The human SWI/SNF-B chromatin-remodeling complex is related to yeast Rsc and localizes at kinetochores of mitotic chromosomes

Yutong Xue*, Julie C. Canman†, Cheol Soon Lee*‡, Zuqin Nie*‡, Dafeng Yang*‡, G. Tony Moreno‡§, Mary K. Young§, E. D. Salmon†, and Weidong Wang*¶

*Laboratory of Genetics, National Institute on Aging, National Institutes of Health, 333 Cassell Drive, TRIAD Center Room 4000, Baltimore, MD 21224; †Department of Biology, University of North Carolina, Chapel Hill, NC 27599; and §Division of Immunology, Beckman Institute of the City of Hope, 1450 Duarte Road, Duarte, CA 91010

Edited by Robert Tjian, University of California, Berkeley, CA, and approved September 26, 2000 (received for review May 9, 2000)

The SWI/SNF family of chromatin-remodeling complexes facilitates gene expression by helping transcription factors gain access to their targets in chromatin. SWI/SNF and Rsc are distinctive members of this family from yeast. They have similar protein components and catalytic activities but differ in biological function. Rsc is required for cell cycle progression through mitosis, whereas SWI/SNF is not. Human complexes of this family have also been identified, which have often been considered related to yeast SWI/SNF. However, all human subunits identified to date are equally similar to components of both SWI/SNF and Rsc, leaving open the possibility that some or all of the human complexes are rather related to Rsc. Here, we present evidence that the previously identified human SWI/SNF-B complex is indeed of the Rsc type. It contains six components conserved in both Rsc and SWI/SNF. Importantly, it has a unique subunit, BAF180, that harbors a distinctive set of structural motifs characteristic of three components of Rsc. Of the two mammalian ATPases known to be related to those in the yeast complexes, human SWI/SNF-B contains only the homolog that functions like Rsc during cell growth. Immunofluorescence studies with a BAF180 antibody revealed that SWI/SNF-B localizes at the kinetochores of chromosomes during mitosis. Our data suggest that SWI/SNF-B and Rsc represent a novel subfamily of chromatin-remodeling complexes conserved from yeast to human, and could participate in cell division at kinetochores of mitotic chromosomes.

ATP-dependent chromatin-remodeling complexes facilitate the opening of chromatin structures to allow transcription and other metabolic reactions to occur on DNA. All such complexes contain an SWI2/SNF2-like ATPase and use the energy of ATP-hydrolysis to disrupt nucleosomes (reviewed in refs. 1–3). SWI/SNF and Rsc are closely related chromatin-remodeling complexes initially discovered in yeast (4–6). Both types of complex engage in transcriptional regulation (7–11). They are similar in structures, sharing two identical subunits and at least four other homologous components (12). Also, both complexes can disrupt nucleosome structures in the presence of ATP, which leads to increased binding of transcription factors to nucleosomes (4, 6, 13, 14). However, the gene cohorts regulated by each complex seem to be distinctive. SWI/SNF is not essential for yeast viability, whereas loss of Rsc is lethal. Also, Rsc is notably required for cell cycle progression through mitosis (6, 15–17), whereas SWI/SNF and other known ATP-dependent chromatin-remodeling complexes are not. However, SWI/SNF is required for yeast to efficiently exit from mitosis by mediating expression of some mitotic genes (18).

We and others have described several human ATP-dependent chromatin-remodeling complexes (19–21). These complexes have been implicated to participate in diverse cellular processes, including transcriptional regulation (22, 23), integration of viral genomes into host chromatin (24), viral DNA replication (25), cell cycle regulation (26, 27) and tumorigenesis (28, 29). They contain either BRG1 or hbrm, two orthologs of yeast SWI2/SNF2-like ATPases. Each particle comprises about 10 subunits named BRG1- or hbrm-associated factors (BAFs), most of which are homologous to components of yeast SWI/SNF and Rsc complexes (20, 30–32). However, no human subunit identified thus far is specifically similar to a subunit of only one yeast complex but not the other. It is therefore unclear which human complex corresponds to yeast SWI/SNF or Rsc. We show here that BAF180, a subunit of the previously detected human SWI/SNF complex B, possesses unique structural motifs conserved in 3 Rsc subunits, suggesting that complex B is related to Rsc. Because the old name "SWI/SNF complex B" incorrectly implies that it is a homolog of SWI/SNF, we have renamed this complex Polybromo, BRG1-associated factors (PBAF) (see below). We also report that PBAF localizes at kinetochores of mitotic chromosomes. This localization of PBAF is consistent with the yeast data that Rsc is required for cell cycle progression through mitosis and suggests that PBAF and Rsc may have a kinetochore function during cell division.

Materials and Methods

Purification of PBAF and Cloning of BAF180. PBAF (SWI/SNF complex B) was purified with a BRG1 antibody-column from human KB cell nuclear extract as described (20). The complex on the antibody beads was either used directly for enzymatic assays or eluted with 0.1 M glycine (pH 2.5) for analysis by SDS/PAGE. The bands containing proteins of interest were sequenced by microspray tandem mass spectrometry (MS/MS) as described (33). The peptides from BAF180 matched several human expressed sequence tags (ESTs) and the chicken Polybromo gene. A cDNA library from Jurkat T cells was screened with the EST DNAs as probes to obtain BAF180 cDNA clones.

A rabbit polyclonal antibody was made against a fusion protein containing amino acid residues of 111–206 of BAF180 fused to the maltose-binding protein (New England Biolabs). The antibody was affinity purified and used for immunoblotting and immunoaffinity purification (20). The load fraction for BAF180 antibody column is the same one used for PBAF purification

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: BAF, BRG1-associated factors, a synonym for human SWI/SNF-A complex; PBAF, Polybromo- and BRG1-associated factors (new name for the SWI/SNF-B complex); EST, expressed sequence tag; BAH, bromo-adjacent homology; HMG, high-mobility-group.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. for BAF180 is AF197569).

‡C.S.L., Z.N., D.Y., and G.T.M. contributed equally to this work.

¶To whom reprint requests should be addressed. E-mail: wangw@grc.nia.nih.gov.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Article published online before print: *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.240208597. Article and publication date are at www.pnas.org/cgi/doi/10.1073/pnas.240208597

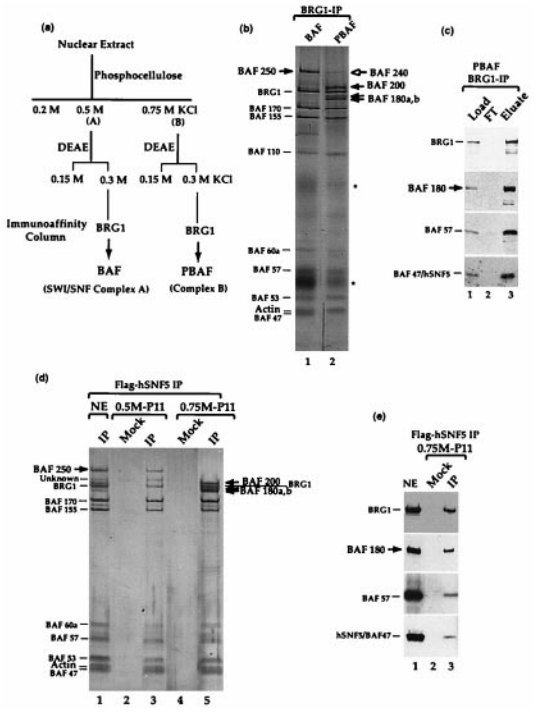

Fig. 1. BAF180 distinguishes two similar human chromatin-remodeling complexes. (*a*) A diagram of the purification scheme for BAF and PBAF complexes (20). Previously assigned names for these complexes are given in parentheses. (*b*) A silver-stained SDS-gel showing BAF and PBAF purified with BRG1 antibody. Unique components are marked with arrows. The band designated as BAF240 (open arrow) was found to be present in substoichiometric amounts by both BRG1 and BAF180 antibodies (see Fig. 3*a*). Some IgG and crosslinked-IgG are indicated with an asterisk. (*c*) Immunoblot analysis of the load, flowthrough (FT), and eluate fractions from the BRG1 antibody column. The doublet bands of BAF180 may represent gene products derived from alternatively spliced mRNA. (*d*) A silver-stained SDS-gel of the human SWI/SNF complex A (lane 3) and B (lane 5) isolated by using a monoclonal antibody against the flag-tagged hSNF5 (34). Immunopurification was performed by using either unfractionated nuclear extract (NE), or fractions from phophocellulose (P11) fractionation of the nuclear extract as indicated on top of the figure (lanes 2–5). Mock purifications (lanes 2 and 4) were done by using extracts derived from regular HeLa cells that do not express the flag-hSNF5. BAF200 may correspond to the protein referred to as p210 in ref. 37. A polypeptide in complex A, marked as ''unknown,'' appears to be present in the mock purification and could be a contaminant. (*e*) Immunoblot analysis of the SWI/SNF complex B from Fig. 1*d*. Nuclear extract (lane 1) was shown as a control.

using BRG1 antibody (Fig. 1*a*). The column was washed with Buffer D [20 mM Hepes (pH 7.9)/0.5 mM EDTA/5% glycerol/1 mM PMSF/0.1 mM DTT] containing 0.5 M KCl, and with Buffer D without any salt. In immunoblot analysis, 2% of the load and flowthough fractions and 5% of the eluted complex (peak fractions) were assayed.

Human SWI/SNF complexes A and B have also been purified from the HeLa cell line that stably expresses flag-tagged hSNF5/BAF47 as described (34). One difference is that our immunopurification was performed after phosphocellulose fractionation of the nuclear extract as indicated in Fig. 1.

Northern blotting was done by using a fragment of BAF180 (nucleotides 1582–2161) as a probe. The human multitissue blot was purchased from CLONTECH.

Characterization of PBAF. Superose 6 gel-filtration chromatography was performed by using Buffer D containing 0.3 M KCl. The load fraction was the DEAE fraction used for PBAF purification by BRG1 antibody column (Fig. 1*a*).

The mononucleosome disruption assay was done as described (13, 33). The complexes bound to the antibody beads were directly used in the assay. The amounts used were comparable by silver staining analysis on gels.

Immunofluorescence. Fixation and staining of Ptk1 cells were done as described (35). Antibodies against BAF180 and dynein intermediate chain (70.1; Sigma) were used at dilutions 1:1,500 and 1:500, respectively. In some experiments, cells were pretreated with nocodazole (10 μM) for 30 min before fixation. Optical sections of fluorescently labeled cells were obtained by using a multimode digital imaging system (36).

Results

BAF180 Distinguishes Two Similar Human Chromatin-Remodeling Complexes. We have previously purified two different human SWI/SNF-related complexes, BAF (also called SWI/SNF-A) and PBAF (SWI/SNF-B)(20) (Fig. 1 *a* and *b*). These complexes can be distinguished, because BAF contains BAF250, whereas PBAF harbors BAF180, -200, and -240. Cloning of BAF250 revealed that it has a structural motif conserved in yeast SWI1 but not any Rsc components (51), suggesting that BAF is more related to SWI/SNF. We purified BAF180 of PBAF and subjected it to mass spectrometry microsequencing. Nine peptides were obtained that match the chicken Polybromo gene and several human EST sequences. Several overlapping human cDNA clones were isolated by using the EST sequences as probes. Sequence analysis of these cDNAs revealed an ORF of 1582 amino acids containing all of the detected peptide sequences. An antibody to a region of the predicted ORF recognized the 180-kDa band of PBAF on immunoblots (Fig. 1*c*), indicating that the cDNA indeed encodes BAF180.

BAF180 Is a Specific Subunit of PBAF Complex. The SWI/SNF complex B (PBAF) obtained from our purification is slightly different from the one described by Kingston and colleagues (19, 37), despite the fact that the purification schemes are similar. Their complex B does not appear to contain any polypeptides near the size of BAF180. The different results between the two labs motivated us to test the possibility whether BAF180 is a contaminant in our procedure. We used the same cell line described by Kingston's group in our purification. This cell line stably expresses flag-tagged hSNF5/BAF47, permitting isolation of human SWI/SNF-related complexes by use of a monoclonal antibody against the flag-epitope (34). The complexes purified by using this antibody resembled those obtained by using BRG1 antibody, with complex B containing polypeptides with gel mobility similar to BAF180 (Fig. 1*d*). Immunoblot analysis confirmed the identity of these polypeptides as BAF180 (Fig. 1*e*). As a control, mock purification by using extracts from cells that lack tagged-hSNF5 failed to yield BAF180 or any other BAFs, indicating that the association between BAF180 and complex B is specific. In addition, complex B purified with a polyclonal antibody against hSNF5 also contains BAF180 (data not shown). These data indicate that BAF180 is not a contaminant, but an intrinsic subunit of PBAF.

We note that another polypeptide, previously referred to as BAF110 in complexes purified with polyclonal antibodies against BRG1 and hSNF5 (20), is absent in complexes isolated with the flag antibody (Fig. 1*d*). It remains to be determined whether BAF110 is a true subunit or a contaminant.

BAF180 Is a Homolog of Chicken Polybromo and Contains Structural Motifs Characteristic of Yeast Rsc. BAF180 is 90% identical to chicken Polybromo (PB1), which was cloned during a search for genes containing bromodomains, but has no known function (38) (Fig. 2*a*). PB1 was found to possess 5 bromodomains, 2 bromo-adjacent homology (BAH) regions, and one high-mobility-group

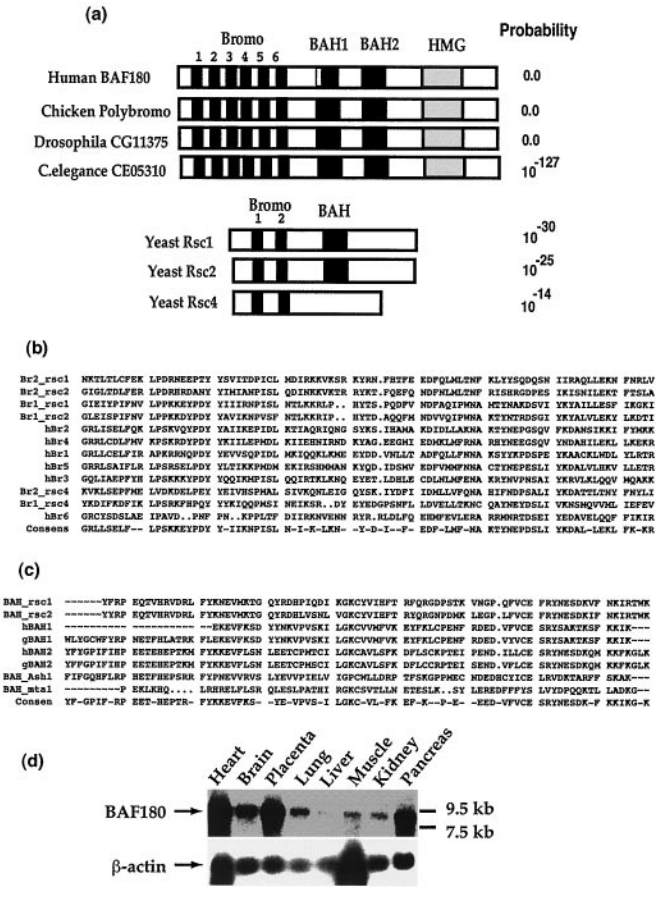

Fig. 2. BAF180 is a human homolog of chicken Polybromo and contains structural motifs similar to yeast Rsc1/Rsc2/Rsc4. (*a*) Schematic representation of domain structures of human BAF180, chicken Polybromo, ORFs from *Drosophila* (CG11375) and *C. elegans* (CE05310), and yeast Rsc1/Rsc2/Rsc4. The BAH1 region of BAF180 is about 20 amino acids shorter than the motifs in other proteins. (*b* and *c*) Alignment of bromodomains (Br) and BAH regions for hBAF180, chicken PB1 (gBAH), and the three yeast Rsc subunits. A consensus sequence is shown at the bottom. The BAH regions of Ash1 (a *Drosophila trithorax* group protein) and mta1 (a subunit of nucleosome remodeling and histone deacetylation chromatin-remodeling complex; ref. 33) are shown for comparison. (*d*) Northern blotting of total RNA from multiple human tissues by using BAF180 or actin DNA as probes.

(HMG) motif. All these features are conserved in BAF180. We noted that both BAF180 and PB1 also have one other bromodomain (hBr6) that diverges somewhat from the standard consensus sequence (Fig. 2*b*). In addition, the BAH1 region of BAF180 is about 20 amino acids shorter at its N terminus (Fig. 2*c*). The mRNA of BAF180 was detected in several human tissues (Fig. 2*d*).

BLAST searches of the complete genome database of *Saccharomyces cerevisiae* detected no yeast gene with the same domain structure as BAF180. However, the same searches revealed that the top three homologs of BAF180 are genes encoding Rsc subunits: *Rsc1*, *Rsc2*, and *Rsc4* (Fig. 2*a*). In addition, Rsc1 and Rsc2 are the only yeast genes that possess both a bromodomain and a BAH region, indicating that they are the closest relatives of BAF180 in this species. Because the number of bromodomains (6) and BAH regions (2) in BAF180 is identical to the total of all three Rsc proteins (Fig. 2 *b* and *c*), we propose that BAF180 is a homolog of all three Rsc subunits combined (yeast Rsc proteins lack the HMG-domain present in BAF180, but Rsc1 and Rsc2 contain AT-hook motifs that can similarly bind the minor-groove of DNA with low sequence-specificity). Notably, the BAH regions and minor-groove binding domains present in PBAF and Rsc are missing in yeast SWI/SNF. The data and analyses thus suggest that PBAF is structurally more related to Rsc than to SWI/SNF. The fact that Rsc1/Rsc2/Rsc4 and BAF180 share many conserved structure motifs further suggests that these proteins are important for functions of their corresponding complexes. Indeed, single mutants of *rsc1* or *rsc2* are nonessential for yeast growth, whereas the double mutant is lethal (39).

The BAF180 Equivalent of *Drosophila melanogaster* and *Caenorhabditis elegans* Is the Closest Relative of Rsc1 and Rsc2 in Their Entire Genomes. It remains to be determined whether BAF180 is the closest human relative of Rsc1, Rsc2, and Rsc4. Because the human genome is not completely assembled, we cannot rule out the possibility that other human genes may be more similar to the three Rsc genes than is BAF180. However, in the completed *Drosophila* and *C. elegans* genomes, sequence searches revealed a single homolog of BAF180 present in each organism: CG11375 and CE05310, respectively (Fig. 2*a*). All structural domains identified in BAF180 are conserved in these two homologs. Importantly, when sequences of Rsc1, Rsc2, and Rsc4 were used to search genomes of these two organisms, CG11375 and CE05310 were also found as the top homologs for all three Rsc genes (data not shown). In fact, no other fly or nematode genes harbor both a bromodomain and a BAH region, suggesting that CG11375 and CE05310 are the closest relatives for Rsc1 and Rsc2. We infer that, during evolution between yeast and *C. elegans*, Rsc1, Rsc2, and Rsc4 became fused to form a single gene. Comparable fusions have been reported for many other proteins. For example, human transcription cofactor TAF250 was shown to be a combined homolog of two yeast TAFs: yTAF145 and BDF1 (40). Based on the observation that some pairs of interacting proteins have homologs in another organism fused into a single polypeptide, a computational method has been developed to predict thousands of interacting protein pairs from genome sequences (41).

PBAF and BAF Share More Identical Subunits Than Yeast SWI/SNF and Rsc. Because BAF180 is a unique subunit of PBAF, immunopurification by using its antibody should isolate PBAF but not BAF. Indeed, the complex isolated with BAF180 antibody exhibits an electrophoretic pattern on a SDS-gel strikingly similar to that of PBAF purified with BRG1 antibody, and different from BAF (compare Fig. 3*a* with Fig. 1*b*). Overall, up to eight PBAF subunits were found to be identical to those in BAF by immunoblotting (Fig. 3*b* and Table 1). The number of identical subunits in human complexes is significantly more than that in yeast SWI/SNF and Rsc, which share only two identical subunits and have more orthologous subunits (at least four). To make certain of this finding, we performed mass spectrometry microsequencing of several PBAF subunits that are known to have orthologs. BAF60 is known to have three different human orthologs, whereas BAF53 has two. BAF complex contains only BAF60a and BAF53a (ref. 30; data not shown). Mass spectrometric analysis confirmed the immunoblot data that hRsc contains only BAF60a, but not BAF60b or BAF60c; it harbors BAF53a but not BAF53b (Table 1 legend). We have also analyzed PBAF purified directly from nuclear extract by using BAF180 antibody and obtained the same results (data not shown). The data thus underscore one difference between human and yeast complexes.

We noticed that BAF47/hSNF5 isolated with BAF180 antibody reacted poorly with its antibody on immunoblot (Fig. 3*b*). However, mass spectrometric analysis of this polypeptide revealed the presence of peptides derived from hSNF5 peptides (Table 1 legend). In addition, PBAF isolated with the monoclonal antibody against flag-hSNF5 contains BAF180 (Fig. 1 *d*

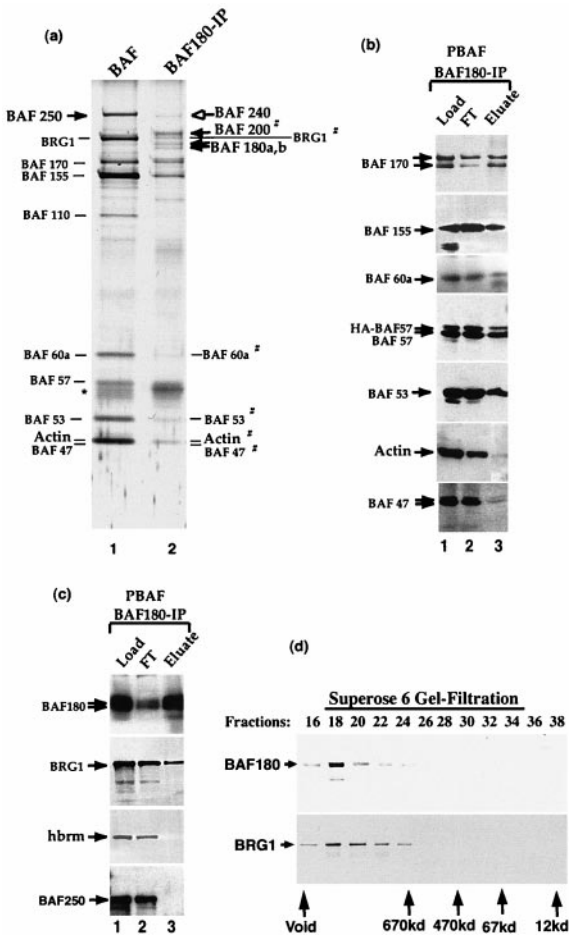

Fig. 3. The PBAF complex contains both common and distinctive subunits compared with BAF. (*a*) A silver-stained SDS-gel showing PBAF purified with BAF180 antibody (lane 2). BAF complex was shown as a control (lane 1). Unique components are marked with arrows. The presence of IgG is indicated by an asterisk. Several polypeptides that have been further identified by mass spectrometry analysis were denoted with #. (*b* and *c*) Immunoblot analysis of the load, flowthrough (FT), and eluate fractions from a BAF180 antibody column. The doublet bands of BAF180 may represent isoforms from alternatively spliced mRNA. (*d*) Immunoblot analysis of elution profile of a Superose 6 gel-filtration chromatography to estimate the molecular mass of PBAF.

and *e*). Furthermore, PBAF purified with a polyclonal antibody against hSNF5 similarly includes BAF180 (data not shown). All these data indicate that hSNF5 is a component of PBAF. It is possible that hSNF5 in PBAF is posttranslationally modified, which reduced the interaction between the antibody and antigen on immunoblot.

PBAF Contains Only BRG1 but Not hbrm. Immunoblot analysis of PBAF purified on a BAF180 antibody column revealed two important differences between PBAF and BAF. First, PBAF contains BAF180 but not BAF250 (Fig. 3*c*), consistent with the silver-staining analysis of the two complexes on SDS gels (see Fig. 3*a*). Second, PBAF contains only BRG1 but not hbrm, a result further confirmed by microsequencing (Table 1 legend). The finding that PBAF contains only BRG1 is in contrast to the BAF complex that can contain either of the two ATPases (51). It should be noted that our previous purification by using an hbrm antibody has obtained BAF180 and PBAF, suggesting that hbrm could associate with BAF180 or PBAF (20). However, that antibody was raised against a region moderately conserved between BRG1 and hbrm (about 60% identity). In light of our current observations, we suspect that the hbrm antibody may have immunoisolated the PBAF complex through antibody crossreactivity with BRG1.

The molecular weight of PBAF was estimated by using Superose 6 gel-filtration chromatography. It fractionates as a complex of about 1.5–2 MDa (Fig. 3*d*), similar to the size of human BAF or yeast SWI/SNF (23, 42). It is somewhat larger than yeast Rsc, which was estimated as a complex of 1 MDa (6).

PBAF Contains an ATP-Dependent Nucleosome Disruption Activity Similar to BAF. PBAF purified with BAF180 antibody was tested for its ATP-dependent nucleosome disruption activity by using the same assay previously used for yeast SWI/SNF, human BAF, and nucleosome remodeling and histone deacetylation chromatin-remodeling complexes (13, 31, 33). In this assay, a 176-bp fragment of 5S ribosomal DNA containing a nucleosome-positioning sequence was assembled into a rotationally phased mononucleosome. DNase I digestion of this nucleosome generates a characteristic 10-bp ladder on denaturing PAGE (Fig. 4, compare lanes 2 with 1). PBAF purified with either BAF180 antibody (lanes 6 and 7) or BRG1 antibody (lanes 8 and 9) strongly disrupts the 10-bp ladder in the presence but not in the absence of ATP. The disrupted pattern is similar to that generated by the BAF complex (lanes 4 and 5). Thus, PBAF and BAF complexes, although distinguishable, share structural as well as catalytic features.

PBAF Is Localized to Kinetochores of Mitotic Chromosomes in the Absence of Microtubules. Rsc is required for cell cycle progression through mitosis in yeast. The mitotic function of Rsc prompted us to investigate whether PBAF may play a similar role in cell division. We analyzed the intracellular localization of PBAF during several phases of cell cycle by indirect immunofluorescence using a BAF180 antibody. Cytoplasmic dynein, which is known to be present at kinetochores and mitotic spindles during cell division (43, 44), was costained and served as a marker. In interphase cells, BAF180 antibody strongly stains nuclei, consistent with the biochemical data for PBAF as a nuclear complex (Fig. 5 *Top*). In prometaphase cells (Fig. 5 *Middle*), PBAF has overlapping colocalization with dynein at some kinetochores and at spindle poles. The kinetochore localization of PBAF was also evident in cells arrested at prometaphase by treatment of a microtubule-destabilizing drug, nocodazole (Fig. 5 *Bottom*). Our data are consistent with a role for PBAF at kinetochores during mitosis. The localization of PBAF at kinetochores was observed only in prometaphase, but not in metaphase, anaphase, telophase, or cytokinesis (data not shown). The association of PBAF with spindle poles has been seen throughout prometaphase and metaphase and under several conditions of cell fixation (data not shown).

Discussion

The SWI/SNF family of chromatin-remodeling complexes includes SWI/SNF and Rsc from yeast, BAF (SWI/SNF-A) and PBAF (SWI/SNF-B) from human. They share at least six homologous subunits (Table 1), and therefore very likely overlap substantially in structure. Most previous studies, however, have inferred a close relation between the human complexes and yeast SWI/SNF. In fact, all these human complexes have been referred to as "SWI/SNF-related" or simply "human SWI/SNF." The importance of our finding is that it demonstrates that PBAF is structurally related to Rsc rather than SWI/SNF. This finding may prompt reconsideration of the interpretation of experiments that used a mixture of BAF and PBAF. Some biological functions previously assigned to "human SWI/SNF" may actually be attributable to the "human Rsc" (PBAF) complex.

BAF180 is likely a combined homolog of yeast Rsc1, Rsc2, and Rsc4. It confers a set of biochemical features to PBAF similar to

Table 1. Human BAF and PBAF components and their yeast orthologs

	Human		Yeast orthologs		
BAFs	BAF	PBAF	SWI/SNF	Rsc	Known domains
BAF250/p270	+	−	SWI1	—	ARID
BAF180	−	+	—	Rsc1, 2, 4	Bromo, BAH, HMG
BRG1	+	+	SWI2	STH1	ATPase, bromo
hbrm	+	−	SWI2	—	ATPase, bromo
BAF170	+	+	SWI3	Rsc8	Myb, Leu-zipper
BAF155	+	+	SWI3	Rsc8	Myb, Leu-zipper
BAF60a	+	+	SWP73	Rsc6	
BAF57	+	+	—	—	HMG, coiled-coil
BAF53	+	+	ARP7, 9	ARP7, 9	Actin-related protein
Actin	+	+	—	—	ATPase, Ca^{++} binding
BAF47	+	+	SNF5	SFH1	

Microsequencing was done for selected PBAF subunits. Twelve peptide sequences were obtained for the 190-kDa band: 10 are specifically from BRG1; 2 from sequences conserved between BRG1 and hbrm; and none is hbrm-specific. All 3 peptide sequences obtained for the 60-kDa band are from BAF60a, but not BAF60b or BAF60c. All 6 peptides for the 53-kDa band are from BAF53a. From the 48-kDa band, 3 peptides are from actin and 5 are from hSNF5/BAF47. BAF250 has been identified as the same protein as p270 (51), which has been independently cloned by Moran and colleagues (48, 49). They found that p270 is related to yeast SWI1 and *Drosophila* Osa and showed that p270 associates with several BAF subunits. Osa has been shown to be a subunit of a *Drosophila* SWI/SNF-related complex (50).

those provided for yeast Rsc by the three Rsc proteins. The same three yeast proteins are the only unique subunits of Rsc that have a homolog in the completed *C. elegans* database (B. Cairns, personal communication). The fact that no other Rsc proteins are conserved in higher eucaryotes is consistent with the notion that the only conserved biochemical markers for this subfamily of complexes are those present in BAF180 and in Rsc1, Rsc2, and Rsc4. Our study thus suggests that Rsc and PBAF represent an evolutionarily conserved subfamily of chromatin-remodeling complexes. SWI/SNF and BAF would represent the other subfamily, with its own distinctive structural motifs (51).

If human PBAF and BAF have functions as distinct as their yeast counterparts, the subunits unique to each complex must confer specificity. Indeed, the domains present in BAF180 are all capable of targeting PBAF to chromatin. The bromodomains and BAH-regions are conserved in yeast Rsc1/Rsc2 and are required for the Rsc functions (39). Structural analysis has suggested that a bromodomain can serve as an acetyl-lysine-binding motif, and may target proteins to acetylated chromatin (45). The function of BAH is currently unknown, but HMG domains can bind DNA or chromatin and play "architectural" roles during formation of higher-order nucleoprotein complexes (46). It is therefore possible that, through these domains, BAF180 anchors PBAF to its specific sites in chromatin.

Distinguishing the two distinct subfamilies of complexes may help to rationalize previously puzzling genetic data. Both

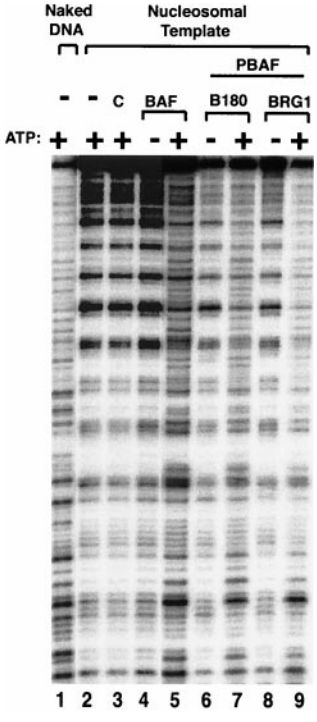

Fig. 4. Human PBAF contains an ATP-dependent mononucleosome disruption activity similar to BAF. An autoradiograph from mononucleosome disruption assay with PBAF purified with either BAF180 (B180) or BRG1 antibodies. The result for BAF is shown for comparison. The complexes and templates used are shown above figure. C, A control using antibody beads without complex loaded. The presence (+) or absence (−) of 1 mM ATP is indicated.

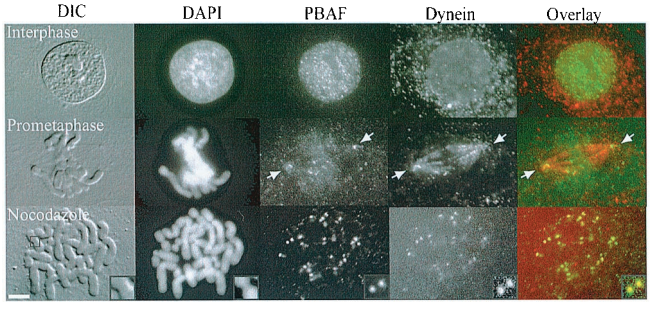

Fig. 5. PBAF localizes at mitotic kinetochores and spindle poles. Immunofluorescence micrographs of cells in interphase (*Top*), prometaphase without nocodazole (*Middle*), and prometaphase in 10 μM nocodazole (*Bottom*). The small inserts in the lower right-hand corners of the bottom row illustrate colocalization of PBAF and dynein at an enlarged kinetochore. The kinetochore that appears in the zoomed *Inset* is marked by a box in the image obtained from differential interference contrast light microscopy (DIC). DNA was visualized by 4′,6-diamidino-2-phenylindole (DAPI) staining. In the overlay column, green represents PBAF staining and red represents dynein. The arrows indicate the spindle poles. Scale bar = 5 μm.

SWI2/SNF2-related ATPases in mammals, brm and BRG1, have been genetically altered in mice and tissue culture cells. Mice in which the mbrm gene was disrupted were viable (26), whereas F9 teratocarcinoma cells deleted for the mBRG1 gene were inviable (47). These results parallel those in yeast, where cells inactivated of SWI2/SNF2 (the ATPase of SWI/SNF) are viable, whereas disruption of STH1 (the ATPase of Rsc) is lethal. The data imply that brm and BRG1 might be the functional equivalents of SWI2/SNF2 and STH1, respectively, in mammalian cells. In accord with these genetic data, we found that both hbrm and BRG1 are present in BAF (51), but only BRG1 is present in PBAF (Fig. 3*c*). Consequently, disrupting the brm gene would abolish only the subset of BAF complexes that have brm, but not those containing BRG1; and all PBAF complexes would remain intact. In contrast, deletion of the BRG1 gene similarly abolished only a subset of BAF. But importantly, it completely eliminated the PBAF complexes, leading to cell death. The data further suggest that mammalian PBAF, like its yeast counterpart Rsc, may be essential for cell growth.

Our observation that PBAF localizes to kinetochores of mitotic chromosomes implies that PBAF may play a role there during mitosis. This notion is consistent with several yeast studies for the related Rsc complex. First, several *rsc* mutants are blocked at G_2/M transition of the cell cycle (15–17). This block depends on the spindle-assembly checkpoint (17), but not the DNA-damage checkpoint (16). Because the spindle checkpoint is usually activated by a failure of chromosome attachment to microtubules or by spindle damage, Rsc is probably required for either kinetochore function, or spindle assembly, or both. Second, an *rsc* mutant is more sensitive to a microtubule-destabilizing drug (17). The increased sensitivity of cells to microtubule-destabilizing drugs is a phenotype commonly observed in mutants of kinetochore components, or mutants of tubulin, microtubule-associated proteins, and components of the spindle checkpoint. Third, an *rsc* mutant exhibits increased frequency of non-disjunction of minichromosomes, which could be caused by defective interactions between kinetochores of paired sister chromatids and spindles (17). Fourth, an *rsc* mutant displays altered chromatin structure at centromeres (17). Two earlier models have been proposed to explain these data. One model proposed that the altered centromeric chromatin structure in the *rsc* mutant may impair kinetochore functions and lead to execution of the spindle checkpoint (17). The second model hypothesized that Rsc may be needed for transcription of genes required for mitosis (16, 17). Our observation appears to favor the first model. It remains to be investigated, however, whether PBAF has additional kinetochore functions other than maintaining centromeric chromatin structure.

We thank Dr. R. Kingston for providing the INI-11 cell line, R. Schiekhattar for several protein fractions, and Dr. Goodwin for providing chicken PB1 antibody. We thank Dr. B. Cairns for communicating unpublished results and advice. Some cells used in this study have been provided by the National Cell Culture Center. We are very grateful to Dr. D. Schlessinger for advice and critical reading of the manuscript. W.W. is a scholar of the Ellison Medical Foundation.

1. Pazin, M. J. & Kadonaga, J. T. (1997) *Cell* **88,** 737–740.
2. Workman, J. L. & Kingston, R. E. (1998) *Annu. Rev. Biochem.* **67,** 545–579.
3. Kornberg, R. D. & Lorch, Y. (1999) *Cell* **98,** 285–294.
4. Cote, J., Quinn, J., Workman, J. L. & Peterson, C. L. (1994) *Science* **265,** 53–60.
5. Cairns, B. R., Kim, Y. J., Sayre, M. H., Laurent, B. C. & Kornberg, R. D. (1994) *Proc. Natl. Acad. Sci USA* **91,** 1950–1954.
6. Cairns, B. R., Lorch, Y., Li, Y., Zhang, M., Lacomis, L., Erdjument-Bromage, H., Tempst, P., Du, J., Laurent, B. & Kornberg, R. D. (1996) *Cell* **87,** 1249–1260.
7. Stern, M., Jensen, R. & Herskowitz, I. (1984) *J. Mol. Biol.* **178,** 853–868.
8. Neigeborn, L. & Carlson, M. (1984) *Genetics* **108,** 845–858.
9. Peterson, C. L. & Herskowitz, I. (1992) *Cell* **68,** 573–583.
10. Yukawa, M., Katoh, S., Miyakawa, T. & Tsuchiya, E. (1999) *Genes Cells* **4,** 99–110.
11. Moreira, J. M. & Holmberg, S. (1999) *EMBO J.* **18,** 2836–2844.
12. Cairns, B. R., Erdjument-Bromage, H., Tempst, P., Winston, F. & Kornberg, R. D. (1998) *Mol. Cell* **2,** 639–651.
13. Owen-Hughes, T., Utley, R. T., Cote, J., Peterson, C. L. & Workman, J. L. (1996) *Science* **273,** 513–516.
14. Lorch, Y., Cairns, B. R., Zhang, M. & Kornberg, R. D. (1998) *Cell* **94,** 29–34.
15. Cao, Y., Cairns, B. R., Kornberg, R. D. & Laurent, B. C. (1997) *Mol. Cell. Biol.* **17,** 3323–3334.
16. Du, J., Nasir, I., Benton, B. K., Kladde, M. P. & Laurent, B. C. (1998) *Genetics* **150,** 987–1005.
17. Tsuchiya, E., Hosotani, T. & Miyakawa, T. (1998) *Nucleic Acids Res.* **26,** 3286–3292.
18. Krebs, J. E., Fry, C. J., Samuels, M. L. & Peterson, C. L. (2000) *Cell* **102,** 587–598.
19. Kwon, H., Imbalzano, A. N., Khavari, P. A., Kingston, R. E. & Green, M. R. (1994) *Nature (London)* **370,** 477–481.
20. Wang, W., Cote, J., Xue, Y., Zhou, S., Khavari, P. A., Biggar, S. R., Muchardt, C., Kalpana, G. V., Goff, S. P., Yaniv, M., *et al*. (1996) *EMBO J.* **15,** 5370–5382.
21. Armstrong, J. A., Bieker, J. J. & Emerson, B. M. (1998) *Cell* **95,** 93–104.
22. Muchardt, C. & Yaniv, M. (1993) *EMBO J.* **12,** 4279–4290.
23. Khavari, P. A., Peterson, C. L., Tamkun, J. W., Mendel, D. B. & Crabtree, G. R. (1993) *Nature (London)* **366,** 170–174.
24. Kalpana, G. V., Marmon, S., Wang, W., Crabtree, G. R. & Goff, S. P. (1994) *Science* **266,** 2002–2006.
25. Lee, D., Sohn, H., Kalpana, G. V. & Choe, J. (1999) *Nature (London)* **399,** 487–491.
26. Reyes, J. C., Barra, J., Muchardt, C., Camus, A., Babinet, C. & Yaniv, M. (1998) *EMBO J.* **17,** 6979–6991.
27. Shanahan, F., Seghezzi, W., Parry, D., Mahony, D. & Lees, E. (1999) *Mol. Cell. Biol.* **19,** 1460–1469.
28. Dunaief, J. L., Strober, B. E., Guha, S., Khavari, P. A., Alin, K., Luban, J., Begemann, M., Crabtree, G. R. & Goff, S. P. (1994) *Cell* **79,** 119–130.
29. Versteege, I., Sevenet, N., Lange, J., Rousseau-Merck, M. F., Ambros, P., Handgretinger, R., Aurias, A. & Delattre, O. (1998) *Nature (London)* **394,** 203–206.
30. Wang, W., Xue, Y., Zhou, S., Kuo, A., Cairns, B. & Crabtree, G. R. (1996) *Genes Dev.* **10,** 2117–2130.
31. Wang, W., Chi, T., Xue, Y., Zhou, S., Kuo, A. & Crabtree, G. R. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 492–498.
32. Zhao, K., Wang, W., Rando, O. J., Xue, Y., Swiderek, K., Kuo, A. & Crabtree, G. R. (1998) *Cell* **95,** 625–636.
33. Xue, Y., Wong, J., Moreno, G. T., Young, M. K., Cote, J. & Wang, W. (1998) *Mol. Cell* **2,** 851–861.
34. Sif, S., Stukenberg, P. T., Kirschner, M. W. & Kingston, R. E. (1998) *Genes Dev.* **12,** 2842–2851.
35. Canman, J. C., Hoffman, D. B. & Salmon, E. D. (2000) *Curr. Biol.* **10,** 611–614.
36. Salmon, E. D., Shaw, S. L., Waters, J., Waterman-Storer, C. M., Maddox, P. S., Yeh, E. & Bloom, K. (1998) *Methods Cell. Biol.* **56,** 185–215.
37. Schnitzler, G., Sif, S. & Kingston, R. E. (1998) *Cell* **94,** 17–27.
38. Nicolas, R. H. & Goodwin, G. H. (1996) *Gene* **175,** 233–240.
39. Cairns, B. R., Schlichter, A., Erdjument-Bromage, H., Tempst, P., Kornberg, R. D. & Winston, F. (1999) *Mol. Cell* **4,** 715–723.
40. Matangkasombut, O., Buratowski, R. M., Swilling, N. W. & Buratowski, S. (2000) *Genes Dev.* **14,** 951–962.
41. Marcotte, E. M., Pellegrini, M., Ng, H. L., Rice, D. W., Yeates, T. O. & Eisenberg, D. (1999) *Science* **285,** 751–753.
42. Peterson, C. L., Dingwall, A. & Scott, M. P. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 2905–2908.
43. Pfarr, C. M., Coue, M., Grissom, P. M., Hays, T. S., Porter, M. E. & McIntosh, J. R. (1990) *Nature (London)* **345,** 263–265.
44. Steuer, E. R., Wordeman, L., Schroer, T. A. & Sheetz, M. P. (1990) *Nature (London)* **345,** 266–268.
45. Dhalluin, C., Carlson, J. E., Zeng, L., He, C., Aggarwal, A. K. & Zhou, M. M. (1999) *Nature (London)* **399,** 491–496.
46. Grosschedl, R., Giese, K. & Pagel, J. (1994) *Trends Genet.* **10,** 94–100.
47. Sumi-Ichinose, C., Ichinose, H., Metzger, D. & Chambon, P. (1997) *Mol. Cell. Biol.* **17,** 5976–5986.
48. Dallas, P. B., Cheney, I. W., Liao, D. W., Bowrin, V., Byam, W., Pacchione, S., Kobayashi, R., Yaciuk, P. & Moran, E. (1998) *Mol. Cell. Biol.* **18,** 3596–3603.
49. Dallas, P. B., Pacchione, S., Wilsker, D., Bowrin, V., Kobayashi, R. & Moran, E. (2000) *Mol. Cell. Biol.* (2000) **20,** 3137–3146.
50. Collins, R. T., Furukawa, T., Tanese, N. & Treisman, J. E. (1999) *EMBO J.* **18,** 7029–7040.
51. Nie, Z., Xue, Y., Yang, D., Zhou, S., Deroo, B. J., Archer, R. K. & Wang, W. (2000) *Mol. Cell. Biol.*, in press.