evolution of the SNF2 family of proteins: subfamilies with distinct sequences and functions. *Nucl. Acids Res.* **23**, 2715-2723.

Elfring, L. K., Deuring, R., McCallum, C. M., Peterson, C. L. and Tamkun, J. W. (1994). Identification and characterization of *Drosophila* relatives of the yeast transcriptional activator SNF2/SWI2. *Mol. Cell. Biol.* **14**, 2225-2234.

Flanagan, J. F. and Peterson, C. L. (1999). A role for the yeast SWI/SNF complex in DNA replication. *Nucl. Acids Res.* **27**, 2022-2028.

Gdula, D. A., Sandaltzopoulos, R., Tsukiyama, T., Ossipow, V. and Wu, C. (1998). Inorganic pyrophosphatase is a component of the *Drosophila* nucleosome-remodeling factor complex. *Genes Dev.* **12**, 3206-3216.

Gelbart, M. E., Rechsteiner, T., Richmond, T. J. and Tsukiyama, T. (2001). Interactions of Isw2 chromatin remodeling complex with nucleosomal arrays: analyses using recombinant yeast histones and immobilized templates. *Mol. Cell. Biol.* **21**, 2098-2106.

Goldmark, J. P., Fazzio, T. G., Estep, P. W., Church, G. M. and Tsukiyama, T. (2000). The Isw2 chromatin remodeling complex represses early meiotic genes upon recruitment by Ume6p. *Cell* **103**, 423-433.

Guschin, D. and Wolffe, A. P. (1999). Transcriptional control: SWItched on mobility. *Curr. Biol.* **9**, 742-746.

Guschin, D., Geiman, T. M., Kikyo, N., Tremethick, D. J., Wolffe, A. P. and Wade, P. A. (2000a). Multiple ISWI ATPase complexes from *Xenopus laevis*. Functional conservation of an ACF/CHRAC homolog. *J. Biol. Chem.* **275**, 35248-35255.

Guschin, D., Wade, P. A., Kikyo, N. and Wolffe, A. P. (2000b). ATP-dependent histone octamer mobilization and histone acetylation mediated by the Mi-2 chromatin remodeling complex. *Biochemistry* **39**, 5238-5245.

Hamiche, A., Sandaltzopoulos, R., Gdula, D. A. and Wu, C. (1999). ATP-dependent histone octamer sliding mediated by the chromatin remodeling complex NURF. *Cell* **97**, 833-842.

Havas, K., Flaus, A., Phelan, M., Kingston, R., Wade, P. A., Lilley, D. M. and Owen-Hughes, T. (2000). Generation of superhelical torsion by ATP-dependent chromatin remodeling activities. *Cell* **103**, 1133-1142.

Hughes, T. R., Marton, M. J., Jones, A. R., Roberts, C. J., Stoughton, R., Armour, C. D., Bennett, H. A., Coffey, E., Dai, H., He, Y. D. et al. (2000).

Functional discovery via a compentium of expression profiles. *Cell* **102**, 109-126.

Ito, T., Bulger, M., Pazin, M. J., Kobayashi, R. and Kadonaga, J. T. (1997). ACF, an ISWI-containing and ATP-utilizing chromatin assembly and remodeling factor. *Cell* **90**, 145-155.

Ito, T., Levenstein, M. E., Fyodorov, D. V., Kutach, A. K., Kobayashi, R. and Kadonaga, J. T. (1999). ACF consists of two subunits, Acf1 and ISWI, that function cooperatively in the ATP-dependent catalysis of chromatin assembly. *Genes Dev.* **13**, 1529-1539.

Jones, M. H., Hamana, N., Nezu, J. and Shimane, M. (2000). A novel family of bromodomain genes. *Genomics* **63**, 40-45.

Kent, N. A., Karabetsou, N., Politis, P. K. and Mellor, J. (2001). In vivo chromatin remodeling by yeast ISWI homologs Iswi1 and Isw2p. *Genes Dev.* **15**, 619-626.

Kikyo, N., Wade, P. A., Guschin, D., Ge, H. and Wolffe, A. P. (2000). Active remodeling of somatic nuclei in egg cytoplasm by the nucleosomal ATPase ISWI. *Science* **289**, 2360-2362.

Krebs, J. E., Fry, C. J., Samuels, M. L. and Peterson, C. L. (2000). Global role for chromatin remodeling enzymes in mitotic gene expression. *Cell* **102**, 587-598.

Kwon, J., Morshead, K. B., Guyon, J. R., Kingston, R. E. and Oettinger, M. A. (2000). Histone acetylation and hSWI/SNF remodeling act in concert to stimulate V(D)J cleavage of nucleosomal DNA. *Mol. Cell* **6**, 1037-1048.

Längst, G., Blank, T. A., Becker, P. B. and Grummt, I. (1997). RNA polymerase I transcription on nucleosomal templates: the transcription termination factor TTF-I induces chromatin remodeling and relieves transcriptional repression. *EMBO J.* **16**, 760-768.

Längst, G., Becker, P. B. and Grummt, I. (1998). TTF-I determines the chromatin architecture of the active rDNA promoter. *EMBO J.* **17**, 3135-3145.

Längst, G., Bonte, E. J., Corona, D. F. and Becker, P. B. (1999). Nucleosome movement by CHRAC and ISWI without disruption or trans-displacement of the histone octamer. *Cell* **97**, 843-852.

LeRoy, G., Orphanides, G., Lane, W. S. and Reinberg, D. (1998). Requirement of RSF and FACT for transcription of chromatin templates in vitro. *Science* **282**, 1900-1904.

LeRoy, G., Loyola, A., Lane, W. S. and Reinberg, D. (2000). Purification and characterization of a human factor that assembles and remodels chromatin. *J. Biol. Chem.* **275**, 14787-14790.

Lorch, Y., Cairns, B. R., Zhang, M. and Kornberg, R. D. (1998). Activated RSC-nucleosome complex and persistently altered form of the nucleosome. *Cell* **94**, 29-34.

Lorch, Y., Zhang, M. and Kornberg, R. D. (1999). Histone octamer transfer by a chromatin-remodeling complex. *Cell* **96**, 389-392.

Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F. and Richmond, T. J. (1997). Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* **389**, 251-260.

Luger, K. and Richmond, T. J. (1998). DNA binding within the nucleosome core. *Curr. Opin. Struct. Biol.* **8**, 33-40.

Martinez-Balbas, M. A., Tsukiyama, T., Gdula, D. and Wu, C. (1998). *Drosophila* NURF-55, a WD repeat protein involved in histone metabolism. *Proc. Natl. Acad. Sci. USA* **95**, 132-137.

Mizuguchi, G., Tsukiyama, T., Wisniewski, J. and Wu, C. (1997). Role of nucleosome-remodeling factor NURF in transcriptional activation of chromatin. *Mol. Cell* **1**, 141-150.

Muchardt, C. and Yaniv, M. (1999). ATP-dependent chromatin remodelling: SWI/SNF and Co. are on the job. *J. Mol. Biol.* **293**, 187-198.

Muchardt, C., Bourachot, B., Reyes, J. C. and Yaniv, M. (1998). Ras transformation is associated with decreased expression of the brm/SNF2α ATPase from the mammalian SWI-SNF complex. *EMBO J.* **17**, 223-231.

Okada, M. and Hirose, S. (1998). Chromatin remodeling mediated by *Drosophila* GAGA factor and ISWI activates fushi tarazu gene transcription in vitro. *Mol. Cell. Biol.* **18**, 2455-2461.

Owen, D. J., Ornaghi, P., Yang, J. C., Lowe, N., Evans, P. R., Ballario, P., Neuhaus, D., Filetici, P. and Travers, A. A. (2000). The structural basis for the recognition of acetylated histone H4 by the bromodomain of histone acetyltransferase gcn5p. *EMBO J.* **19**, 6141-6149.

Pazin, M. J. and Kadonaga, J. T. (1997). SWI2/SNF2 and related proteins: ATP-driven motors that disrupt protein-DNA interactions? *Cell* **88**, 737-740.

Pazin, M. J., Bhargava, P., Geiduschek, E. P. and Kadonaga, J. T. (1997). Nucleosome mobility and the maintenance of nucleosome positioning. *Science* **276**, 809-812.

Pennings, S., Meersseman, G. and Bradbury, M. E. (1991). Mobility of positioned nucleosomes on 5S rDNA. *J. Mol. Biol.* **220**, 101-110.

Pennings, S., Meersseman, G. and Bradbury, M. E. (1994). Linker histones H1 and H5 prevent the mobility of positioned nucleosomes. *Proc. Natl. Acad. Sci. USA* **91**, 10275-10279.

Peoples, R. J., Cisco, M. J., Kaplan, P. and Francke, U. (1998). Identification of the WBSCR9 gene, encoding a novel transcriptional regulator, in the Williams-Beuren syndrome deletion at 7q11.23. *Cytogenet. Cell Genet.* **82**, 238-246.

Peterson, C. L. (2000). ATP-dependent chromatin remodeling: going mobile. *FEBS Lett.* **476**, 68-72.

Phelan, M. L., Schnitzler, G. R. and Kingston, R. E. (2000). Octamer transfer and creation of stably remodeled nucleosomes by human SWI-SNF and its isolated ATPases. *Mol. Cell. Biol.* **20**, 6380-6389.

Poot, R. A., Dellaire, G., Hulsmann, B. B., Grimaldi, M. A., Corona, D. F., Becker, P. B., Bickmore, W. A. and Varga-Weisz, P. D. (2000). HuCHRAC, a human ISWI chromatin remodelling complex contains hACF1 and two novel histone-fold proteins. *EMBO J.* **19**, 3377-3387.

Schnitzler, G., Sif, S. and Kingston, R. E. (1998). Human SWI/SNF interconverts a nucleosome between its base state and a stable remodeled state. *Cell* **94**, 17-27.

Trachtulcova, P., Janatova, I., Kohlwein, S. D. and Hasek, J. (2000). *Saccharomyces cerevisiae* gene ISW2 encodes a microtubule-interacting protein required for premeiotic DNA replication. *Yeast* **16**, 35-47.

Tsukiyama, T. and Wu, C. (1995). Purification and properties of an ATP-dependent nucleosome-remodeling factor. *Cell* **83**, 1011-1020.

Tsukiyama, T., Daniel, C., Tamkun, J. and Wu, C. (1995). ISWI, a member of the SWI2/SNF2 ATPase family, encodes the 140 kDa subunit of the nucleosome-remodeling factor. *Cell* **83**, 1021-1026.

Tsukiyama, T., Palmer, J., Landel, C. C., Shiloach, J. and Wu, C. (1999). Characterization of the imitation switch subfamily of ATP-dependent chromatin-remodeling factors in *Saccharomyces cerevisiae*. *Genes Dev.* **13**, 686-697.

Ura, K., Hayes, J. J., and Wolffe, A. P. (1995). A positive role for nucleosome mobility in the transcriptional activity of chromatin templates: restriction by linker histones. *EMBO J.* **14**, 3752-3765.

Ura, K., Araki, M., Saeki, H., Masutani, C., Ito, T., Iwai, S., Mizukoshi, T., Kaneda, Y. and Hanaoka, F. (2001). ATP-dependent chromatin remodeling facilitates nucleotide excision repair of UV-induced DNA lesions in synthetic dinucleosomes. *EMBO J.* **20**, 2004-2014.

Van Holde, K. E. and Yager, T. D. (1985). Nucleosome motion: evidence and models. In *Structure and Function of the Genetic Apparatus*. (eds C. Nicolini and P. O. P. Tso). pp. 35-53. New York: Plenum Press.

Varga-Weisz, P. D. and Becker, P. B. (1998). Chromatin-remodeling factors: machines that regulate? *Curr. Opin. Cell Biol.* **10**, 346-353.

Varga-Weisz, P. D., Blank, T. A. and Becker, P. B. (1995). Energy-dependent chromatin accessibility and nucleosome mobility in a cell-free system. *EMBO J.* **14**, 2209-2216.

Varga-Weisz, P. D., Wilm, M., Bonte, E., Dumas, K., Mann, M. and Becker, P. B. (1997). Chromatin-remodelling factor CHRAC contains the ATPases ISWI and topoisomerase II. *Nature* **388**, 598-602.

Vignali, M., Hassan, A. H., Neely, K. and Workman, J. (2000). ATP-dependent chromatin-remodeling complexes. *Mol. Cell. Biol.* **20**, 1899-1910.

Wade, P. A., Gegonne, A., Jones, P. L., Ballestar, E., Aubry, F. and Wolffe, A. P. (1999). Mi-2 complex couples DNA methylation to chromatin remodelling and histone deacetylation. *Nat. Genet.* **23**, 62-66.

Wall, G., Varga-Weisz, P. D., Sandaltzopoulos, R. and Becker, P. B. (1995). Chromatin remodeling by GAGA factor and heat shock factor at the hypersensitive *Drosophila* hsp26 promoter in vitro. *EMBO J.* **14**, 1727-1736.

Whitehouse, I., Flaus, A., Cairns, B. R., White, M. F., Workman, J. L. and Owen-Hughes, T. (1999). Nucleosome mobilization catalysed by the yeast SWI/SNF complex. *Nature* **400**, 784-787.

Winston, F. and Carlson, M. (1992). Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. *Trends Genet.* **8**, 387-391.

Workman, J. L. and Kingston, R. E. (1998). Alteration of nucleosome structure as a mechanism of transcriptional regulation. *Annu. Rev. Biochem.* **67**, 545-579.

Zhang, Y., Ng, H. H., Erdjument-Bromage, H., Tempst, P., Bird, A. and Reinberg, D. (1999). Analysis of the NuRD subunits reveals a histone deacetylase core complex and a connection with DNA methylation. *Genes Dev.* **13**, 1924-1935.