Acf1, the largest subunit of CHRAC, regulates ISWI-induced nucleosome remodelling

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The **chromatin accessibility complex (CHRAC)** was originally defined biochemically as an ATP-dependent 'nucleosome remodelling' activity. Central to its activity is the ATPase ISWI, which catalyses the transfer of histone octamers between DNA segments *in cis*. In addition to ISWI, four other potential subunits were observed consistently in active CHRAC fractions. We have now identified the p175 subunit of CHRAC as Acf1, a protein known to associate with ISWI in the ACF complex. Interaction of Acf1 with ISWI enhances the efficiency of nucleosome sliding by an order of magnitude. Remarkably, it also modulates the nucleosome remodelling activity of ISWI qualitatively by altering the directionality of nucleosome movements and the histone 'tail' requirements of the reaction. The Acf1-ISWI heteromer tightly interacts with the two recently identified small histone fold proteins CHRAC-14 and CHRAC-16. Whether topoisomerase II is an integral subunit has been controversial. Refined analyses now suggest that topoisomerase II should not be considered a stable subunit of CHRAC. Accordingly, CHRAC can be molecularly defined as a complex consisting of ISWI, Acf1, CHRAC-14 and CHRAC-16.

Keywords: CHRAC/chromatin structure/ISWI/
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Introduction

The packaging of the eukaryotic genome into chromatin has important implications for fundamental nuclear processes such as DNA replication (DePamphilis, 1999), transcription (Pollard and Peterson, 1998; Varga-Weisz and Becker, 1998; Wolffe and Hayes, 1999; Strahl and Allis, 2000), repair (Schlissel, 2000) and recombination (Moggs and Almouzni, 1999; Smerdon and Conconi, 1999). Repressive chromatin structure can be rendered permissive for interaction of regulatory factors through the action of remodelling machines that modulate the structure of the nucleosome (Kadonaga, 1998; Kingston and Narlikar, 1999; Vignali *et al.*, 2000). One type of

'remodelling' machine requires continuous ATP hydrolysis for its activity. Central to their function are ATPases of the SWI2/SNF2 superfamily that are found in all eukaryotes, from yeast to man (Eisen *et al.*, 1995). The currently characterized enzymes define three distinct families of nucleosome remodelling machines: SWI2/SNF2-like, ISWI-like and Mi2-like (Boyer *et al.*, 2000; Brehm *et al.*, 2000; Guschin *et al.*, 2000). Nucleosome remodelling factors driven by the ATPase ISWI have first been isolated by biochemical fractionation of *Drosophila* embryo extracts. Three distinct ISWI-containing complexes with different protein composition and functional characteristics have been isolated: the nucleosome remodelling factor (NURF; Tsukiyama and Wu, 1995), the chromatin accessibility complex (CHRAC; Varga-Weisz *et al.*, 1997) and the ATP-dependent chromatin assembly and remodelling factor (ACF; Ito *et al.*, 1997). For the NURF complex, so far two ISWI-associated proteins have been identified: the WD repeat protein NURF-55, which can also be found in the chromatin assembly factor CAF-1 (Martinez-Balbas *et al.*, 1998), and the inorganic pyrophosphatase NURF-38 (Gdula *et al.*, 1998). Following a chromatin assembly activity, Kadonaga and colleagues discovered ACF, a two-subunit complex in which ISWI associates with the 185 kDa Acf1. Acf1 contains a bromodomain, two PHD fingers and a putative heterochromatin-targeting domain (Ito *et al.*, 1999; Poot *et al.*, 2000). ACF-like complexes have also been found in *Xenopus* and human cells (Bochar *et al.*, 2000; Guschin *et al.*, 2000; Poot *et al.*, 2000). CHRAC was isolated as a factor with two ATP-dependent activities: a remodelling activity increasing the accessibility of nucleosomal arrays and a nucleosome-spacing activity able to convert irregular successions of nucleosomes into regular arrays (Varga-Weisz *et al.*, 1997). These two phenomena may be explained by our recent discovery of ATP-dependent nucleosome sliding, defined as relocation of intact histone octamers on DNA *in cis* without *trans*-displacement (Längst *et al.*, 1999).

CHRAC was characterized initially as a five-subunit complex consisting of ISWI, topoisomerase II (topo II) and three polypeptides with mol. wts of 175, 16 and 14 kDa (Varga-Weisz *et al.*, 1997). We recently identified the two smallest proteins, CHRAC-14 and CHRAC-16, as integral subunits of CHRAC (Corona *et al.*, 2000). These two histone fold proteins interact with each other presumably via a histone-like 'handshake' motif (Arents *et al.*, 1991). Their developmental regulation suggests a specialized role in early embryonic development (Corona *et al.*, 2000).

We have now cloned and identified the 175 kDa subunit of CHRAC. This protein is identical to Acf1, which is also a component of the ACF complex. A modified purification protocol for CHRAC and interaction studies confirm our previous notion of a functional complex consisting of

Fig. 2. Size exclusion chromatography of CHRAC, native topo II and recombinant topo II. Size standards are depicted by arrows. (A) Pooled CHRAC hydroxyapatite fractions (21–24) were separated on a Superose 6 column. Western blot analyses showed that CHRAC eluted with a mol. wt of ~ 600 kDa. (B) Superose 6 chromatography of topo II-containing hydroxyapatite fractions (28–32). (C) Superose 6 chromatography of purified recombinant *Drosophila* topo II. (D) Analysis of recombinant topo II by MALS coupled to a Superdex S-200 sizing column.

fractionation on different chromatography resins. In highly purified fractions obtained from the hydroxyapatite column, we observed a perfect co-fractionation of Acf1, ISWI, CHRAC-16 and CHRAC-14 (Figure 1B). These co-purified with CHRAC activity as measured by the nucleosome sliding assay (data not shown). However, we found that topo II eluted at a higher potassium phosphate concentration (Figure 1B). To separate CHRAC from topo II, it was critical to elute the hydroxyapatite column with a shallow potassium phosphate gradient [i.e. 20 column volumes (CVs)]. We next estimated the size of CHRAC and topo II by gel filtration. Distinct fractions for CHRAC (HAP fractions 21–24) and topo II (HAP fractions 28–32) were pooled, concentrated and subjected to Superose 6 size exclusion chromatography (Figure 2A and B). Acf1, ISWI, CHRAC-16 and CHRAC-14 co-eluted perfectly with an estimated size of ~ 660 kDa (Figure 2A), in agreement with the size of human, topo II-less CHRAC (Poot *et al.*, 2000) and our previous CHRAC analysis (Varga-Weisz *et al.*, 1997). The co-fractionation of CHRAC-16 and CHRAC-14 with ISWI and Acf1 following an entirely different purification scheme confirmed our recent analysis (Corona *et al.*, 2000). Topo II eluted in a broad peak corresponding to mol. wts of 500–600 kDa (Figure 2B). The predominant active form of *Drosophila* topo II is a dimer with a theoretical mol. wt of ~ 300 kDa (Shelton *et al.*, 1983). In order to clarify whether the increased size estimate obtained by gel filtration was due to interaction of topo II with other factors, we expressed full-length *Drosophila* topo II in yeast and characterized the purified, active enzyme by gel filtration and multiple angle light scattering (MALS). Topo II migrated on the Superose 6 column as a 600 kDa protein

complex (Figure 2C). MALS coupled to a Superdex 200 column revealed a molecular mass of $\sim 3.4 \times 10^5$ g/mol (Figure 2D) consistent with a topo II dimer. The simplest interpretation of these results is that topo II is not an integral subunit of CHRAC but co-fractionates with CHRAC during gel filtration due to its unusual shape.

To substantiate further the association of Acf1 with CHRAC, we performed immunoprecipitation experiments. Antibodies against ISWI, CHRAC-14 and CHRAC-14/16 specifically immunoprecipitated Acf1 from *Drosophila* nuclear extracts while the pre-immune serum did not (Figure 3). Taken together, our results demonstrate that Acf1 is a bona fide subunit of CHRAC. This leads us to a molecular definition of CHRAC, composed of Acf1, ISWI and the two histone fold proteins CHRAC-16 and CHRAC-14.

Acf1 alters the directionality of ISWI-induced nucleosome sliding

A heterodimeric complex of Acf1 and ISWI previously has been termed 'ACF' (Ito *et al.*, 1999). In this context, Acf1 significantly increases the activity of ISWI in chromatin assembly. Since we identified Acf1 also as a component of CHRAC, we wished to evaluate the impact of Acf1 on ISWI-induced nucleosome sliding. We previously observed that the directionality of nucleosome sliding differed depending on whether the reaction was catalysed by ISWI alone or by CHRAC (Längst *et al.*, 1999). Flag-tagged ISWI and Acf1 were expressed from baculovirus vectors in insect cells (Hamiche *et al.*, 1999; Ito *et al.*, 1999), affinity purified (Figure 4) and assayed for nucleosome sliding (Figure 5). In agreement with our previous results, catalytic amounts (2–3 fmol) of ISWI

Fig. 3. Immunoprecipitation of the CHRAC components from *Drosophila* nuclear extract. Protein from crude extract was immunoprecipitated with anti-ISWI, anti-CHRAC-14 and anti-CHRAC-14/16 antibodies as well as pre-immune serum. Aliquots of the immunoprecipitated material were resolved by SDS-PAGE and detected by western blotting using the indicated antisera.

Fig. 4. Purification of recombinant ISWI, Acf1 and ISWI/Acf1. The indicated factors (Flag-ISWI, Acf1-Flag or Flag-ISWI + Acf1) were expressed via a baculovirus system and purified by α-Flag immunoaffinity chromatography. Two different concentrations for each protein were resolved by 6% SDS-PAGE and stained with Coomassie blue. Arrows depict the positions of Acf1 and ISWI.

moved a mononucleosome from the centre of a 248 bp rDNA fragment to the fragment end (Figure 5A). No mobility was observed when the end-positioned nucleosome was exposed to ISWI (see Figure 5B). In contrast to the movement generated by ISWI, CHRAC catalyses nucleosome sliding from the end to the centre of the DNA fragment (Längst *et al.*, 1999). Strikingly, CHRAC-type directionality of nucleosome sliding was also obtained if Acf1 was added to ISWI, either after separate expression (data not shown) or by co-expression of both proteins in Sf9 cells. While Acf1 alone was inactive for nucleosome sliding, it boosted ISWI activity by at least an order of magnitude such that 10-fold lower enzyme concentrations (0.3–0.5 fmol) were required for nucleosome mobilization. Most importantly, Acf1 changed the directionality of sliding such that end-positioned nucleosomes moved to central positions (Figure 5B).

In order to determine whether Acf1 had an additional effect on the kinetics of nucleosome mobility under those conditions, we performed a time course of nucleosome mobility (Figure 6A). The amounts of enzymes were chosen such that we expected complete mobilization of the nucleosome after 90 min (10-fold less ACF than ISWI). At any given time point throughout the reaction, the ratio of nucleosomes that had been mobilized to those that had not moved was determined. Nucleosome movement in the two reactions proceeded with similar speed, indicating that ACF was about an order of magnitude more efficient in nucleosome mobilization than ISWI alone. This could be explained most readily if Acf1 stimulated the ATPase activity of ISWI. To determine whether this was the case, we compared the enzymes in standard ATPase assays (Figure 6B). ISWI alone showed a robust (7-fold)

Fig. 5. Nucleosome sliding by recombinant ISWI/Acf1 (= ACF) and ISWI. (A) Nucleosomes positioned at the centre of the 248 rDNA fragment were incubated with the indicated amounts of affinity-purified Acf1-Flag, ACF or Flag-ISWI. Nucleosomes were then analysed on a native 4.5% polyacrylamide gel. Terminal or central nucleosome positions are indicated to the left. Lane (-) indicates the untreated centre nucleosome. (B) End-positioned nucleosomes were incubated with the indicated amounts of affinity-purified Flag-ISWI, ACF or Acf1-Flag. Nucleosome analysis and figure labelling are described in (A).

nucleosome stimulation of ATPase. This response to a nucleosomal structure remained unaltered if Acf1 was added, either after separate expression (Figure 6B, ISWI + Acf1) or through co-expression (Figure 6B, ACF). While Acf1 alone did not show any sign of ATPase activity (Figure 6B, Acf1), it also did not stimulate the ATPase of ISWI significantly (7-fold stimulation over the free DNA level in all cases). This conclusion was reached under a number of different conditions and times, and with different enzyme preparations.

ACF requires the histone H4 tails for nucleosome mobilization

Recently, we found that deletion of the H4 N-termini completely abolished the ability of CHRAC to slide nucleosomes, whereas removal of any other histone tail had only minor effects (Clapier *et al.*, 2001). In contrast, ISWI-induced sliding not only required the histone H4 N-termini (like CHRAC), but was also impaired if any of the other tails were deleted (Clapier *et al.*, 2001). Since Acf1 modulated the directionality of nucleosome sliding to resemble that of CHRAC, we tested the histone tail dependence of ACF-induced nucleosomal sliding. As expected, we found that deletion of the H4 tail completely abolished the remodelling activity of ACF (Figure 7). Removal of any other histone tail, however, had only little influence on the sliding activity of ACF. This result reinforces our notion of a qualitative alteration of the

nucleosome remodelling activity of ISWI by Acf1, which points to altered interaction with the nucleosomal substrate.

Fig. 6. Functional comparison of ISWI and ACF. (A) Nucleosome sliding was monitored during a time course of reactions containing either 2 fmol of ACF and end-positioned nucleosome, or 25 fmol of F-ISWI with a centrally positioned nucleosome substrate. The reactions were analysed by native gel electrophoresis as before. Signals corresponding to the moved and unmoved nucleosome fraction were quantified by a PhosphorImager (FujiFilm BAS-1500) and the fraction of moved nucleosomes plotted as a function of time. (B) ATPase assays: 400 fmol each of F-ISWI or ACF were tested for ATPase in the presence of 100 ng of naked DNA (open bars) or 100 ng of nucleosomal DNA reconstituted by salt gradient dialysis (black bars). A low background of hydrolysed ATP in the absence of enzyme was subtracted. The results have been reproduced qualitatively under various circumstances, although the absolute numbers vary as a function of ATP batch, age and enzyme preparation.

Discussion

Molecular definition of CHRAC

CHRAC was isolated initially as a biochemical activity containing five proteins (Varga-Weisz *et al.*, 1997). In these preparations, two ATPases were identified that potentially could be involved in a nucleosome remodelling reaction: ISWI and topo II. ISWI later proved to be the catalytic core of CHRAC and related complexes (Corona *et al.*, 1999; Längst *et al.*, 1999). Specific inhibition of topo II had no effect on the remodelling reaction (data not shown; Varga-Weisz *et al.*, 1997), but the remarkable co-purification of topo II and ISWI and their consistent co-immunoprecipitation under various conditions suggested that CHRAC combined the activity of both ATPases. While co-purification of topo II with the human homologue of ISWI, hSNF2, was also observed by those who purified human ISWI complexes (discussed in LeRoy *et al.*, 2000; Poot *et al.*, 2000), other researchers failed to detect topoisomerase in association with ISWI (Bochar *et al.*, 2000; Guschin *et al.*, 2000). To clarify the controversial issue of whether topo II is a bona fide subunit of CHRAC, we purified the complex according to a different scheme and found that hydroxyapatite chromatography separated topo II from ISWI/CHRAC without affecting the gel filtration properties of either of the two entities. MALS demonstrated that a dimer of recombinant topo II with a mass of 340 kDa indeed migrates with an apparent mol. wt of ~600 kDa, providing a satisfactory explanation for the co-fractionation of topo II with CHRAC on a Superose 6 column.

Recently, we identified two small histone fold proteins, CHRAC-14 and CHRAC-16, as integral components of CHRAC (Corona *et al.*, 2000). We have now discovered that the 175 kDa subunit of CHRAC is identical to Acf1. Acf1 is known to associate with ISWI to form ACF, a factor purified from *Drosophila* embryo extracts following its activity in the assembly of regularly spaced nucleosomes (Ito *et al.*, 1997). CHRAC therefore differs from ACF by the presence of the two small histone fold factors (Corona *et al.*, 2000). Complexes resembling either CHRAC or ACF have also been identified in human cells (LeRoy *et al.*, 2000; Poot *et al.*, 2000). The relationship between the two entities remains to be established, and will certainly become clearer when the functional impact of the association of CHRAC-14 and CHRAC-16 with ACF is resolved.

Fig. 7. Histone tail requirement for nucleosome mobilization by ACF. Recombinant ACF complex was incubated with end-positioned nucleosomes reconstituted with either wild-type histones (intact) or, alternatively, a histone mixture consisting of three wild-type histones and one tailless variant (Δ) as indicated. The reactions were analysed by native polyacrylamide gel electrophoresis. Positions of nucleosomes and free DNA are indicated. Reactions contained 0.1, 0.2, 0.5, 0.75, 1 and 2 fmol of ACF (the 2 fmol sample was omitted in the panel 'intact').

Acf1-like factors are commonly associated with ISWI

Acf1 is a member of a growing family of proteins with similar domain architecture (Bochar *et al.*, 2000; Jones *et al.*, 2000; Poot *et al.*, 2000) including WSTF, one of the genes invariantly deleted in William–Beuren syndrome patients (Lu *et al.*, 1998; Peoples *et al.*, 1998). In addition to several other sequence similarities, these factors contain a prominent C-terminal bromodomain and one or two PHD fingers. Acf1-like factors so far have been found exclusively in association with ISWI. Complexes consisting of just ISWI and an Acf1-like factor were purified from *Drosophila* (Ito *et al.*, 1997), human (Bochar *et al.*, 2000; LeRoy *et al.*, 2000) and yeast cells (Goldmark *et al.*, 2000). One of the ISWI-containing remodelling factors in *Xenopus* extracts contains an unknown protein (p175) in addition to ISWI and Acf1 (Guschin *et al.*, 2000). CHRAC, defined as a four-subunit complex consisting of ISWI, Acf1, CHRAC-14 and CHRAC-16, has been identified so far in human and fly cells (Corona *et al.*, 2000; Poot *et al.*, 2000; this report). Although ISWI can function as a remodelling ATPase in certain *in vitro* assays (Corona *et al.*, 1999; Längst *et al.*, 1999), it has never been purified from a native source on its own. These data suggest that ISWI and an Acf1-like factor constitute a functional core module with which other proteins may associate to generate a family of diverse remodelling machines, potentially tailored to specific functions.

Functional role of Acf1-like factors

Acf1 was first identified as a protein associated with ISWI to form the nucleosome assembly and spacing factor ACF (Ito *et al.*, 1997). While either recombinant ISWI or Acf1 alone was only poorly active in the assembly system of Kadonaga and co-workers, the reconstitution of ACF from the two subunits increased the *in vitro* nucleosome assembly activity by some 30-fold (Ito *et al.*, 1999). We observed earlier that ISWI, expressed in a bacterial system, could, in principle, function autonomously in various cell-free remodelling assays (Corona *et al.*, 1999; Längst *et al.*, 1999; Clapier *et al.*, 2001). The direct comparison of the activity of factors expressed under similar conditions from baculovirus vectors shows that Acf1 enhances ISWI-induced nucleosome mobility by about an order of magnitude. In addition, the association of Acf1 has a striking qualitative effect as it alters the directionality of nucleosome sliding triggered by ISWI and affects the sensitivity of the ATPase towards deletion of the histone N-termini on the nucleosomal substrate. ISWI and Acf1 approach the nucleosome in a co-ordinated manner, leading to a new quality of interaction, such that Acf1 does not simply enhance the action of ISWI, as could have been concluded from the analysis of Ito *et al.* (1999) of ACF as a nucleosome assembly factor. Whether Acf1 interacts with DNA directly, effectively hindering the sliding of the nucleosome to the fragment end, remains to be explored. Upon association of ISWI with Acf1, 10-fold lower enzyme concentrations and correspondingly fewer ATP hydrolysis events are required to move a nucleosome as compared with free ISWI alone. It is possible that ACF has a higher affinity for the nucleosomal substrate, due to interaction domains contributed by Acf1 (see below). A decreased off-rate may lead to a higher processivity of the

enzyme, converting the energy of ATP hydrolysis more effectively into directional nucleosome sliding. Testing this and alternative hypotheses will require more quantitative measurements of the parameters of the nucleosome sliding reaction.

The PHD finger and bromodomain are likely to be involved in Acf1 activity. PHD fingers are protein interaction surfaces found in many chromatin-bound regulators (Pascual *et al.*, 2000). Bromodomains are equally abundant among nuclear regulators. They are a hallmark of the remodelling ATPases of the SWI2/SNF2 type, but are absent in ISWI. Bromodomains are known interactors of acetylated histone H4 N-termini (Jacobson *et al.*, 2000; Owen *et al.*, 2000). Nucleosome remodelling by ISWI critically depends on the integrity of the H4 tail on the nucleosomal substrate (Clapier *et al.*, 2001). It is possible that tandem PHD fingers and a bromodomain form a cooperative interaction unit, as has been suggested recently for the KRAB proteins (Schultz *et al.*, 2001). It will be interesting to see whether Acf1 interacts directly with the H4 tail during nucleosome mobilization and whether histone acetylation modulates this process. The function of the N-terminal WAC domain of Acf1, which has been implicated in targeting proteins to heterochromatin (Ito *et al.*, 1997; Poot *et al.*, 2000), is unknown.

Speculations on the biological function of CHRAC

Although CHRAC was first perceived due to its activity to render nucleosomal DNA accessible (Varga-Weisz *et al.*, 1997), it was soon discovered that CHRAC and the related ACF may have an important role in the assembly of regular nucleosomal arrays *in vitro*. Nucleosome mobility is not restricted to the assembly phase, but can also be observed within an ordered nucleosomal array (Varga-Weisz *et al.*, 1995; Längst *et al.*, 1999). CHRAC may be involved mainly in the assembly and maintenance of nucleosomal arrays with dynamic properties. Several observations are in line with such a function. (i) The *in vitro* phenomenology shows that CHRAC and ACF can catalyse the assembly of dynamic nucleosomal arrays (Corona *et al.*, 1999; Ito *et al.*, 1999; Längst *et al.*, 1999). (ii) The restricted expression of ISWI, Acf1 and the CHRAC-14/16 pair during *Drosophila* embryonic development correlates with the time of most intense nuclear division (Ito *et al.*, 1999; Corona *et al.*, 2000). (iii) Proteins with similarity to CHRAC-14 and CHRAC-16 have been found to associate with human DNA polymerase ϵ (Li *et al.*, 2000). (iv) Mutation of ISWI in male flies leads to a striking abnormality of the structure of the male X chromosome, which is marked and perhaps sensitized by specific acetylation of the histone H4 N-terminus (Deuring *et al.*, 2000), although the additional presence of ISWI in NURF complicates the interpretation of the mutant phenotype. The outcome of rendering nucleosomes mobile may depend on the circumstances: *in vitro*, CHRAC can facilitate SV40 replication by promoting the access of T antigen to a nucleosomal origin (Alexiadis *et al.*, 1998). In contrast, an ACF-like complex contributes to the targeted repression in yeast, presumably by modulating nucleosome positions in the promoters of meiosis-specific genes (Goldmark *et al.*, 2000).

To understand fully a complex remodelling machine like CHRAC remains a formidable task. Now that all

subunits have been molecularly defined, the reconstitution of CHRAC from recombinant subunits will constitute a major milestone on this track.

Materials and methods

Mass spectrometry and peptide sequencing

Native CHRAC was purified from 3-h-old *Drosophila* embryos (Varga-Weisz *et al.*, 1997). Mass spectrometry was carried out essentially as described elsewhere (Varga-Weisz *et al.*, 1997; Corona *et al.*, 2000). Proteins were trypsinized in the gel (Shevchenko *et al.*, 1996). Extracted peptides were purified on a 100 nl R2 Poros microcolumn and eluted in 1 µl of 60% methanol/5% formic acid into a nanoelectrospray capillary. The peptides were sequenced on an API III triple quadrupole instrument (PE-Sciex) equipped with an upgraded collision cell and the nanoelectrospray ion source (Wilm and Mann, 1996). Details are available upon request.

Cloning and sequencing of Acf1

The peptide LAAVEINRK allowed us to identify an STS (embl-AL023453) that was used to design the PCR primers STS-forw., CAAAAGAAGACCCTGGT; and STS-rev., CATCTGCAATTCGTG-CT. These amplified a 400 bp fragment, which was used by the Resource Center of the German Human Genome Project (DHGP) to screen the *Drosophila melanogaster* embryo cDNA library no. 603. The screen identified clones J2175, D1339 and E1459.

Highly degenerate oligonucleotides obtained by backtranslation of amino acid sequences were used to screen the P1 *Drosophila* high-density filters (Genome Systems Inc.) under tetramethylammonium chloride (TMAC) conditions (Honore and Madsen, 1997). A RACE kit (Roche) was used according to the manufacturer's instructions to reverse transcribe mRNA from *Drosophila* embryos (16 h) primed by oligonucleotide p175-RT: GTTTGAAGTCTCCATC. The resulting cDNA was poly(A) tailed by terminal transferase and subjected to the first round of PCR using oligonucleotides p175-1 (ACTGCCACACCGTTG-AGT) and anchor-dT. The second round of PCR amplification was performed with oligonucleotides p175-2 (GATGTAGCAGCAGAA-GACCT) and the PCR anchor primer. The complete sequence, which deviates at seven positions from the previous Acf1 sequence (Ito *et al.*, 1999), has the DDBJ/EMBL/GenBank accession No. AJ 238397.

Antibodies

Polyclonal antibodies against Acf1 (peptide RPNKGDPSAELDLP and peptide SALPKNRKDLRVYL) were raised in rabbits and purified on protein A-Sepharose columns. The anti-ISWI and the anti-topo II antisera were generously provided by J.Tamkun and D.Arndt-Jovin, respectively. The generation and purification of the anti-CHRAC-14/16 antibody are described elsewhere (Corona *et al.*, 2000).

Purification of CHRAC

Chromatographic materials were purchased from Amersham-Pharmacia (Mono Q HR 5/5, Superose HR 10/30, Superdex S-200) and from Bio-Rad (Bio-Rex 70, Bio-Gel HT hydroxyapatite). Extractions, dialyses and chromatographies were carried out at 4°C. Crude nuclear extract was prepared from 200 g of 0- to 12-h-old *Drosophila* embryos (Nightingale *et al.*, 1998) and fractionated on a Bio-Rex 70 cation exchange column equilibrated in CB-150 [CB: 10 mM HEPES pH 7.6, 1 mM MgCl₂, 0.5 mM EGTA, 0.1 mM EDTA, 10% glycerol, 0.05% NP-40, 1 mM dithiothreitol (DTT), 0.2 mM phenylmethylsulfonyl fluoride (PMSF) and protease inhibitors; CB-150: CB + 150 mM KCl]. The 0.5 M KCl eluate of the Bio-Rex 70 column was dialysed in CB-150 and processed on a Mono Q HR 5/5 column. Bound material was eluted with 20 CVs of linear gradient from CB-150 to CB-500. CHRAC fractions as determined by western blotting (eluting at 320 mM KCl) were pooled, concentrated and applied to a Superose 6 HR 10/30 size exclusion column in CB-400. Fractions containing CHRAC were then loaded directly on a 0.8 ml Bio-Gel HT hydroxyapatite column equilibrated in CB-100 without NP-40. The resin was washed with 4 CVs of CB-100 containing 10 mM potassium phosphate at pH 7.2 (KPi) followed by 4 CVs of CB-100/100 mM KPi. Elution was performed with 20 CVs of linear KPi gradient from CB-100/100 mM KPi to CB-100/500 mM KPi. Fractions containing CHRAC (300 mM KPi) and topo II (380 mM KPi) were pooled separately, concentrated and chromatographed on Superose 6 HR 10/30 as described above.

Immunoprecipitations and western blot analysis

Immunoprecipitations were performed in buffer HEMG-250 (25 mM HEPES pH 7.6, 250 mM KCl, 12.5 mM MgCl₂, 0.5 mM EDTA, 0.5 mM EGTA, 10% glycerol, 0.1% NP-40, 1 mM DTT, 0.2 mM PMSF and protease inhibitors) at 4°C. The indicated antisera were added to 150 µl of *Drosophila* nuclear extract and incubated for 4 h with gentle rotation. To this, equilibrated protein A beads (Roche) were added and incubation was continued for 1 h. Beads were washed three times with 1 ml of HEMG-250 and twice with 1 ml of HEMG-100. Immunoprecipitated material was separated by SDS-PAGE, transferred to Hybond-P (Amersham-Pharmacia) and probed with the relevant antibodies as described (Corona *et al.*, 2000).

Purification of recombinant proteins

The baculovirus stocks for the expression of Acf1 and C-terminally Flag-tagged Acf1 were kindly provided by Dr J.Kadonaga. Full-length Acf1 proteins and ACF were expressed and purified (Ito *et al.*, 1999) with modifications as previously described (Brehm *et al.*, 2000). The ISWI-expressing baculovirus was a gift from Dr C.Wu (Hamiche *et al.*, 1999). *Drosophila* topo II cDNA was cloned into pESC-URA (Stratagene). Expression in *Saccharomyces cerevisiae* and purification of the recombinant protein followed standard procedures (Knudsen *et al.*, 1996). The molar mass was determined by MALS using a dawn EOS system (Wyatt Technology) coupled to a Superdex S-200 column (Folta-Stogniew and Williams, 1999). A 20 µg aliquot of purified recombinant topo II was separated on a Superdex S-200 column equilibrated in BC-400 (25 mM HEPES pH 7.6, 400 mM NaCl, 0.1 mM EDTA, 0.5 mM EGTA, 1 mM Mg₂Cl₂, 10% glycerol). The recorded signal was integrated and analysed using the ASTRA analysis software (Wyatt Technology).

Nucleosome mobility assays

Mononucleosome assemblies from purified *Drosophila* histones and polyglutamic acid (PGA; Sigma P4886) and nucleosome mobility assays were as described previously (Längst *et al.*, 1999). Briefly, nucleosomes were assembled on a radiolabelled 248 bp rDNA fragment and central and end positions were separated by polyacrylamide gel electrophoresis. Gel slices containing the positioned nucleosomes were crushed and the nucleosomes were eluted for 1 h at 4°C in 350 µl of 0.5× TE, 5 mM NaCl, containing 150 µg/ml chicken egg albumin (CEA; Sigma). Mobility assays were performed in a final volume of 10 µl in buffer EX-80 (10 mM HEPES pH 7.6, 80 mM KCl, 2.5 mM MgCl₂, 10% glycerol, 10 mM glycerophosphate, 1 mM DTT, 0.2 mM PMSF) for 90 min at 28°C. Typical reactions contained 60 fmol of isolated mononucleosomes, purified factors (F-ISWI: 1.2–30 fmol; Acf1-F: 1.6–50 fmol; ACF: 0.09–1.5 fmol), 1 mM ATP and 200 ng/µl CEA. Reactions were terminated by the addition of 100 ng of unlabelled nucleosomal DNA and further incubation for 5 min at 28°C. The reaction was then electrophoresed again on native 4.5% polyacrylamide gels in 0.5× TBE.

ATPase assay

ATPase assays (Corona *et al.*, 1999) contained 100 ng of free or oligonucleosomal DNA, reconstituted by salt gradient dialysis; 400 fmol of either ISWI or ISWI/Acf1 complexes were analysed. Hydrolysed phosphate was separated from unreacted ATP by thin-layer chromatography and spots subsequently were quantified by PhosphorImager (FujiFilm BAS-1500).

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