

PBAF chromatin-remodeling complex requires a novel specificity subunit, BAF200, to regulate expression of selective interferon-responsive genes

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PBAF and BAF are two chromatin-remodeling complexes of the SWI/SNF family essential for mammalian transcription and development. Although these complexes share eight identical subunits, only PBAF can facilitate transcriptional activation by nuclear receptors in vitro. Here we show that these complexes have selectivity in mediating transcription of different interferon-responsive genes. The selectivity by PBAF requires a novel subunit, BAF200, but not the previously described PBAF-specificity subunit, BAF180 (Polybromo). Our study provides in vivo evidence that PBAF and BAF regulate expression of distinct genes, and suggests that BAF200 plays a key role in PBAF function.

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The regulation of temporal and spatial gene expression is crucial for normal development and differentiation in higher eukaryotes. This is ultimately achieved through delicate cross-talk between the chromatin and the transcriptional apparatus. Reorganization of chromatin structure facilitates or inhibits the access of transcription factors to their target genes, leading to transcriptional activation or repression. Several groups of chromatin-remodeling complexes are responsible for this process (Kornberg and Lorch 1999; Levine and Tjian 2003). One such group is the SWI/SNF family of remodeling enzymes, which uses the energy of ATP hydrolysis to disrupt the interactions between DNA and histones to make the nucleosomal DNA more accessible (Vignali et al. 2000). The remodeling could also trigger histone oc-

tomers to "slide" on DNA (Hamiche et al. 1999; Whitehouse et al. 1999), or to dissociate from DNA (Lorch et al. 1999). Because many promoters have defined nucleosome structures, the repositioning or loss of nucleosomes in a regulatory region could lead to either activation or repression of transcription.

SWI/SNF complexes are evolutionarily conserved from yeast to human, and have been implicated to participate in many biological processes, such as transcriptional regulation, tumorigenesis, development, and differentiation (Vignali et al. 2000). They can be grouped into two major subfamilies: One includes human BAF (BRG1 or hbrm-associated factor), *Drosophila* BAP, and yeast SWI/SNF; the other comprises human PBAF (Polybromo-associated BAF), *Drosophila* PBAP, and yeast RSC (Cairns et al. 1994, 1996; Cote et al. 1994; Kwon et al. 1994; Wang et al. 1996; Papoulas et al. 1998; Nie et al. 2000; Xue et al. 2000; Mohrmann et al. 2004). These two subfamilies of complexes have similar subunit compositions, but appear to have different functions (Angus-Hill et al. 2001; Lemon et al. 2001). For example, human BAF and PBAF complexes share eight identical subunits, and are distinguished by the presence of only two unique subunits: BAF180 (also called Polybromo) for PBAF and BAF250a for BAF (Nie et al. 2000; Xue et al. 2000). Despite such similarities, only PBAF, but not BAF, is capable of facilitating ligand-dependent transcriptional activation by nuclear receptors in vitro (Lemon et al. 2001). Whether and how these complexes selectively regulate expression of different genes in vivo remain largely unclear. Here, we present in vivo evidence that PBAF and BAF do have selectivity in regulating interferon-responsive genes. Moreover, we have identified a second specificity subunit for PBAF, termed BAF200, and demonstrated that BAF200, but not BAF180, is required for PBAF to mediate expression of an interferon-responsive gene, suggesting that BAF200 is an important targeting subunit of PBAF.

Results and Discussion

BAF 200 is a new intrinsic subunit of PBAF complex

We and others have described purification of the two major human SWI/SNF complexes, BAF (hSWI/SNF-A) and PBAF (hSWI/SNF-B) (Kwon et al. 1994; Wang et al. 1996; Nie et al. 2000; Xue et al. 2000; Lemon et al. 2001). These two complexes share as many as eight common subunits (BRG1, BAF170, BAF155, BAF60a, BAF57, BAF53, actin, and hSNF5/INI1), but are distinguished by the presence of two specificity subunits: BAF180 present only in PBAF, and BAF250a exclusively present in BAF (Nie et al. 2000; Xue et al. 2000; Lemon et al. 2001). We also noticed the presence of a 200-kDa polypeptide, referred to as BAF200, in PBAF complex purified by antibodies against BRG1, BAF180, and hSNF5 (Wang et al. 1996; Xue et al. 2000). The fact that this polypeptide is immunopurified by multiple PBAF antibodies strongly suggests that it could be a new component of PBAF.

To obtain large amounts of BAF200 for identification, we used an improved immunoprecipitation (IP) protocol to directly isolate PBAF from HeLa nuclear extract using an antibody against BAF180 (the previous purification used 0.75 M phosphocellulose fraction of HeLa nuclear

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extract). The purity of PBAF complex isolated by the new protocol is comparable to that obtained by the previous method (Fig. 1A), and a 200-kDa polypeptide (BAF200) was similarly detected. This polypeptide was identified by mass spectrometry as a hypothetical protein of 1835 amino acid residues, with the approved gene symbol of ARID2 (AT-rich interactive domain 2; aliases: KIAA1557, DKFZp686G052, FLJ30619; gene accession ID: NP_689854).

We also used mass spectrometry to analyze the 200-kDa polypeptide in the PBAF complex immunoprecipitated by the Flag antibody from HeLa cells stably expressing Flag-tagged hSNF5/INI1 as described (Xue et al. 2000). The results confirmed that this polypeptide is ARID2. The findings that ARID2 is present in PBAF immunoprecipitated by two independent antibodies indicate that it is

an integral component of PBAF. A polyclonal antibody raised against ARID2 also specifically recognized the 200-kDa polypeptide in the PBAF complex isolated by antibodies to either BAF180 or Flag-hSNF5 (Fig. 1B,C), providing further confirmation for ARID2 as BAF200.

To rule out the possibility that BAF200 may indirectly associate with PBAF complex through DNA, IP was carried out in the presence of ethidium bromide (EtBr), a DNA-intercalating drug that dissociates proteins from DNA. The amount of BAF200 in PBAF was unaffected by the presence of EtBr (Fig. 1A,B), indicating that BAF200 forms a complex with BAF180 independent of DNA.

We also analyzed the Superose 6 gel-filtration profile of BAF200 in HeLa nuclear extract, and compared it with that of other BAF proteins. The results showed that the profile of BAF200 was identical to that of the PBAF-specific subunit, BAF180, which supports the notion that these two proteins are unique components of the PBAF complex (Fig. 1D). As a comparison, the profiles of BAF200 and BAF180 only partially overlapped with those of BRG1, BAF57, and hSNF5. Because the latter three proteins are shared by many other SWI/SNF complexes (including BAF, which is more abundant than PBAF), it is not too surprising that their profiles are different from those of BAF200 and BAF180.

The expression of BAF200 mRNA was detected by Northern blot analysis in multiple human tissues that have been examined (data not shown). This property resembles that of BAF180, as well as some other BAF genes.

BAF200 is a new specificity subunit of PBAF and absent in BAF

In our previously purified PBAF and BAF complexes from fractionated HeLa nuclear extract, BAF200 was detected only in PBAF, but not BAF, by silver-stained SDS-gel analysis (Nie et al. 2000; Xue et al. 2000). To exclude the possibility that BAF200 may also be a subunit of BAF complex but was dissociated during fractionation of HeLa nuclear extract, we directly immunoprecipitated PBAF and BAF from unfractionated extract under mild washing conditions (0.3 M salt) using either BAF180 (PBAF) or BAF250a (BAF) antibodies, respectively. Consistent with previous data, BAF200 was detected in only PBAF, but not BAF (Fig. 1E).

We also performed reciprocal IP to determine whether BAF200-associated polypeptides contain PBAF or BAF components. Because our BAF200 antibody was inefficient in IP (data not shown), we established a HeLa cell line stably expressing BAF200 tagged with a Flag epitope. The BAF200-associated polypeptides immunoprecipitated by the Flag antibody contained the PBAF-specific subunit, BAF180, but not the BAF-specific subunit, BAF250a (Fig. 1F), consistent with the data above that BAF200 is a new specific subunit of PBAF complex. The polypeptides also included components that are shared by many SWI/SNF complexes, such as BRG1, BAF170, BAF155, BAF57, and hSNF5/INI1. As a control, mock IP using nuclear extract from HeLa cells that do not express Flag-BAF200 yielded no PBAF components. Taken together, our data demonstrate that BAF200, like BAF180, is a specific subunit of the PBAF complex.

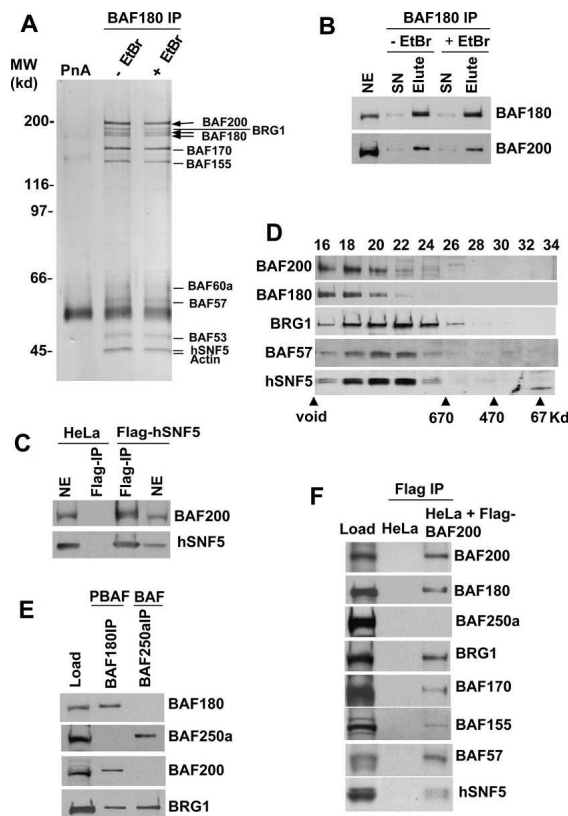


Figure 1. BAF200 is a new unique subunit of PBAF. (A) A silver-stained SDS gel shows the PBAF complex obtained from immunoprecipitation (IP) by a BAF180 antibody with HeLa nuclear extract in the presence (+) or absence (-) of ethidium bromide (EtBr). Mock IP was done by using protein A beads (PnA) alone with the extract. (B) Immunoblotting shows the presence of BAF200 in the complex purified by BAF180 antibody. The nuclear extract (NE), supernatant (SN), and eluted fraction from IP are indicated. (C) Immunoblotting shows the presence of BAF200 in PBAF immunoprecipitated by a Flag antibody from the nuclear extract of INI1 cells stably expressing Flag-hSNF5/INI1 (Xue et al. 2000). Mock IP was done using HeLa cells. (D) Immunoblotting shows the Superose 6 gel-filtration profiles of BAF200, BAF180, BRG1, BAF57, and hSNF5 in HeLa extract. (E) Immunoblotting shows the presence of BAF200 in the PBAF complex immunoprecipitated by a BAF180 antibody, but not in the BAF complex isolated by a BAF250a antibody. (F) Immunoblotting shows that PBAF components are coimmunoprecipitated with Flag-BAF200 by a Flag antibody from an extract of HeLa cells stably expressing Flag-BAF200.

BAF200, but not BAF180, is essential for the stability of PBAF complex in vivo

Previous studies have shown that the absence of certain yeast SWI/SNF subunits results in reduced stability of other SWI/SNF components, suggesting that these subunits may be critical for the assembly of the complex (Peterson et al. 1994). We examined the role of BAF200 in PBAF assembly by siRNA knockdown. Depletion of BAF200 in HeLa cells by either siRNA oligos (Fig. 2A) or a vector-based siRNA (Fig. 2B) resulted in not only reduced level of BAF200, but also that of BAF180. As a comparison, depletion of BAF200 had no significant effect on the level of other PBAF subunits that are shared with the BAF complex. Because BAF180 is the only defining subunit of the PBAF complex other than BAF200 (Xue et al. 2000; Lemon et al. 2001), our results suggest that BAF200 is essential for the stability of PBAF complex in vivo, perhaps by recruiting BAF180 into the complex.

We also knocked down BAF180 by siRNA in HeLa cells and found that the level of BAF200 was comparable to cells treated with a scrambled control oligo (Fig. 2C). Moreover, a breast cancer cell line (HCC1143) with a biallelic mutation in the BAF180 gene and an undetectable level of BAF180 protein (R. Parsons, unpubl.) expressed BAF200 at a level indistinguishable to that of HeLa and another breast cancer cell line MCF7 (Fig. 2D), suggesting that BAF180 is dispensable for the stability of BAF200, and may also be dispensable for assembly of BAF200 into PBAF (see below).

BAF200 is a homolog of Drosophila BAP170 and contains several conserved domains

A search of the GenBank database revealed that BAF200 (ARID2) has sequence orthologs in mouse, chicken (*Gal-*

lus gallus), *Drosophila*, *Anopheles gambiae*, and *Caenorhabditis elegans*, but not in yeast. The *C. elegans* ortholog of BAF200 (NP_495679) has been analyzed by a genome-wide siRNA study, and found to be essential for embryonic development (Kamath et al. 2003). Its *Drosophila* ortholog, BAP170, was recently identified as a component of the PBAP chromatin-remodeling complex (Mohrmann et al. 2004). *Drosophila* has two SWI/SNF-related complexes, BAP and PBAP, which appear to correspond to human BAF and PBAF, respectively. Both BAP170 and a fly homolog of BAF180 (Polybromo) were identified as the defining subunits for PBAP, whereas a homolog of BAF250a (*Osa*) is the defining subunit for BAP. The fact that BAF200 and its homolog are defining subunits in both human PBAF and *Drosophila* PBAP complexes suggests that these proteins may have conserved functions.

Drosophila BAP170 and some of its orthologs in other species have been found to contain a conserved AT-rich DNA interaction domain (ARID), multiple LXXLL motifs (which may mediate protein-protein interaction between cofactors and nuclear hormone receptors) (Savkur and Burris 2004), proline- and glutamine-rich regions, and two C2H2 Zn-fingers (which may interact with either DNA or proteins) (Mohrmann et al. 2004). We noticed that all these domains are also conserved in human BAF200 (Supplementary Fig. 1A). In addition, BAF200 and its orthologs contain a putative sequence-specific DNA-binding domain conserved among the regulatory factor X (RFX) family of proteins (Supplementary Fig. 1B). This domain is structurally similar to winged helix proteins, and can interact with DNA at the major groove (Gajiwala and Burley 2000). The presence of multiple potential DNA and protein interaction domains in BAF200 makes it a potential targeting subunit of the PBAF complex.

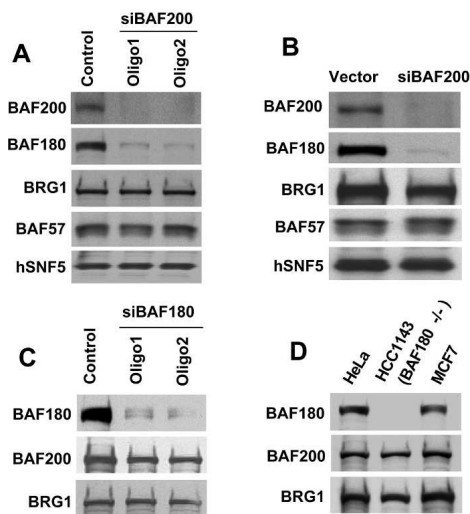


Figure 2. BAF200, but not BAF180, is essential for the stability of PBAF complex in vivo. (A) Immunoblotting shows that depletion of BAF200 by siRNA oligos reduced the level of BAF180 protein. A nonspecific siRNA oligo was used as a control. (B) Immunoblotting shows that the level of BAF180 protein was decreased when BAF200 was depleted by vector-based siRNA. The empty vector was used as a control. (C) Immunoblotting shows that the level of BAF200 is not significantly affected by depleting BAF180 in HeLa cells. (D) Immunoblotting shows that the expression of BAF200 in a BAF180-deficient cell line, HCC1143, is comparable to that of HeLa or MCF7 cells.

PBAF and BAF are selectively required for expression of distinct IFN- α -inducible genes

SWI/SNF complexes were shown to directly regulate expression of multiple interferon-responsive genes, based on chromatin-IP and other studies (Agalioti et al. 2002; Huang et al. 2002; Liu et al. 2002; Pattenden et al. 2002; Cui et al. 2004). However, because these results are based on analyses of subunits that are shared among various SWI/SNF complexes, it remains unclear which complex (BAF or PBAF) participates in such regulation. We attempted to make this distinction by depleting the subunits uniquely present in BAF or PBAF, which should inactivate only their corresponding complex but not the other. Between the two unique subunits of PBAF (BAF180 and BAF200), we first chose to deplete BAF200 because its loss also triggers concomitant destabilization of BAF180 (Fig. 2A,B). Thus, depletion of BAF200 could lead to complete inactivation of PBAF.

We screened eight SWI/SNF-dependent interferon- α -inducible genes (Liu et al. 2002; Cui et al. 2004) in HeLa cells that were stably knocked down of BAF200 by a vector-based siRNA (Fig. 2B). Induction of the IFITM1 gene was strongly inhibited (Fig. 3A). The same result was also observed when BAF200 was knocked down by two different siRNA oligos (Fig. 3B). Notably, the induction of this gene remains undisturbed in HeLa cells depleted of BAF250a, the BAF-specific subunit (Fig. 3C,D).

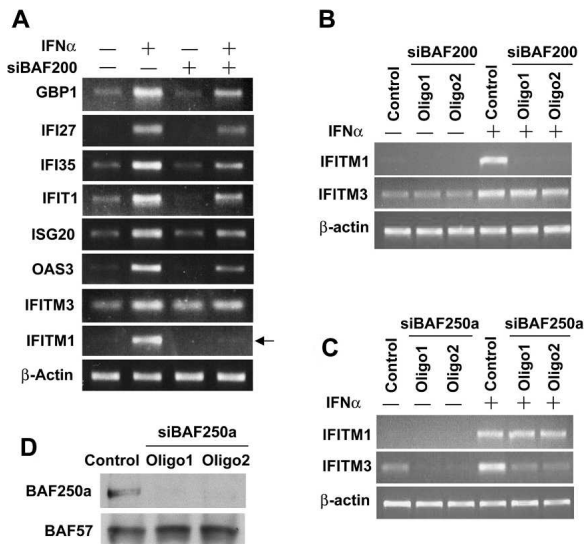


Figure 3. PBAF and BAF complexes are selectively required for expression of distinct IFN- α -inducible genes. (A) RT-PCR shows that HeLa cells depleted of BAF200 by vector-based siRNA display drastically reduced expression of the IFITM1 gene. β -Actin was included as a control. (B) RT-PCR shows that HeLa cells depleted of BAF200 by siRNA oligos exhibited strong inhibition of IFITM1 gene expression, but a normal level of IFITM3 gene. (C) RT-PCR shows that HeLa cells depleted of BAF250a by siRNA oligos have significantly reduced expression of IFITM3 gene under both basal and IFN- α -induced conditions. (D) Immunoblotting shows that BAF250a was efficiently depleted by siRNA oligos in HeLa cells. A nonspecific siRNA oligo was included as a control. Immunoblotting of BAF57 was used as a loading control.

These results indicate that IFITM1 gene expression specifically depends on PBAF but not BAF.

We also searched for IFN- α -inducible genes that depend on BAF but not PBAF. We noticed that the expression levels of IFITM3 gene under basal and IFN- α -induced conditions were largely unaffected in HeLa cells depleted of BAF200 by vector-based siRNA (Fig. 3A), suggesting that expression of this gene is PBAF-independent, and may be dependent on other SWI/SNF complexes, such as BAF. Further analysis revealed that IFITM3 gene expression under both basal and IFN- α -induced conditions was strongly inhibited in HeLa cells depleted of BAF250a by siRNA oligos (Fig. 3C), but remained unaltered in cells depleted of BAF200 by siRNA oligos (Fig. 3B). These data demonstrate that although IFITM1 and IFITM3 are both IFN- α -inducible genes, they depend on distinct SWI/SNF complexes for optimal expression: The IFITM1 gene is PBAF-dependent, whereas IFITM3 is BAF-dependent.

The PBAF-specific subunit BAF180 is dispensable for IFITM1 gene expression

We found that knocking down BAF180 in HeLa cells by two different siRNA oligos (Fig. 2C) had no significant effect on induction of the IFITM1 gene by IFN- α (Fig. 4A). This is somewhat surprising because BAF180 is also a specific subunit of PBAF complex (Xue et al. 2000), and has been shown to be essential for expression of several genes (Wang et al. 2004). One possible explanation is that BAF180 was not completely removed by siRNA, so that the residual amount of this protein is still able to medi-

ate induction of the IFITM1 gene. To address this possibility, we analyzed IFITM1 gene induction in the BAF180-deficient cell line HCC1143, and the same cell line stably expressing ectopically introduced BAF180 (Fig. 4B). The results show that IFITM1 gene induction was normal in the HCC1143 cells, and this induction was unaffected by reintroduction of BAF180 into these cells (Fig. 4C). These results suggest that the expression of the IFITM1 gene is independent of BAF180.

BAF200 forms a complex in the absence of BAF180 and mediates the expression of the IFITM1 gene

The data above showed that in both BAF180-depleted HeLa cells and BAF180-deficient HCC1143 cells, the expression of BAF200 remains normal (Fig. 2C,D), which correlates with the normal induction of the IFITM1 gene (Fig. 4A,C), implying that BAF200 may be able to form a complex in the absence of BAF180 that mediates induction of IFITM1 and perhaps other genes. Consistent with this notion, BAF200 from the extract of HCC1143 cells cofractionated with PBAF subunits BRG1 and BAF57 in a complex of near 1 Mda by Superose 6 gel-filtration chromatography (Fig. 5A). The peak of BAF200 was shifted only two fractions compared with that of HeLa (from 18 to 20) (cf. Fig. 1D), hinting that a significant portion of PBAF complex remains intact without BAF180. Indeed, BAF200 from HCC1143 extract coimmunoprecipitated with BRG1 and BAF57 (Fig. 5B). Biochemical purification of PBAF from HCC1143 cells (Fig. 5C) revealed that it has nearly all the components as the PBAF from HeLa cells, including BAF200, BRG1, and other subunits (Fig. 5D). One important difference is that it lacks BAF180, but contains a novel polypeptide with similar molecular weight as BAF180 (marked with an asterisk). This polypeptide is not BAF180 based on immunoblotting (data not shown), and its relevance to PBAF function is under investigation. Nevertheless, these data together demonstrate that BAF200 can form a new version of PBAF complex absent of BAF180.

The immediate question is whether this complex is still functional. Suppression of BAF200 by siRNA in

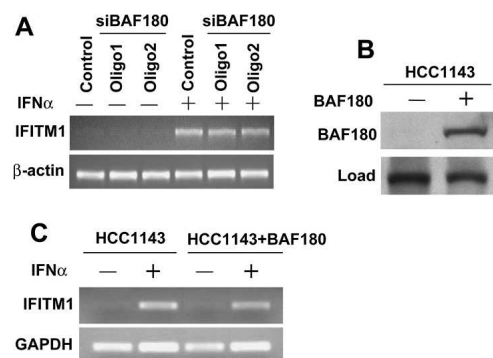


Figure 4. BAF180 is dispensable for IFITM1 gene expression. (A) RT-PCR shows that depletion of BAF180 by siRNA oligos in HeLa cells has no significant effect on induction of the IFITM1 gene by IFN- α . (B) Immunoblotting shows that BAF180 is absent in HCC1143 cells, and present after reintroduction of BAF180 cDNA. (C) RT-PCR shows that induction of the IFITM1 gene is comparable in BAF180-deficient HCC1143 cells, and the same cells stably expressing ectopically introduced BAF180. GAPDH was used as a control.

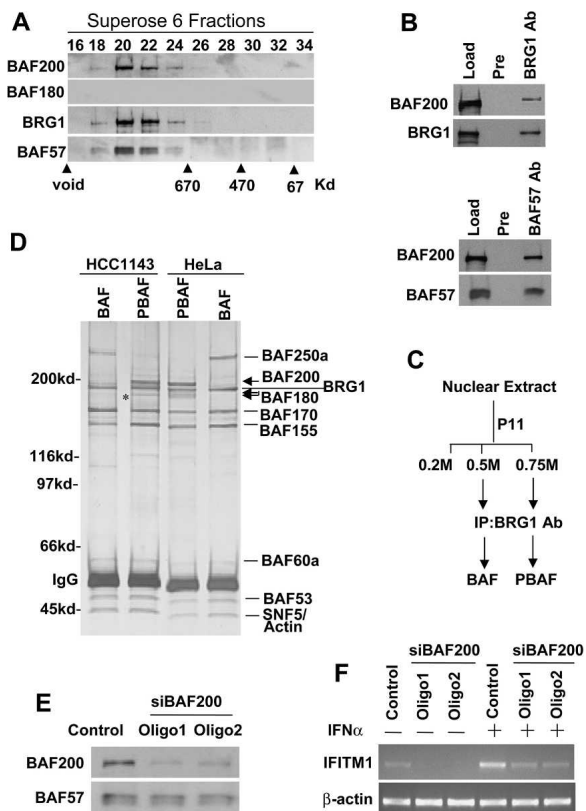


Figure 5. BAF200 forms a complex with BRG1 and mediates IFITM1 gene expression in the absence of BAF180. (A) Immunoblotting shows that BAF200 from the extract of BAF180-deficient HCC1143 cells cofractionates with BRG1 and BAF57. (B) Immunoblotting shows that BAF200 in the extract of HCC1143 cells coimmunoprecipitates with BRG1 and BAF57. The preimmune serum (Pre) was used as a control. (C) A diagram of the purification scheme for PBAF and BAF complexes. (D) A silver-stained SDS-gel shows PBAF and BAF complexes immunoprecipitated with BRG1 antibody from the extract of HCC1143 cells and HeLa cells. An “unknown” polypeptide in the PBAF complex of HCC1143 cells was labeled with an asterisk. (E) Immunoblotting shows that BAF200 expression is suppressed by siRNA oligos in HCC1143 cells. BAF57 was used as a loading control. (F) RT-PCR shows that HCC1143 cells depleted of BAF200 by siRNA oligos have reduced the expression of the IFITM1 gene under both basal and IFN- α -induced conditions.

HCC1143 cells (Fig. 5E) reduced IFITM1 gene expression at both basal and IFN- α -induced levels (Fig. 5F), which is similar to the data from HeLa cells (cf. Fig. 3B). The results suggest that this new version of PBAF is still functional and capable of mediating the IFITM1 gene expression.

Although several mammalian genes have been identified that depend on the human SWI/SNF family of complexes for expression, for the majority of these genes, it remains unclear which particular complex (BAF or PBAF, or both) regulates their expression. Without such knowledge, it would be difficult to address the underlying mechanism of how each gene is targeted by a specific complex. In this study, we show that the two major human SWI/SNF complexes selectively regulate transcription of two interferon-responsive genes in vivo: PBAF, but not BAF, is required for expression of the IFITM1 gene, whereas BAF, but not PBAF, is required for the IFITM3 gene. The two related complexes in yeast (SWI/SNF and RSC) have previously been found to regulate

expression of nonoverlapping genes (Angus-Hill et al. 2001). Moreover, the related complexes in *Drosophila* (BAP and PBAP) localize on different regions in polytene chromosomes (Mohrmann et al. 2004). Together, these data suggest that through millions of years of evolution, the SWI/SNF complexes have maintained their selectivity in regulating expression of nonoverlapping genes.

How is each complex directed to its correct target genes? Because the human BAF and PBAF share the majority of their subunits, their targeting should be accomplished primarily through the specificity subunits that distinguish them. Here we show that PBAF has a second specificity subunit, BAF200, which possesses several conserved domains that are distinct from those in BAF180. It is therefore conceivable that these different domains in BAF200 and BAF180 can target PBAF to different genes through protein–protein or protein–DNA interactions. Consistent with this notion, we show that BAF200 is essential for expression of the IFITM1 gene, whereas BAF180 is not, indicating that at least for this gene, the targeting subunit should be BAF200, but not BAF180. On the other hand, mice inactivated of BAF180 have been found to display severe defects in cardiac chamber maturation, and BAF180 is required for normal expression of several important genes (Wang et al. 2004), suggesting that in the latter case, the targeting subunit could be BAF180. However, it remains possible that BAF200 is also the essential targeting subunit for the latter genes, wherein BAF180 plays an essential but non-targeting role. Distinguishing these possibilities would require inactivating BAF200 without interfering with other subunits of PBAF. This will be difficult because complete elimination of BAF200 results in concomitant destabilization of BAF180. One therefore has to create specific mutants in BAF200 that eliminate its targeting function, but do not destabilize BAF180. Nevertheless, our study suggests that genes that require BAF180 for expression represent only a subset of PBAF-dependent genes, and a complete account of all PBAF-dependent genes will require identification of those that require BAF200 for expression.

Materials and methods

Purification and cloning of BAF200

PBAF complex was directly immunoprecipitated with a BAF180 antibody from HeLa nuclear extract by using an IP protocol as described (Xue et al. 2003). Before IP, the extract was incubated with or without ethidium bromide (EtBr, 100 μ g/mL) for 1 h. The PBAF complex was purified from Flag-hSNF5/INI1 cells as described (Xue et al. 2000). The superose 6 gel-filtration analysis has been described (Xue et al. 2000). The band corresponding to 200 kDa on the Coomassie blue-stained SDS gel was excised and subjected to mass spectrometric analysis as described (Xue et al. 2003). A cDNA clone (PR1374_A05) encoding the large part of this protein (ARID2) was purchased from OriGene, Inc. The missing sequence was generated by PCR using another cDNA clone (AB2553_E11) from OriGene as a donor, and the two fragments were assembled into the cDNA with a full-length open reading frame.

siRNA experiment

For vector-based siRNA, HeLa cells were transfected with either the BAF200 siRNA construct (Supplemental Material) or a control vector by using Superfect (QIAGEN), and selected in 1 μ g/mL of puromycin for 5 d. The remaining cells were then extracted with a lysis buffer (10 mM Tris-HCl at pH 7.5, 1% Triton X-100, 0.1% SDS, 0.1% sodium deoxycholate, 300 mM NaCl, 1 mM EDTA) and subsequently analyzed by immunoblotting. Sometimes, cells were treated with IFN- α (500 U/mL) for 8 h prior to harvest. For oligonucleotide-based siRNA, HeLa or

HCC1143 cells were transfected with the different siRNA oligos (Supplemental Material) by using Oligofectamine (Invitrogen). After 2 d, the cells were lysed for immunoblotting, or treated with IFN- α (500 U/mL) for 8 h followed by RT-PCR analysis as described [Liu et al. 2001].

Other experimental procedures are described in the Supplemental Material.

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