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Purification and biochemical heterogeneity of the mammalian SWI–SNF complex

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We have purified distinct complexes of nine to 12 proteins [referred to as BRG1-associated factors (BAFs)] from several mammalian cell lines using an antibody to the SWI2–SNF2 homolog BRG1. Microsequencing revealed that the 47 kDa BAF is identical to INI1. Previously INI1 has been shown to interact with and activate human immunodeficiency virus integrase and to be homologous to the yeast SNF5 gene. A group of BAF47-associated proteins were affinity purified with antibodies against INI1/BAF47 and were found to be identical to those co-purified with BRG1, strongly indicating that this group of proteins associates tightly and is likely to be the mammalian equivalent of the yeast SWI–SNF complex. Complexes containing BRG1 can disrupt nucleosomes and facilitate the binding of GAL4–VP16 to a nucleosomal template similar to the yeast SWI–SNF complex. Purification of the complex from several cell lines demonstrates that it is heterogeneous with respect to subunit composition. The two SWI–SNF2 homologs, BRG1 and hbrm, were found in separate complexes. Certain cell lines completely lack BRG1 and hbrm, indicating that they are not essential for cell viability and that the mammalian SWI–SNF complex may be tailored to the needs of a differentiated cell type.

Keywords: BRG1/hbrm/INI1/SNF/SWI

Introduction

In *Saccharomyces cerevisiae*, the products of five genes, SWI1 (ADR6), SWI2 (SNF2), SWI3, SNF5 and SNF6, are required for the activation of discrete transcriptional responses such as mating and sucrose fermentation (Neigeborn and Carlson, 1984; Stern *et al.*, 1984; Breeden and Nasmyth, 1987; for reviews see Winston and Carlson, 1992; Carlson and Laurent, 1994; Peterson and Tamkun, 1995). Both genetic and biochemical studies have indicated that these five proteins function together as a multisubunit complex. For example, single and double SWI–SNF mutants have similar phenotypes (Peterson and Herskowitz, 1992). Each of the five SWI–SNF gene products participates in the regulation of the same set of genes (reviewed by Peterson and Herskowitz, 1992; Winston and Carlson, 1992). These proteins co-fractionate through many chromatographic columns and co-elute from a sizing column with an apparent molecular weight of 2×10^6 Da (Cairns *et al.*, 1994; Peterson *et al.*, 1994). Immunoprecipitation by antibodies against different SWI–SNF proteins precipitates all five proteins, indicating that they are indeed subunits of one complex. Biochemical purification of the complex has shown that it consists of 11 subunits, including TFGIII and SNF11 (Cairns *et al.*, 1994; Côté *et al.*, 1994; Lewin, 1994; Peterson and Tamkun, 1995; Treich *et al.*, 1995).

The function of the SWI–SNF complex appears to involve interactions with chromatin (for reviews see Winston and Carlson, 1992; Carlson and Laurent, 1994; Wolffe, 1994; Peterson and Tamkun, 1995). Several genetic suppressors of *swi/snf* mutants have been found in components of chromatin, such as H2A, H2B, H3 and H4 (Hirschhorn *et al.*, 1995; Prelich and Winston, 1993; Kruger *et al.*, 1995). The chromatin structure of the SUC2 promoter is altered in *swi/snf* mutants, a change that is independent of transcription (Hirschhorn *et al.*, 1992). In addition, the purified yeast SWI–SNF complex has an ATP-dependent activity that enables it to disrupt mononucleosomes and facilitate the binding of GAL4 derivatives to the nucleosome core *in vitro* (Côté *et al.*, 1994). Thus, one model for the function of the SWI–SNF complex is that it facilitates the ability of transcriptional activators to overcome the repressive effects of chromatin (Travers, 1992).

Several lines of evidence suggest that higher eukaryotes contain homologs of the yeast SWI–SNF complex. First, transcription activation by the rat glucocorticoid receptor (GR), the *Drosophila* bicoid factor, and ftz have been shown to be dependent on SWI–SNF function when expressed ectopically in yeast (Laurent and Carlson, 1992; Peterson and Herskowitz, 1992; Yoshinaga *et al.*, 1992). In addition, *brahma* (*brm*) a *Drosophila* homolog of the SWI2 gene (Kennison and Tamkun, 1988; Tamkun *et al.*, 1992), suppresses mutations in *Polycomb*, a repressor of several homeotic genes thought to act by regionally compacting chromatin (reviewed by Moehrle and Paro, 1994). Thus, *brm* itself may be involved in modification of the chromatin structure. *brm* is required for the proper

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expression of several homeotic genes and segmentation genes (Tamkun *et al.*, 1992; Brizuela *et al.*, 1994). Both its maternal function and zygotic function are required for *Drosophila* embryogenesis. Flies lacking either function die at embryonic stages. The ATPase domains of SWI2 and *brm* are functionally interchangeable, suggesting that they play similar roles in transcriptional activation. *brm* appears to be the closest *Drosophila* relative of SWI2. More recently, a more distant SWI2–SNF2 homolog, ISWI, has been demonstrated to be in a complex capable of remodeling chromatin (Elfring *et al.*, 1994; Tsukiyama and Wu, 1995; Tsukiyama *et al.*, 1995).

In mammals, a systematic search for SWI2-like genes has not been conducted. However, at least seven different proteins have thus far been described as having SWI2-like ATPase motifs (Okabe *et al.*, 1992; Troelstra *et al.*, 1992; Delmas *et al.*, 1993; Khavari *et al.*, 1993; Muchardt and Yaniv, 1993; Gibbons *et al.*, 1995; Sheridan *et al.*, 1995). The two closest relatives, BRG1 and hbrm, have been implicated as functional homologs of SWI2 from several lines of evidence (Khavari *et al.*, 1993; Muchardt and Yaniv, 1993; Chiba *et al.*, 1994). These two genes are >70% identical to each other, and are homologous to SWI2 throughout the entire gene. Replacement of the ATPase domain of SWI2 with that of BRG1 can partially rescue the *swi2⁻* phenotype. BRG1 is present in a high molecular weight complex of 2 MDa (Khavari *et al.*, 1993), and thus similar in size to the yeast complex. An apparent dominant negative mutant of BRG1, created by disrupting the ATPase domain, produces moderate suppression of the activity of certain promoters in co-transfection studies (Khavari *et al.*, 1993). Recently, Kwon *et al.* (1994) reported the partial purification of two BRG1-containing complexes from HeLa nuclear extracts by following BRG1 immunoblot reactivity. They showed that their partially purified fractions contain an activity similar to the yeast SWI–SNF complex that can disrupt nucleosomes and facilitate the binding of GAL4 derivatives and TBP to a nucleosomal core (Imbalzano *et al.*, 1994; Kwon *et al.*, 1994). However, because the complex used in these studies was only partially pure (we estimate 1–5%) and the ISWI complex, which appears to be more abundant than the SWI–SNF complex, can also disrupt nucleosomes to allow the binding of transcription factors as well as restriction enzymes (Tsukiyama and Wu, 1995), these studies did not in fact demonstrate that the BRG1-containing complex had nucleosomal disruption activity.

Here we report the complete purification of distinct complexes containing either BRG1 or hbrm by immunoaffinity purification using antibodies to different subunits of the mammalian SWI–SNF complex. Our purified complex differs from the partially purified fractions from HeLa cells reported by Kwon *et al.* (1994) in that the affinity columns allow an additional 500- to 1000-fold purification estimated from Western blots of the BRG1 and hbrm subunits. The 47 kDa subunit of our purified complex was found to be the mammalian homolog of SNF5 [INI1, a gene originally cloned by its ability to interact with human immunodeficiency virus (HIV) integrase (Kalpana *et al.*, 1994); also named hSNF5 (Muchardt *et al.*, 1995)]. Unlike the yeast complex, the mammalian complex is surprisingly heterogeneous with respect to its subunit composition within the same cells or from different cell lines.

Results

Purification of BRG1-associated factors (BAFs)

We purified the BRG1-associated proteins from several mammalian tissues and cell lines using a combination of conventional chromatography and immunoaffinity purification (Figure 1a; see also Materials and methods). The affinity purification was essential because we found that biochemical methods gave ~1–5% of purity, as judged by Western blotting of the fractions with antibodies to the different components and by comparison with the silver-staining profile of each purification step (data not shown). Rabbit polyclonal antibodies were raised against different regions of BRG1 (see Materials and methods). They were used to follow the BRG1 immunoreactivity after each column and to make immunoaffinity columns as the final purification step. Consistent with previous data by Kwon *et al.* (1994), BRG1 immunoreactivity was detected in both P11 0.50 M (referred to as complex A) and P11 0.75 M (referred to as complex B) KCl fractions (data not shown). We found that highly stringent conditions (0.50 M KCl and 0.2 M $GuHCl_2$) could be used to wash the immunoaffinity columns without significant loss of a group of proteins that were present at roughly similar levels, as judged by silver staining. We referred to these proteins as BAFs. These proteins were then eluted from the antibody columns under mildly acidic conditions (pH 2.5, 0.1 M glycine). The purification of complex A from a rat hepatocyte cell line, FAO, revealed a group of 11 BAFs, ranging in molecular weight from 250 to 47 kDa (Figure 1b, lane 1).

Identification of BAF47 as INI1/hSNF5

The identities of these BAF polypeptides were investigated by microsequencing after trypsin digestion. Three peptide sequences were obtained for BAF47 of complex A from rat liver (see the legend to Figure 2), and all of them matched the human INI1 protein. INI1 was identified previously by the yeast two-hybrid system using HIV integrase as the bait (Kalpana *et al.*, 1994). It contains a region that is 35–50% identical to the yeast SNF5 gene (Laurent *et al.*, 1990), and was suggested as its human homolog (Kalpana *et al.*, 1994; Muchardt *et al.*, 1995). A *Drosophila* homolog of SNF5, SNR1, has been cloned by low-stringency hybridization and shown to interact with the *Drosophila* SWI2 homolog, hbrm (Dingwall *et al.*, 1995). Interestingly, we found that INI1 and SNR1 are homologous not only to SNF5, but also to a yeast open reading frame, L8543.4 (PIR access number S53399). This predicted yeast protein (for convenience referred to as SNF5b) does not contain the long N-terminal glutamine-rich region or the C-terminal proline-rich region present in SNF5 but absent in INI1 or SNR1. Therefore, SNF5b is more closely related to INI1/BAF47 or SNR1. The sequence alignment revealed a conserved region that is >30% identical and >50% similar among all these genes (Figure 2a). Previous studies have revealed at least two other homologs for SWI2–SNF2 in yeast: STH1 (Laurent *et al.*, 1992) and an open reading frame homologous to the ISWI family of proteins (Tsukiyama *et al.*, 1995). The presence of multiple SNF5 genes in yeast raises the possibility that there are different forms of the SWI–SNF complex in yeast.

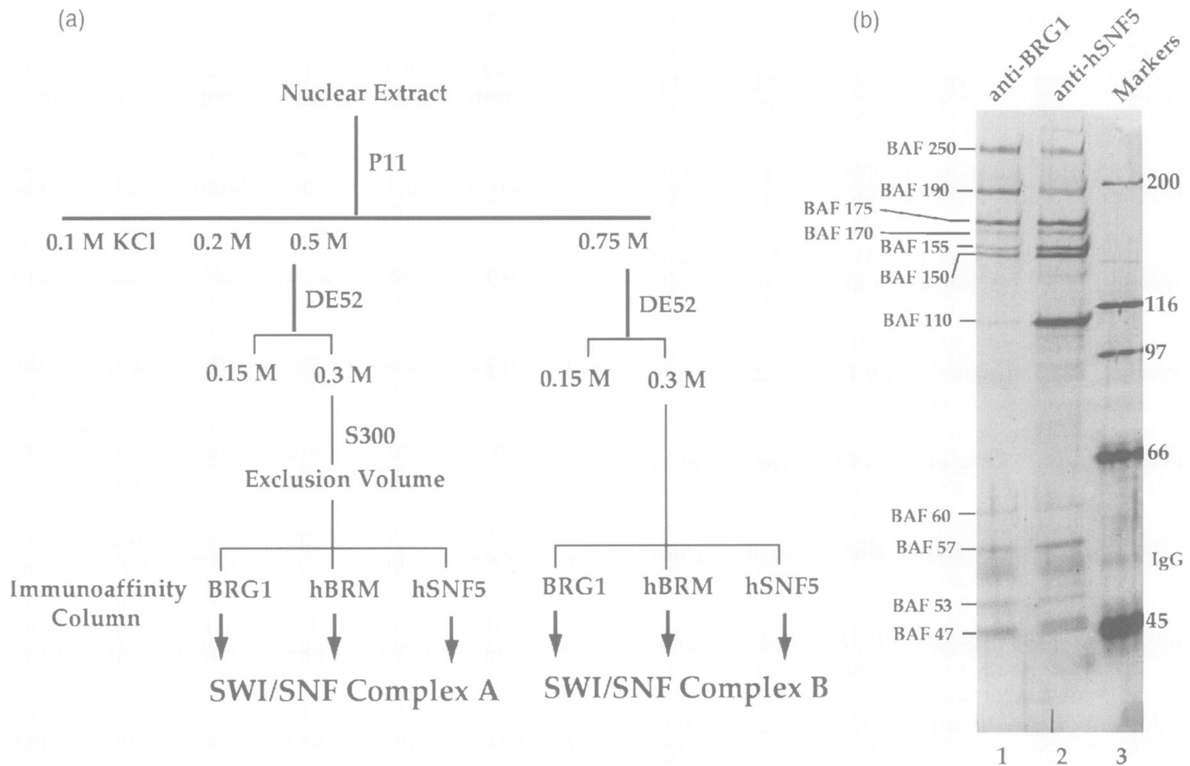

Fig. 1. Purification of the mammalian SWI–SNF complex. (**a**) Purification scheme for mammalian SWI–SNF complexes. (**b**) The silver-stain gel of the purified SWI–SNF complex from a rat hepatocyte cell line, FAO (see Materials and methods). The antibodies against BRG1 (BrgC) or hSNF5/INI1/BAF47 were used in immunoaffinity purifications and are marked at the top of each lane. The eluted polypeptides were named BAFxx (see text; xx refers to the apparent molecular weight). The band between BAF53 and BAF57 is Ig heavy chain (IgG). We noticed a polypeptide with an apparent molecular weight close to 300 kDa in some elutes of INI1/hSNF5 antibody column. This band is not seen in elutes from BRG1 or hbrm antibody columns. Preliminary data suggest that INI1/hSNF5 may also be present in a different complex.

To demonstrate further that BAF47 is INI1, an INI1 antibody was used to analyze the column fractions containing BRG1 by Western blots. INI1 immunoreactivity co-fractionated with BRG1 on every column we used (Figure 2b, lanes 1–4; including single-stranded DNA cellulose, Mono Q and Superose 6; data not shown). INI1 was tightly retained on two different BRG1 affinity columns using the J1 and BrgC antibodies (Figure 2b, lanes 5 and 6), and the association can withstand highly stringent washing conditions (0.5 M KCl and 0.2 M $GuHCl_2$). An affinity column made with INI1 antibody quantitatively depletes BRG1 from the load fraction (lanes 7–9). The binding of BRG1 to the INI1 column is also strong enough to resist the same stringent washing conditions. Furthermore, recombinant INI1 protein has a similar mobility to BAF47, as judged by SDS–PAGE (data not shown). These data strongly indicate that INI1 (BAF47) is the mammalian version of SNF5.

BAFs co-associate with BAF47

Evidence that all of the proteins shown in Figure 1b are likely to be components of a mammalian SWI–SNF complex was obtained by preparing an anti-INI1/BAF47 affinity column and independently purifying the proteins associating with INI1/BAF47. Each of the proteins purified from fraction A with the BRG1 antibody is also present in the complex purified with the hSNF5 antibody (Figure 1b, compare lane 1 with lane 2). The purification of a similar group of proteins with two non-crossreacting antibodies strongly indicates that these proteins associate tightly in the presence of 0.5 M NaCl and 0.2 M $GuHCl_2$ used to wash the affinity column. Based on the observations that (i) the BRG1-associated proteins include a homolog of SNF5, (ii) proteins purified with BRG1 and hSNF5 antibodies show identical mobility on SDS–PAGE gels, and (iii) either the BRG1 or the INI1/BAF47 affinity column will deplete the other component, we tentatively assume that the proteins displayed in Figure 1b, lanes 1 and 2, define a mammalian SWI–SNF complex. We noticed that the amount of BAF110 is more in complexes purified with INI1/BAF47 antibody than in those purified with BRG1 antibody. This could be because the displacement of BAF110 by the anti-BRG1 antibodies makes it less apparent in the final complex.

Purification of distinct forms of SWI–SNF complex from human YT cell line

To determine if SWI–SNF complexes from other mammalian cell lines have a similar composition, we purified the complex from a human lymphoid cell line, YT. Purification from the A fraction (Figure 1a) using either the BRG1 antibody or the INI1/hSNF5 antibody yielded nine polypeptides with apparent molecular weights of 250, 190, 170, 155, 110, 60, 57, 53 and 47 (Figure 3, left panel, lanes 2 and 3). The mobilities of these subunits appear to be the same as those in FAO cells (see Figure 5b and Table I). However, the fraction lacks BAF175 and BAF150, suggesting that different forms of SWI–SNF complex could be present in different mammalian cells. As a control, if the BRG1 terminal peptide used for

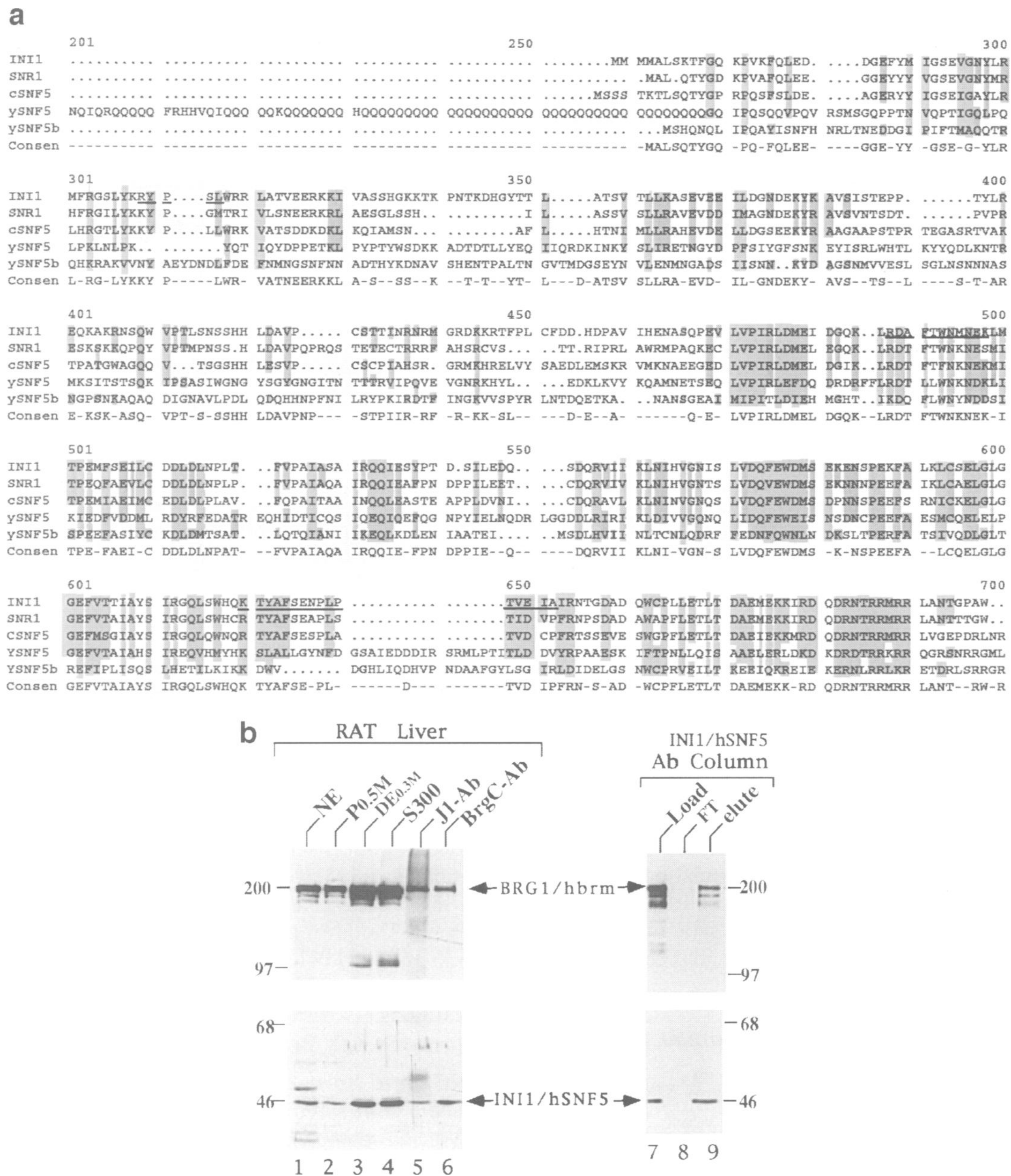

Fig. 2. BAF47/INI1 is homologous to *S.cerevisiae* SNF5, SNF5b, *D.melanogaster* SNR1 and *C.elegans* cSNF5. Microsequencing of three peptide sequences after tryptic digestion of BAF47 showed that all three are identical to the predicted sequence of human INI1 gene. (**a**) The alignment of INI1/hSNF5 (Kalpana *et al.*, 1994) to its *Drosophila* homolog (SNR1; Dingwall *et al.*, 1995), a *Caenorhabditis elegans* open reading frame (cSNF5, R07E5.3; Wilson *et al.*, 1994), and two yeast genes, SNF5 and SNF5b (PIR access number S53399, L8543.4). The peptide sequences obtained by microsequencing within INI1 were underlined. The alignment was performed using the Pileup and Pretty programs of UWGCG. (**b**) Left panel: Western blot of different rat liver fractions containing BRG1 using either BRG1 (top) or INI1/hSNF5 (bottom) antibodies. Each fraction is marked on the top of each lane. J1 and BrgC are two different immunoaffinity columns made with antibodies against different regions of BRG1. The arrows indicate the presence of BRG1 and hbrm or INI1/hSNF5. hbrm also co-fractionates with INI1 (data not shown). Right panel: Western blot of load, flowthrough (FT) and elute fractions of the immunoaffinity column made by INI1/hSNF5 antibodies. The BRG1 antibody J1 was used to assay the top part of the filter and the INI1/SNF5 antibody to assay the bottom. The arrows indicate BRG1 and hbrm or INI1/hSNF5. Some minor bands are probably degradation products of BRG1 and hbrm.

immunization was added prior to affinity purification, binding of the BAFs was blocked (Figure 3, lane 1).

Immunopurification from the B fraction of YT cells yielded 11 polypeptides (complex B, Figure 3, right panel). Nine polypeptides had the same mobilities as those in complex A, suggesting that these are common components of both complexes. BAF250 is present only in complex A, whereas three novel subunits, BAF200, BAF180a and BAF180b, are present only in complex B. An additional 120 kDa polypeptide was seen in the immunopurified B complex using anti-hSNF5/INI1 antibody but not BRG1 antibody (Figure 3, lane 7). Both complexes A and B eluted at the same peak fraction on a Superose 6 column, corresponding to a molecular weight of 2 MDa, the same size as the complex in the crude nuclear extract (data not shown; Khavari *et al.*, 1993), suggesting that they are similar in size and shape. The fact that both complexes have the same molecular weight as the complexes in the

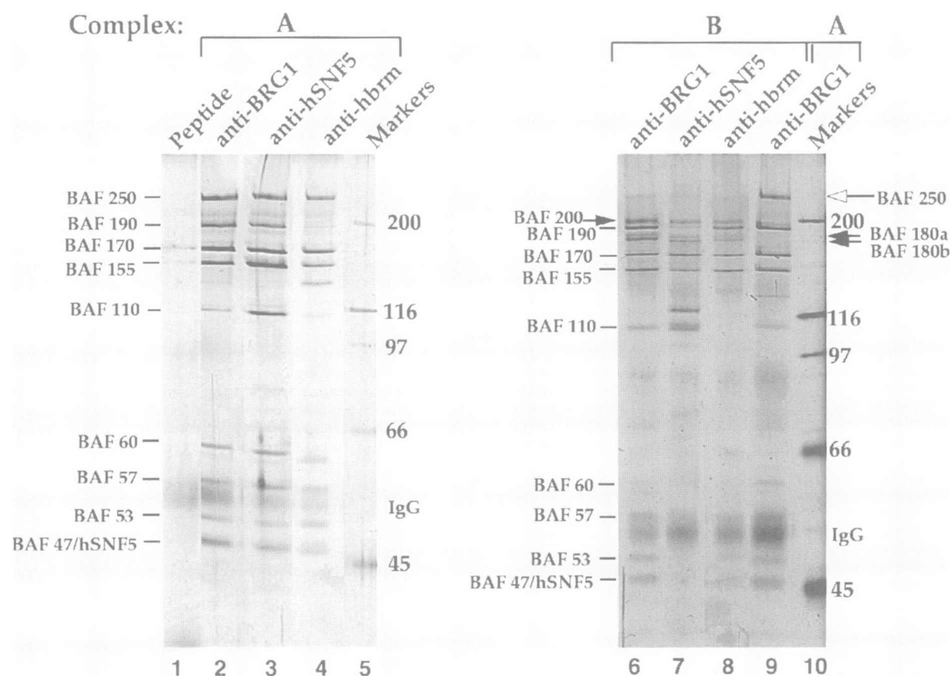

Fig. 3. Purification of two distinct SWI–SNF complexes, A and B. The silver-stain gel of the purified SWI–SNF complexes A and B from a human lymphoid cell line, YT (see Materials and methods). The antibodies against BRG1 (BrgC), hbrm or hSNF5/INI1/BAF47 were used in immunoaffinity purification and are indicated at the top of each lane. Lane 1 (peptide), the BRG1 immunoaffinity column was blocked with its peptide antigen prior to immunopurification. The eluted polypeptides were named BAFxx (see text; xx refers to the apparent molecular weight). The hollow arrow indicates the subunit present only in complex A. The solid arrow indicates the subunits only in complex B. The band between BAF53 and BAF57 is Ig heavy chain (IgG).

crude nuclear extract argues against the possibility that these complexes were derived from degradation or partial dissociation of a single complex during our biochemical purification. The presence of unique subunits in each complex suggests that each complex has a different function or different specificity.

The hbrm-associated proteins are similar to BRG1-associated proteins

hbrm has been identified by Muchardt and Yaniv (1993) as a second mammalian homolog of yeast SWI2 and *Drosophila brahma.* hbrm and BRG1 are >70% identical throughout their length, suggesting that they could perform similar functions and may both be subunits of mammalian SWI–SNF complexes. To identify and purify a potential hbrm-containing complex, a rabbit polyclonal antibody was raised against the N-terminus of hbrm and was used for immunoblotting and immunopurification. Like BRG1, hbrm is also present in a high molecular weight complex of 2 MDa, as determined using a Superose 6 sizing column (data not shown). Its immunoreactivity co-purifies with both BRG1 and INI1/hSNF5 through every column tested (P11, DE52, S300, single-stranded DNA, Mono Q and Superose 6), suggesting that the hbrm complex may have a similar composition to the BRG1 complex. Indeed, purification using the hbrm antibody yielded a very similar pattern of proteins to those generated by the BRG1 or INI1/hSNF5 antibodies (Figure 3, lanes 4 and 8). The INI1/hSNF5 antibody also recognizes the 47 kDa polypeptide eluted from the hbrm antibody column (data not shown; see below), suggesting that BRG1 and hbrm may associate with similar or identical sets of proteins, including INI1/hSNF5. BAF190 is most probably composed of both BRG1 and hbrm because it reacts with their antibodies on Western blotting and is absent in complexes purified from cell lines lacking BRG1 and hbrm (see below). The level of BAF190 is low in the elute from the hbrm antibody column (lane 4) because it binds tightly to the column and is not efficiently eluted under these conditions.

BRG1 and hbrm are in separate complexes with hSNF5 that have overlapping activities

SWI–SNF complexes from both humans and yeast have apparent molecular weights of 2 MDa (Khavari *et al.*, 1993; Peterson *et al.*, 1994). The total molecular weights of individual subunits of either the yeast complex or the mammalian complex can be calculated as ~1 MDa (Cairns *et al.*, 1994; Côté *et al.*, 1994; this study). This raises the possibility that the complex contains more than one copy of each subunit and thus may contain both BRG1 and hbrm. We tested this hypothesis using a BRG1-specific antibody to immunoblot the complex purified with an hbrm antibody column. To overcome any crossreactivity between BRG1 and hbrm antibodies, hbrm was epitope-tagged with the HA epitope and was used to stably transfect mouse DT cells (a ras-transformed 3T3 cell line that lacks hbrm but contains normal amounts of BRG1; Muchardt *et al.*, 1996). Clones that stably express tagged hbrm were selected. One clone (DT21) was grown in large quantities and used to purify the hbrm complex utilizing the 12CA5 monoclonal antibody to the HA-tagged hbrm. BRG1 was not detected in the elute, whereas hSNF5 was detected (Figure 4, lane 3). Thus, purified hbrm complexes do not contain detectable amounts of

BRG1, indicating that BRG1 and hbrm are not present in the same complex.

Despite the observation that BRG1 and hbrm appear to be in different complexes, the subunit compositions of the complexes purified with the BRG1 and hbrm antibodies are quite similar (Figure 3), suggesting that they may have similar activities. hbrm has been shown to stimulate activation by GR in co-transfection studies. We tested whether BRG1 had the same activity as hbrm by co-transfection of BRG1, GR and a GR-induced luciferase reporter gene into SW13 cells. This cell line contains no detectable BRG1 but does contain complexes with most other subunits (Figure 3 and Table I). Figure 5 shows that wild-type BRG1 enhances the activation of GR 5-fold, whereas a point mutant of BRG1, which replaces 706K of ATP binding motif to Arg, stimulates GR activation by <2-fold, indicating that BRG1 has at least one overlapping activity with hbrm.

Distinct forms of the SWI–SNF complex are present in different cell lines

BRG1 and hbrm were found in a wide range of tissues and cell types (Khavari *et al.*, 1993; Muchardt and Yaniv, 1993). INI1/hSNF5 protein is also present in a variety of cell types (Figure 6a). Interestingly, two human cell lines, SW13 and C33A, which contain no detectable hbrm and

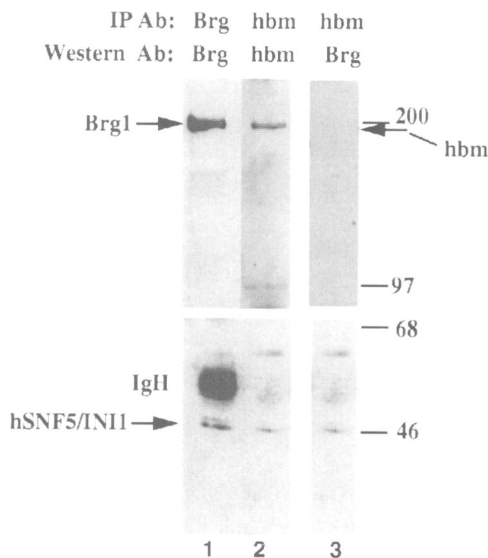

Fig. 4. BRG1 and hbrm associate with SNF5 in separate complexes. A Western blot analysis of the complex immunopurified with BRG1- and hbrm-specific antibodies. hbrm is tagged with Flu antigen and stably transfected mouse DT cells. 12CA5 monoclonal antibody was then used to specifically purify the tagged hbrm complex from these cells. The antibody used for immunopurification (IP) or Western blotting is marked at the top of each lane. All lanes were blotted with INI1/hSNF5 antibody. The arrow indicates the presence of corresponding protein.

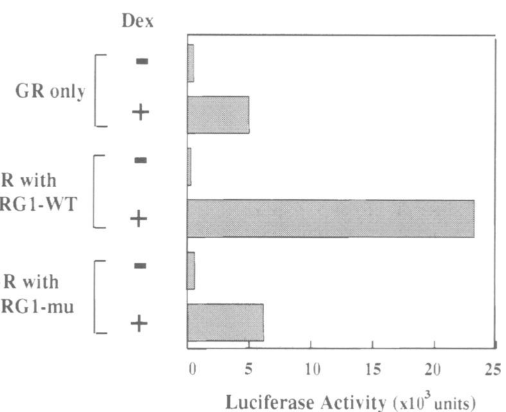

Fig. 5. hbrm and BRG1 complexes have similar activities in co-transfection studies. The co-transfection of BRG1 and GR into SW13 cells stimulates GR activity. The GR expression vector and its reporter, $(GRE)_3$-luciferase plasmid, were co-transfected alone with either wild type (BRG1-WT) or a mutant (BRG1-mu) BRG1 expression vector into SW13 cells. The BRG1 expression vectors have been described previously (Khavari *et al.*, 1993). The graph represents the average of luciferase activities of three independent transfection experiments. A number of other promoters and activators have been tested using the same assay, including AP1, NFAT, HNF-1a, cytomegalovirus and EF-1a. The results were all negative (data not shown).

Table I. Summary of the subunit composition of SWI–SNF complexes A and B from several mammalian cell lines

	Complex A									Complex B		
	YT	C33	SW13	HeLa	Jurkat	DT	C2F3	PC12	FAO	YT	HeLa	SW13
BAF250	+		+	+	+	+	+	+	+			
BAF210			+									
BAF200										+	+	+
hbrm/BRG1	+			+	+	+	+	+	+	+	+	low
BAF180a										+	+	
BAF180b										+	+	
BAF175									+			
BAF170	+	low	low	+	+	+	low	+	+	+	+	+
BAF160		+	+			+	+	+				+
BAF158								+				
BAF155	+	+	+	+	+	+	+	+	+	+	+	+
BAF150							low	+	+			
BAF110	+	+	+	+	+	+	+	+	+	+	+	
BAF60	+	+	+	+	+	+	+	+	+	+	+	+
BAF57	+	+	+	+	+	+	+	+	+	+	+	+
BAF53	+			+	+	+	+	+	+	+	+	
INI1/hSNF5	+	+	+	+	+	+	+	+	+	+	+	+

Data derived from complexes A and B (silver-stain gels in Figure 6b and data not shown). The presence of each subunit (BAF) in each complex of each cell line is indicated.

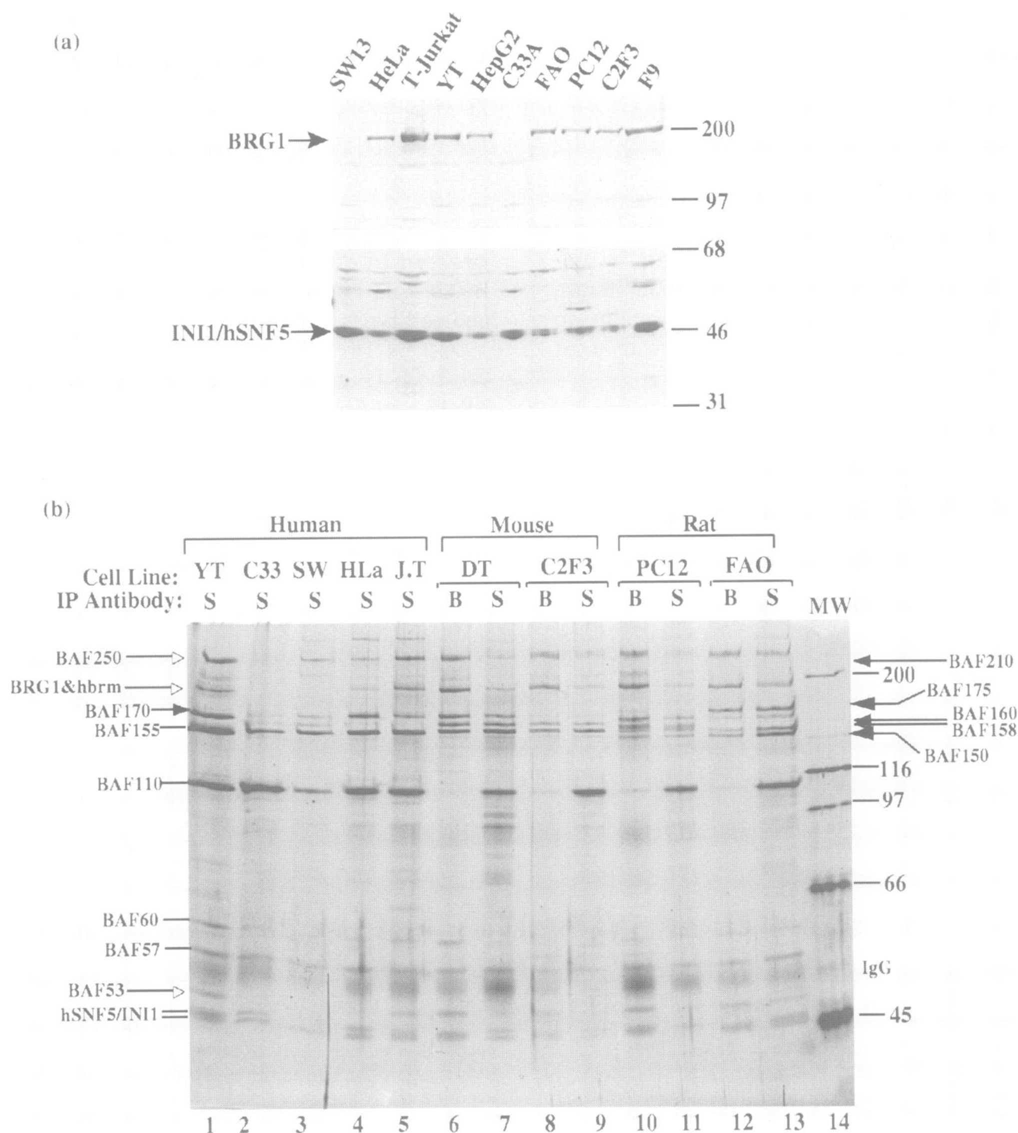

Fig. 6. Multiple forms of SWI–SNF complex A are present in different mammalian cell lines. (**a**) A Western blot of nuclear extracts from several mammalian cell lines using either BRG1 (top) or INI1/hSNF5 antibodies (bottom). The cell lines are marked on top of each lane. (**b**) Silver-stained gel of complex A purified from nine different mammalian cell lines. The A complexes shown for the five human cell lines were purified by the INI1/hSNF5 antibody column because both C33A and SW13 cells lack BRG1 and hbrm in this fraction. The A complexes of HeLa and Jurkat T cells were purified with the BrgC affinity column and the result is consistent with this figure. Their patterns are indistinguishable from each other and are identical to complex A of YT cells (data not shown). The lines indicate polypeptides present in all forms of complex. The hollow arrows indicate polypeptides that only co-purify with hbrm and BRG1. The solid arrows indicate polypeptides that vary when BRG1 and hbrm are present. The abbreviations used for the cell lines are: SW, SW13 cells; HLa, HeLa cells; and JT, Jurkat T cells. The antibodies used for the immunopurifications are abbreviated as: S, hSNF5 antibody; and B, BRG1 antibody. They are marked at the top of each lane. We noticed a polypeptide with an apparent molecular weight close to 300 kDa present in some elutes of the INI1/hSNF5 antibody column. This band is not seen in elutes from BRG1 or hbrm antibody columns. Preliminary data suggest that INI1/hSNF5 may also be present in a different complex.

BRG1 (Muchardt and Yaniv, 1993; Dunaief *et al.*, 1994), have normal amounts of INI1/hSNF5. This suggests that these cells may contain distinct forms of the SWI–SNF complex with INI1/hSNF5 but without BRG1 or hbrm. We purified complex A from these cells using the INI1/hSNF5 antibody column. Immunopurification from SWI3 or C33A cells using BRG1 antibodies did not yield any proteins, reaffirming the specificity of our antibodies (data not shown). As expected, complex A purified with anti-INI1 from either C33A or SW13 cells lacks BAF190, supporting our conclusion that BAF190 represents BRG1 and hbrm (Figure 6b, compare lanes 2 and 3 with lane 1).

As YT, C33 and SW13 cells are derived from different tissues, the data imply that different tissues may contain tissue-specific forms of SWI–SNF complex. As an initial step to identify potential tissue-specific subunits, we purified complex A from several additional cell lines and found that there were clear differences in subunit composition and stoichiometry for these complexes (Figure 6b, lanes 4–13; summary in Table I). The number of subunits in complex A ranged from seven in C33 cells to 12 in PC12 cells. BAF47 (INI1/hSNF5), BAF57, BAF110 and BAF155 are present in all cell lines. Complex B, although more difficult to purify, also varies in subunit composition (Table I).

These different complexes do not arise from proteolytic

degradation, because Western blotting indicates that BRG1 and hbrm, which have very high molecular weights of 190 kDa, are intact in the crude extract as well as in purified fractions (data not shown). Microsequencing and cloning of several small subunits of complex A revealed that they are not degradation products of larger subunits (data not shown). The molecular weight of the complex is ~2 MDa from several cell lines. The differences in molecular weights of the subunits are not caused by the species difference of the cell lines because the complex from mouse DT cells is almost identical to that from human HeLa, Jurkat and YT cells, except for an additional subunit, BAF160. This extra subunit can also be seen in human C33 and SW13 cells.

The mammalian SWI–SNF complex contains mononucleosome disruption activity similar to the yeast complex

The purified yeast SWI–SNF complex contains a DNA-dependent ATPase activity (Cairns *et al.*, 1994; Côté *et al.*, 1994), as well as an ATP-dependent mononucleosome disruption activity, which results in the enhanced binding of GAL4 derivatives to nucleosomal DNA (Côté *et al.*, 1994). Fractionated extracts from HeLa cells containing BRG1 immunoreactivity have also been shown to have nucleosome disrupting activities (Imbalzano *et al.*, 1994; Kwon *et al.*, 1994). To determine if the purified BRG1 complex does indeed have a nucleosome-disrupting activity or if the activity in the previously reported partially pure cellular factions is caused by the related NURF complex (Tsukiyama and Wu, 1995; Tsukiyama *et al.*, 1995), we assayed the nucleosome disruption activity of the purified BRG1 complex. Because we have not been able to efficiently elute the complex without disrupting it, we elected to determine its activity while on the antibody beads after thorough washing. Our purified SWI–SNF complex did not contain detectable hSNF2L, a human homolog of ISWI (Okabe *et al.*, 1992), indicating that it is probably free of the mammalian version of the NURF complex. However, the load or flowthrough fractions of our affinity column do contain abundant immunoreactivity of hSNF2L (Figure 8).

A 154 bp template containing one GAL4 binding site 32 bp from one end was used (Côté *et al.*, 1994) to assay nucleosome disruption activity. Incorporation of the template into a nucleosome core inhibits GAL4–VP16 binding by at least 100-fold (Figure 7, compare lanes 14 and 15 with lanes 2 and 3). The purified mammalian SWI–SNF complex A or B from YT cells strongly disrupts the characteristic 10 bp DNase I ladder of the nucleosome core (compare lanes 5 and 9 with lane 2). The patterns of disruption are indistinguishable from each other and very similar to that of the yeast SWI–SNF complex (Côté *et al.*, 1994). In addition, each complex strongly enhanced the binding of GAL4–VP16 to the nucleosomal core, as revealed by enhanced DNase I footprinting of the protein to its binding site (lane 5 and 9). Both activities require the presence of hydrolyzable ATP (see lanes 4 and 8 for reactions with [γ-S]ATP), which is also consistent with this property of yeast SWI–SNF complex. The immunizing peptide blocked the purification of the nucleosome disruption activity, further implicating BRG1-associated com-

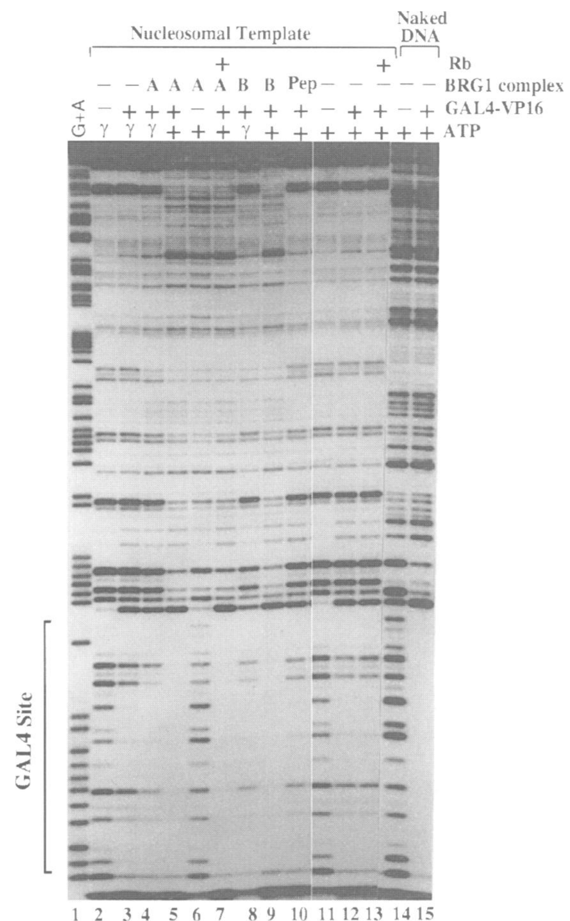

Fig. 7. The purified mammalian SWI–SNF complexes can disrupt nucleosomes and facilitate the binding of GAL4–VP16 to mononucleosomes. A DNase I footprint of reconstituted nucleosomal cores in the presence or absence of 100 nM GAL4–VP16 (G4), 1 mM ATP or [γ-S]ATP, BRG1 complex A or B on beads (3 μl), and recombinant GST–RB, as indicated at the top of each lane (see Materials and methods). A 154 bp probe containing a single GAL4 site at 32 bp from one end was used. Binding was analyzed by DNase I footprinting. The GAL4 binding site is indicated with a bracket.

plexes in the nucleosome disruption activity (compare lane 9 with lane 10).

The retinoblastoma protein (RB) has been reported to form a complex with BRG1 and to induce cell cycle arrest (Dunaief *et al.*, 1994). It has also been suggested that RB interacts with hbrm to potentiate activation by GR (Singh *et al.*, 1995). We found by Western blotting (data not shown) that RB is not a subunit of our purified complexes, probably because of our stringent washing conditions. We also tested whether a recombinant GST–RB fusion protein enhances or represses the activities of our purified complex *in vitro*, but did not observe any effects (lanes 7 and 13).

Mammalian cells contain nucleosome disruption activities other than SWI–SNF complex

A complex with a similar nucleosome remodeling activity to SWI–SNF complex has been purified recently from *Drosophila*. One subunit of this complex was identified as ISWI, which also belongs to the SWI2–SNF2 family of proteins (Tsukiyama and Wu, 1995; Tsukiyama *et al.*, 1995). A mammalian cDNA, hSNF2L, is highly homologous to the *Drosophila* ISWI gene (Okabe *et al.*, 1992;

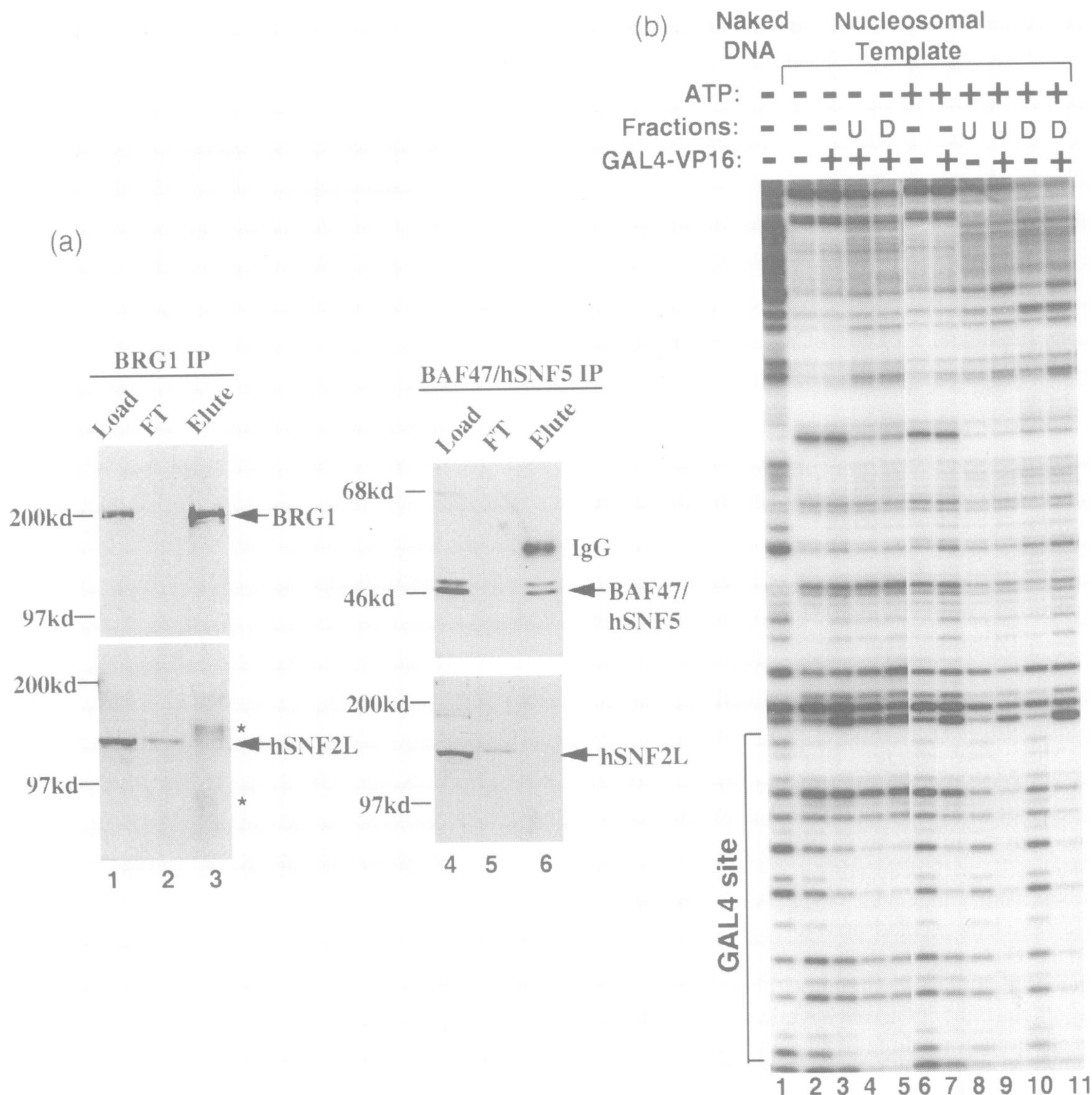

Fig. 8. Mammalian cells contain nucleosome disruption activities other than the SWI–SNF complex. (**a**) A Western blot showing the presence of hSNF2L in the load and flowthrough (FT) fractions of the anti-BRG1 or BAF47/hSNF5 column but not in the elute of the columns. The presence of BRG1, hSNF2L and BAF47/hSNF5 was indicated by arrows. Crosslinked IgG (denoted by an asterisk) was present in the elute fractions of the antibody column and was recognized by the secondary antibody. The protein concentration of the flowthrough fraction was 3-fold less than that of the load fraction, and the same volumes of each fraction were assayed by immunoblotting. Thus the level of hSNF2L in the flowthrough fraction was underestimated compared with the load fraction. (**b**) The mononucleosome disruption assay using a fraction depleted of BRG1–hbrm complex by the BAF47/hSNF5 antibody column. The presence of either depleted extract (D) or undepleted control extract (U) is indicated. The presence or absence of GAL4–VP16 and ATP is shown at the top of each lane. The GAL4 binding site was marked by a bracket. The amount of protein of load or flowthrough fraction was normalized so that equal amount of protein (3 μg) were tested in the assay.

Tsukiyama *et al.*, 1995) and is probably a subunit of the ISWI-like complex in mammalian cells. Using an antibody against hSNF2L (a generous gift from Dr Robert Roeder), we found that hSNF2L has overlapping but not identical chromatographic properties as the BRG1 complex through several ion-exchange columns (data not shown). It is not present in our complexes purified using either a BRG1 or a BAF47/hSNF5 antibody column (Figure 8a, lanes 3 and 6), but is readily detectable in either the load or the flowthrough fractions of the affinity columns (lanes 1, 2, 4 and 5; note that the protein concentration of the flowthrough fractions is 3-fold less than that of the load fraction, and therefore the hSNF2L level in the flowthrough fraction is an underestimate compared with the load). The result is consistent with the *Drosophila* data, i.e. that ISWI and *brm* are present in different complexes.

The presence of hSNF2L in the BRG1-depleted fractions prompted us to test if these depleted fractions still contain the nucleosome disruption activities. Indeed, the nucleosome disruption activity of the depleted fraction is indistinguishable from that of the undepleted fraction (Figure 8b, compare lanes 8 and 9 with lanes 10 and 11). The results indicate that the major nucleosome disruption activities in mammalian cells are not associated with BRG1 (BAF190) or BAF47 (INI1/hSNF5). The ISWI complex or perhaps additional, as yet uncharacterized, complexes could be such major nucleosome disruption activities. These complexes may obfuscate interpretation of the results of a

previous partial purification of the human SWI–SNF complex (Kwon *et al.*, 1994) in which these proteins very likely co-purified with the BRG1-associated activity.

Discussion

Purification of mammalian SWI–SNF complexes

Our data demonstrate that (i) mammalian cells contain of a group of BRG1-associated proteins that are similar in complexity to those seen in the yeast SWI–SNF complex (Cairns *et al.*, 1994; Côté *et al.*, 1994) and (ii) the highly purified mammalian complex has an *in vitro* nucleosome disruption activity similar to that of the yeast SWI–SNF complex (Côté *et al.*, 1994) and the *Drosophila* ISWI complex (Tsukiyama and Wu, 1995; Tsukiyama *et al.*, 1995). Furthermore, this complex contains a homolog of SNF5 (BAF47 or INI1), a component of the yeast complex. Purification using INI1/hSNF5 antibody yielded the same group of proteins as BRG1 or hbrm antibodies. These results indicate that the BAFs we have purified represent the mammalian counterpart of the SWI–SNF complex. A previous partial biochemical purification (Kwon *et al.*, 1994) did not differentiate between BRG1-associated activity, hbrm-associated activity and ISWI activity (Tsukiyama and Wu, 1995) because of the relative impurity of the final fractions used for analysis. In addition, our preliminary analysis of peptides obtained from BAF155/170 indicates that they are encoded by separate genes which are homologs of SWI3 (W.Wang, Y.Xue, S.Zhou, A.Kuo, B.R.Cairns and G.R.Crabtree, manuscript submitted). BAF60 is a homolog of SWP73, which was independently shown to be a component of the yeast SWI–SNF complex (B.Cairns, R.Kornberg and K.Yamamoto, personal communication). Thus the BAF complex we have purified contains at least five homologs of yeast SWI–SNF proteins and hence appears to be the mammalian version of the SWI–SNF complex.

From the purification of several cell lines and tissues, we estimated that there are ~2000 SWI–SNF complexes per mammalian cell. This is ~10-fold higher than the estimated number of yeast complexes per cell (Côté *et al.*, 1994). There are between 10 000 and 60 000 genes expressed in most differentiated tissues (Axel *et al.*, 1976), indicating that the SWI–SNF complex should not be required for every expressed gene. Hence, the complexes are most likely to be required for the expression of only a subset of genes in each cell type or only during activation rather than maintenance. In this sense, the mammalian complex may be like the yeast complex that is necessary only for certain newly activated genes (Neigeborn and Carlson, 1984; Stern *et al.*, 1984; Breeden and Nasmyth, 1987). There are at least six other mammalian genes that have been described as having SWI2–SNF2-like domains (Okabe *et al.*, 1992; Troelstra *et al.*, 1992; Delmas *et al.*, 1993; Khavari *et al.*, 1993; Muchardt and Yaniv, 1993; Gibbons *et al.*, 1995; Sheridan *et al.*, 1995). There are also several nucleosome remodeling activities that have been described as similar to that of the SWI–SNF2 complex (Pazin *et al.*, 1994; Tsukiyama *et al.*, 1994; Tsukiyama and Wu, 1995; Wall *et al.*, 1995). These complexes and activities could function in concert with the mammalian SWI–SNF complex.

Biochemical heterogeneity of mammalian SWI–SNF complexes

We find that the mammalian BAF complex is more diverse than the yeast SWI–SNF complex. While only a single SWI–SNF complex has been reported in yeast, we were able to purify two biochemically distinct mammalian BAF or SWI–SNF complexes, referred to as A and B, from several mammalian cell lines, such as YT, HeLa and SW13. These complexes share several common subunits, but also contain unique subunits. Both can disrupt nucleosomes *in vitro*, but may have distinct functions in living cells.

In addition, we find that the mammalian SWI–SNF complex is biochemically heterogeneous in different cell lines. These alternative complexes do not arise from the degradation or partial dissociation of a single complex because (i) each complex was ~2 MDa in molecular weight and (ii) Western blotting with antibodies to BRG1, hbrm or BAF47 detected no evidence of degradation of these components. For example, 12 different subunits are present in SWI–SNF complex A in PC12 cells, 11 in FAO cells, nine in YT, HeLa or Jurkat T cells, and only seven in C33 cells. Because these complexes are purified from cell lines derived from different tissues, these different SWI–SNF complexes could be involved in chromatin remodeling for tissue-specific genes. However, these differences should be interpreted with caution because cell lines are often selected for growth in culture and these differences could be related to selective pressure in culture rather than cell type-specific differences. In addition, non-essential genes are rapidly methylated and not expressed after cells are placed in culture (Antequera *et al.*, 1990), and the lack of certain components could simply indicate that these subunits are non-essential for growth in cell culture. Because certain cell lines such as SW13 grow normally without either BRG1 or hbrm, these subunits are not essential for transcription or cell division.

A question thus arises as to whether yeast also contains more than one SWI–SNF complex. Interestingly, we find that INI1/hSNF5 matches not only the yeast SNF5 gene, but also a second yeast open reading frame, as revealed by the Genome Sequencing Project (Figure 2a). Using other known SWI–SNF proteins to search the yeast genome database revealed the presence of open reading frames which are homologs of SWI2 and SWI3 (data not shown). The previous identification of STH1, a SNF2–SWI2-like protein (Laurent *et al.*, 1992) also suggests that yeast contains a second complex similar in composition to the SWI–SNF complex.

Multiple chromatin remodeling activities associated with BRG1, hbrm and ISWI

Recently the *Drosophila* NURF activity (Tsukiyama *et al.*, 1994) was purified and found to be distinct from the SWI–SNF complex in that it contains ISWI, a homolog of SNF2–SWI2, and has nucleosome-dependent ATPase activity rather than DNA-dependent ATPase activity (Tsukiyama and Wu, 1995; Tsukiyama *et al.*, 1995). Both the ISWI complex and the SWI–SNF complex remodel chromatin in nucleosome disruption assays *in vitro*. Although the *Drosophila* SWI–SNF complex has not been purified, the *Drosophila* NURF complex is smaller in size and simpler in subunit composition than the mammalian

SWI–SNF complex. It has only four subunits and is 500 kDa in size, in contrast to the nine to 12 subunits and 2 MDa in size we find in the mammalian SWI–SNF complex. However, the NURF complex is much more abundant than the SWI–SNF complex (100 00 versus 2000 complexes/cell). Thus the NURF complex could be involved in the transcription of most if not all genes, whereas the SWI–SNF complex is only involved in the activation of a subset of genes. A close relative of ISWI in mammalian cells, hSNF2L, has been described previously (Okabe *et al.*, 1992; Tsukiyama *et al.*, 1995), suggesting the existence of NURF complex in mammalian cells. We found abundant mononucleosome disruption activity in the flowthrough fractions from the INI1/hSNF5 affinity columns that lack both BRG1 and hbrm, but do contain the mammalian homolog of ISWI, hSNF2L (Figure 8). This activity may be accounted for by the mammalian NURF complex or some other chromatin remodeling activity (Pazin *et al.*, 1994; Wall *et al.*, 1995).

We found that the SWI2–SNF2 homologs, BRG1 and hbrm, are in separate complexes. Although we have been unable to purify sufficient quantities of hbrm complex to address its *in vitro* activity, the transfection of either BRG1 or hbrm aids activation by the GR (Yoshinaga *et al.*, 1992), suggesting that they have similar activities. Thus three different chromatin remodeling activities based on ISWI (hSNF2L) (Okabe *et al.*, 1992), hbrm and BRG1 could co-exist in mammalian cells.

Our purification of the mammalian SWI–SNF complex indicates that it is surprisingly heterogeneous and may be tailored to the needs of a differentiated cell type. These observations are consistent with earlier studies in *Drosophila* in which *brm* shows distinct and specific developmental functions (Brizuela *et al.*, 1994). Isolation of the genes encoding the specialized components of the mammalian SWI–SNF complex will be required to understand the function of this complex in different cell types.

Materials and methods

Fractionation of nuclear extracts

Nuclear extracts were prepared as described previously (Gorski *et al.*, 1986). For analytical fractionation, 200 mg in 0.1 M buffer D (0.1 M KCl, 20 mM HEPES, pH 7.9, 0.25 mM EDTA, 20% glycerol and 0.1% Tween 20) were loaded onto a 30 ml P11 column (Whatmann). The column was washed with 0.2 M buffer D, and eluted sequentially with 0.50 and 0.75 M buffer D. The presence of BRG1 and hbrm was monitored by Western blotting using antibodies against BRG1 and hbrm, respectively. They co-fractionated on every conventional column we examined. The majority of BRG1 and hbrm was found to be present in the 0.50 M fraction, whereas a 5- to 10-fold lower amount of immunoreactivity was present in the 0.75 M fraction. The 0.50 M fraction was diluted to 0.15 M, and then loaded onto a 10 ml DEAE-52 (Whatmann) column. The column was washed with 0.15 M buffer D and eluted with 0.3 M buffer D. The elute was loaded directly onto a 60 ml S-300 column (Pharmacia). The fractions containing BRG1 and hbrm were collected and used for immunoaffinity purification. In some experiments, the S-300 fractions containing BRG1 were purified further by a single-stranded DNA cellulose column (US Biochemicals), Mono Q (Pharmacia) and Superose 6 column (Pharmacia). For the single-stranded DNA column, the fractions were loaded in 0.1 M buffer D and eluted with 0.3 M buffer D. For the Mono Q column, the fractions were loaded in 0.1 M buffer D and eluted with a linear gradient of 0.1–0.5 M buffer D. For the Superose 6 column, the fractions were run in 0.3 M buffer D.

Nuclear extracts from cell cultures were prepared as described previously (Dignam *et al.*, 1983). They were similarly fractionated through P11 and DE52 columns but with smaller bed volumes. At least 10 mg nuclear extract was needed for each cell line. We usually started with 20–30 mg nuclear extract. The S-300 column was found to be unnecessary and was not included in the purification. The 0.3 M DE52 fraction was used directly for immunoaffinity purification. We found that the purification of SWI–SNF complex from tissue sources was more susceptible to protease degradation (data not shown), whereas purification from cultured cell lines was much easier and the results were highly reproducible.

Immunoaffinity purification of BRG1 and hbrm complexes

Three rabbit polyclonal antibodies were made against BRG1. The J1 antibody was raised against a glutathione-*S* transferase (GST)–BRG1 fusion protein (BRG1 amino acids 1086–1307; Khavari *et al.*, 1993). The BrgC antibody was raised against the 50 amino acid C-terminal region of BRG1 fused to the bacterial maltose binding protein (MBP; New England Biolabs). Brgsp antibody was raised against a non-conserved region between BRG1 and hbrm (amino acids 217–270). One rabbit polyclonal antibody for hbrm was raised against a fusion protein of GST to the N-terminal 283 amino acids of hbrm. One rabbit polyclonal antibody was raised against INI1/hSNF5. A MBP fusion protein containing the C-terminal 250 amino acids of INI1 was used as immunogen.

hbrm was also epitope tagged at the N-terminus with the 12 amino acid HA-antigen and was used to stably transfect mouse DT cells (Muchardt *et al.*, 1996). One clone, DT21, which stably expresses tagged hbrm was used for the purification of hbrm complex employing the 12CA5 monoclonal antibody.

All these antibodies work on Western blotting as well as immunoprecipitations. They were affinity purified using the corresponding antigens as affinity ligands, cross-linked to Protein A–Sepharose (Pharmacia) with DMP and used for affinity purification of the complexes containing BRG1, hbrm and INI1/hSNF5. 300–500 μg antibodies were crosslinked to 1 ml Protein A–Sepharose. The fractionated nuclear extract (S-300 fraction of rat liver or DE52 fraction of tissue culture cells) was incubated with antibody–Protein A beads for 4–5 h in 0.15 M buffer D at 4°C (the ratio of the extract to beads was 10:1 by volume). The beads were washed twice with each of the following buffer: 0.5 M buffer D; 0.2 M guanidine hydrochloride in 0.1 M buffer D; 0.1 M buffer D. The exception was for the 12CA5 monoclonal antibody column. The 0.2 M guanidine hydrochloride washing disrupted the interaction between the complex and antibody. It was replaced by washing four times with 0.5 M buffer D and twice with 0.1 M buffer D. The proteins were eluted with 0.1 M glycine, pH 2.5 into 1/10 volume of neutralizing buffer of 1.0 M Tris–HCl, pH 8.0. Between 10 and 20 μg BRG1 and hbrm complex were obtained from 200 mg nuclear extract.

Using Western blotting with the BRG1 and BAF47 antibodies, we estimated that the overall purification of SWI–SNF complex was ~8000- to 10 000-fold using nuclear extracts from different cell lines. Two ion-exchange columns, P11 and DE52, gave ~3- and 5-fold purification, respectively. The affinity column g27 provided ~500- to 1000-fold purification. We found that the purification procedure described by Kwon *et al.* (1994) gave ~150- to 400-fold purification: P11, 3-fold; single-stranded DNA cellulose, 3- to 5-fold; green dye column, 5-fold; and Mono Q, 3- to 5-fold. The Superose 6 sizing column diluted SWI–SNF complex too much and could only be used for analytical purposes.

For microsequencing, the proteins were separated on 8% SDS–PAGE gels and transferred to polyvinylidine difluoride membrane. The protein bands were visualized with Ponseau-S staining and excised. The BAF47 band was digested with trypsin overnight. The peptides were separated by reverse-phase HPLC and sequenced using an automatic peptide sequencer.

Sometimes, the BrgC antibody beads were blocked with the peptide antigen to serve as controls. The 50 amino acid peptide corresponding to the C-terminal region of BRG1 was synthesized at Stanford PAN facility. BrgC antibody beads was blocked with the peptide (1 mg/ml, 10 bed volume) at room temperature for 2 h, before immunopurification.

Cell cultures

A number of cell lines were used as sources of SWI–SNF complexes. Five human cell lines were employed. YT is a natural killer cell line. SW13 is an adrenal cortex carcinoma-derived cell line. C33A is a cervical epithelium cell line. HeLa is derived from human cervical carcinoma. These four cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum (CS).

Jurkat T cells is a T cell leukemia cell line and was maintained in RPMI supplemented with 10% CS. Two rat cell lines were also used: FAO is a highly differentiated hepatoma cell line and was maintained in DMEM supplemented with 10% CS; and PC12 is an adrenal pheochromocytoma cell line and was maintained in DMEM supplemented with 6% fetal CS (FCS) and 6% horse serum. Two mouse cell lines were used: DT is a ras-transformed NIH3T3 cell line and was grown in DMEM with 10% CS; and C2F3 is a myogenic cell line and was grown in DMEM supplemented with 5% FCS and 15% CS.

Transfection and luciferase assays

The SW13 cells were maintained and transfected as described previously (Muchardt and Yaniv, 1993; Dunaief *et al.*, 1994). Briefly, 1 μg GR expression vector and 1 μg $(GRE)_3$-luciferase reporter were transfected by electroporation with or without 10 μg BRG1 expression vector, BRG1-WT or BRG1-mutant (Khavari *et al.*, 1993). After 24 h, dexamethasone was added to a final concentration of 10^{-6} mM. The cells were harvested 24 h later and a luciferase assay was performed.

Mononucleosome disruption assays

Nucleosomal core reconstitution on labeled probes was performed by the octamer transfer method using HeLa H1-depleted oligonucleosomes as described previously (Côté *et al.*, 1994). The production of a 154 bp probe containing one GAL4 site 32 bp from the end using plasmid pBEND401G1 has been described by Côté *et al.* (1994). GAL4(1–147)–VP16 was purified from bacteria as described previously (Lin *et al.*, 1988). Mononucleosome disruption and enhanced GAL4–VP16 binding were assayed by DNase I footprinting, essentially as described previously (Côté *et al.*, 1994) except for the following modifications. Binding reactions were performed in 20 μl of 20 mM HEPES (pH 7.5), 50 mM NaCl, 3 mM $MgCl_2$, 1 mM $ZnCl_2$, 2 mM dithiothreitol, 5% glycerol, 0.2 mM phenylmethylsulfonyl fluoride, 200 mg/ml bovine serum albumin and 5000 c.p.m. reconstituted nucleosomal cores (0.25 ng probe and 37 ng cold donor nucleosome cores). Typically 3–9 μl SWI–SNF complex on beads were used in binding reactions, keeping the total volume at 20 μl. About 10–20 ng complex were present on 1 μl of beads, unless otherwise indicated in the text. Reactions were incubated at 30°C for 60 min with frequent mixing. Naked DNA controls used probes that had instead been added at the final dilution step of the octamer transfer protocol (mock reconstituted). The GST–RB recombinant protein was purified as described previously (Ewen *et al.*, 1993).

For the nucleosome disruption assay using BRG1–hbrm-depleted fractions, 300 μg fractionated extract after the DE52 column were incubated twice with BAF47/hSNF5 antibody beads for 4 h at 4°C. The supernatant was found to be quantitatively depleted of BRG1, hbrm or hSNF5/BAF47 (Figures 2b and 8; data not shown), but contained hSNF2L when assayed by a Western blot. 3 μg of either depleted or undepleted extract were used in the mononucleosome disruption assay.

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References

Antequera,F., Boyes,J. and Bird,A. (1990) High levels of *de novo* methylation and altered chromatin structure at CpG islands in cell lines. *Cell*, **62**, 503–514.

Axel,R., Feigelson,P. and Schutz,G. (1976) Analysis of the complexity and diversity of mRNA from chicken liver and oviduct. *Cell*, **7**, 247–254.

Breeden,L. and Nasmyth,K. (1987) Cell cycle control of the yeast HO gene: cis- and trans-acting regulators. *Cell*, **48**, 389–397.

Brizuela,B.J., Elfring,L., Ballard,J., Tamkun,J.W. and Kennison,J.A. (1994) Genetic analysis of the *brahma* gene of *Drosophila melanogaster* and polytene chromosome subdivisions 72AB. *Genetics*, **137**, 803–813.

Cairns,B.R., Kim,Y.J., Sayre,M.H., Laurent,B.C. and Kornberg,R.D. (1994) A multisubunit complex containing the SWI1/ADR6, SWI2/SNF2, SWI3, SNF5, and SNF6 gene products isolated from yeast. *Proc. Natl Acad. Sci. USA*, **91**, 1950–1954.

Carlson,M. and Laurent,B.C. (1994) The SNF/SWI family of global transcriptional activators. *Curr. Opin. Cell Biol.*, **6**, 396–402.

Chiba,H., Muramatsu,M., Nomoto,A. and Kato,H. (1994) Two human homologues of *Saccharomyces cerevisiae* SWI2/SNF2 and *Drosophila brahma* are transcriptional coactivators cooperating with the estrogen receptor and the retinoic acid receptor. *Nucleic Acids Res.*, **22**, 1815–1820.

Côté,J., Quinn,J., Workman,J.L. and Peterson,C.L. (1994) Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. *Science*, **265**, 53–60.

Delmas,V., Stokes,D.G. and Perry,R.P. (1993) A mammalian DNA-binding protein that contains a chromodomain and an SNF2/SWI2-like helicase domain. *Proc. Natl Acad. Sci. USA*, **90**, 2414–2418.

Dignam,J.D., Lebowitz,R.M. and Roeder,R.G. (1983) Accurate transcription initiated by polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res.*, **11**, 1475–1489.

Dingwall,A.K., Beek,S.J., McCallum,C.M., Tamkun,J.W., Kalpana,G.V., Goff,S.P. and Scott,M.P. (1995) The *Drosophila* snr1 and brm proteins are related to yeast SWI/SNF proteins and are components of a large protein complex. *Mol. Biol. Cell*, **6**, 777–791.

Dunaief,J.L., Strober,B.E., Guha,S., Khavari,P.A., Alin,K., Luban,J., Begemann,M., Crabtree,G.R. and Goff,S.P. (1994) The retinoblastoma protein and BRG1 form a complex and cooperate to induce cell cycle arrest. *Cell*, **79**, 119–130.

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.

Ewen,M.E., Sluss,H.K., Sherr,C.J., Matsushime,H., Kato,J. and Livingston,D.M. (1993) Functional interactions of the retinoblastoma protein with mammalian D-type cyclins. *Cell*, **73**, 487–497.

Gibbons,R.J., Picketts,D.J., Villard,L. and Higgs,D.R. (1995) Mutations in a putative global transcriptional regulator cause X-linked mental retardation with α-thalassemia (ATR-X syndrome). *Cell*, **80**, 837–845.

Gorski,K., Carneiro,M. and Schibler,U. (1986) Tissue-specific *in vitro* transcription from the mouse albumin promoter. *Cell*, **47**, 767–776.

Hirschhorn,J.N., Brown,S.A., Clark,C.D. and Winston,F. (1992) Evidence that SNF2/SWI2 and SNF5 activate transcription in yeast by altering chromatin structure. *Genes Dev.*, **6**, 2288–2298.

Hirschhorn,J.N., Bortvin,A.L., Ricupero-Hovasse,S.L. and Winston,F. (1995) A new class of histone H2A mutations in *Saccharomyces cerevisiae* causes specific transcriptional defects *in vivo*. *Mol. Cell. Biol.*, **15**, 1999–2009.

Imbalzano,A.N., Kwon,H., Green,M.R. and Kingston,R.E. (1994) Facilitated binding of TATA-binding protein to nucleosomal DNA [see comments]. *Nature*, **370**, 481–485.

Kalpana,G.V., Marmon,S., Wang,W., Crabtree,G.R. and Goff,S.P. (1994) Binding and stimulation of HIV-1 integrase by a human homolog of yeast transcription factor SNF5 [see comments]. *Science*, **266**, 2002–2006.

Kennison,J.A. and Tamkun,J.W. (1988) Dosage-dependent modifiers of *polycomb* and *antennapedia* mutations in *Drosophila*. *Proc. Natl Acad. Sci. USA*, **85**, 8136–8140.

Khavari,P.A., Peterson,C.L., Tamkun,J.W., Mendel,D.B. and Crabtree, G.R. (1993) BRG1 contains a conserved domain of the SWI2/SNF2 family necessary for normal mitotic growth and transcription. *Nature*, **366**, 170–174.

Kruger,W., Peterson,C.L., Sil,A., Coburn,G., Arents,G., Moudrianakis, E.N. and Herskowitz,I. (1995) Amino acid substitutions in the structured domains of histones H3 and H4 partially relieve the requirement of the yeast SWI/SNF complex for transcription. *Genes Dev.*, **9**, 2770–2779.

Kwon,H., Imbalzano,A.N., Khavari,P.A., Kingston,R.E. and Green,M.R. (1994) Nucleosome disruption and enhancement of activator binding by a human SW1/SNF complex [see comments]. *Nature*, **370**, 477–481.

Laurent,B.C. and Carlson,M. (1992) Yeast SNF2/SWI2, SNF5 and SNF6 proteins function coordinately with the gene-specific transcriptional activators GAL4 and Bicoid. *Genes Dev.*, **6**, 1707–1715.

Laurent,B.C., Treitel,M.A. and Carlson,M. (1990) The SNF5 protein of *Saccharomyces cerevisiae* is a glutamine- and proline-rich transcriptional activator that affects expression of a broad spectrum of genes. *Mol. Cell. Biol.*, **10**, 5616–5625.

Laurent,B.C., Yang,X. and Carlson,M. (1992) An essential *Saccharomyces cerevisiae* gene homologous to SNF2 encodes a helicase-related protein in a new family. *Mol. Cell. Biol.*, **12**, 1893–1902.

Lewin,B. (1994) Chromatin and gene expression: constant questions, but changing answers. *Cell*, **79**, 397–406.

Lin,Y.S., Carey,M.F., Ptashne,M. and Green,M.R. (1988) GAL4 derivatives function alone and synergistically with mammalian activators *in vitro*. *Cell*, **54**, 659–664.

Moehrle,A. and Paro,R. (1994) Spreading the silence: epigenetic transcriptional regulation during *Drosophila* development. *Dev. Genet.*, **15**, 478–484.

Muchardt,C. and Yaniv,M. (1993) A human homologue of *Saccharomyces cerevisiae* SNF2/SWI2 and *Drosophila brm* genes potentiates transcriptional activation by the glucocorticoid receptor. *EMBO J.*, **12**, 4279–4290.

Muchardt,C., Sardet,C., Bourachot,B., Onufryk,C. and Yaniv,M. (1995) A human protein with homology to *Saccharomyces cerevisiae* SNF5 interacts with the potential helicase hbrm. *Nucleic Acids Res.*, **23**, 1127–1132.

Muchardt,C., Reyes,J.-C., Bourachot,B., Legouy,E. and Yaniv,M. (1996) The hbrm and BRG-1 proteins, components of the human SNF/SWI complex, are phosphorylated and excluded from the condensed chromosomes during mitosis. *EMBO J.*, **15**, 3394–3402.

Neigeborn,L. and Carlson,M. (1984) Genes affecting the regulation of SUC2 gene expression by glucose repression in *Saccharomyces cerevisiae*. *Genetics*, **108**, 845–858.

Okabe,I., Bailey,L.C., Attree,O., Srinivasan,S., Perkel,J.M., Laurent,B.C., Carlson,M., Nelson,D.L. and Nussbaum,R.L. (1992) Cloning of human and bovine homologs of SNF2/SWI2: a global activator of transcription in yeast *S. cerevisiae*. *Nucleic Acids Res.*, **20**, 4649–4655.

Pazin,M.J., Kamakaka,R.T. and Kadonaga,J.T. (1994) ATP-dependent nucleosome reconfiguration and transcriptional activation from preassembled chromatin templates. *Science*, **266**, 2007–2011.

Peterson,C.L. and Herskowitz,I. (1992) Characterization of the yeast SWI1, SWI2, and SWI3 genes, which encode a global activator of transcription. *Cell*, **68**, 573–583.

Peterson,C.L. and Tamkun,J.W. (1995) The SWI–SNF complex: a chromatin remodeling machine? *Trends Biochem. Sci.*, **20**, 143–146.

Peterson,C.L., Dingwall,A. and Scott,M.P. (1994) Five SWI/SNF gene products are components of a large multisubunit complex required for transcriptional enhancement [see comments]. *Proc. Natl Acad. Sci. USA*, **91**, 2905–2908.

Prelich,G. and Winston,F. (1993) Mutations that suppress the deletion of an upstream activating sequence in yeast: involvement of a protein kinase and histone H3 in repressing transcription *in vivo*. *Genetics*, **135**, 665–676.

Sheridan,P.L., Schorpp,M., Voz,M.L. and Jones,K.A. (1995) Cloning of an SNF2/SWI2-related protein that binds specifically to the SPH motifs of the SV40 enhancer and to the HIV-1 promoter. *J. Biol. Chem.*, **270**, 4575–4587.

Singh,P., Coe,J. and Hong,W. (1995) A role for retinoblastoma protein in potentiating transcriptional activation by the glucocorticoid receptor. *Nature*, **374**, 562–565.

Stern,M., Jensen,R. and Herskowitz,I. (1984) Five SWI genes are required for expression of the HO gene in yeast. *J. Mol. Biol.*, **178**, 853–868.

Tamkun,J.W., Deuring,R., Scott,M.P., Kissinger,M., Pattatucci,A.M., Kaufman,T.C. and Kennison,J.A. (1992) *brahma*: a regulator of *Drosophila* homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. *Cell*, **68**, 561–572.

Travers,A.A. (1992) The reprogramming of transcriptional competence. *Cell*, **69**, 573–575.

Treich,I., Cairns,B.R., de los Santos,T., Brewster,E. and Carlson,M. (1995) SNF11, a new component of the yeast SNF–SWI complex that interacts with a conserved region of SNF2. *Mol. Cell. Biol.*, **15**, 4240–4248.

Troelstra,C., van Gool,A., de Wit,J., Vermeulen,W., Bootsma,D. and Hoeijmakers,J.H. (1992) ERCC6, a member of a subfamily of putative helicases, is involved in Cockayne's syndrome and preferential repair of active genes. *Cell*, **71**, 939–953.

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

Tsukiyama,T., Becker,P.B. and Wu,C. (1994) ATP-dependent nucleosome disruption at a heat-shock promoter mediated by binding of GAGA transcription factor [see comments]. *Nature*, **367**, 525–532.

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.

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.

Wilson,R. *et al.* (1994) 2.2 Mb of contiguous nucleotide sequence from chromosome III of *C.elegans*. *Nature*, **368**, 32–38.

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

Wolffe,A.P. (1994) Transcription: in tune with the histones. *Cell*, **77**, 13–16.

Yoshinaga,S.K., Peterson,C.L., Herskowitz,I. and Yamamoto,K.R. (1992) Roles of SWI1, SWI2, and SWI3 proteins for transcriptional enhancement by steroid receptors. *Science*, **258**, 1598–1604.

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