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# Subunits of the Yeast SWI/SNF Complex Are Members of the Actin-related Protein (ARP) Family*

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**The yeast SWI/SNF chromatin remodeling complex is comprised of 11 tightly associated polypeptides (SWI1, SWI2, SWI3, SNF5, SNF6, SNF11, SWP82, SWP73, SWP59, SWP61, and SWP29). We have used matrix-assisted laser desorption ionization time-of-flight mass spectrometry to identify the genes that encode the SWP59 and SWP61 subunits. Surprisingly, we find that SWP59 and SWP61 are encoded by the *ARP9* and *ARP7* genes, respectively, which encode members of the actin-related protein (ARP) family. Sequence analyses have shown that ARP9 and ARP7 are 24–26% identical (48–51% similar) to yeast actin and that they are likely to maintain the overall actin fold. Deletion of either the *ARP9* or *ARP7* gene causes typical *swi/snf* phenotypes, including growth defects on media containing galactose, glycerol, or sucrose as sole carbon sources. *ARP9* and *ARP7* are also required for expression of an *HO-lacZ* fusion gene and for full transcriptional enhancement by the GAL4 activator. The identification of two ARP family members as crucial subunits of the SWI/SNF complex suggests that the complex may contain a total of three different ATPase subunits; furthermore, the similarity of ARP7 and ARP9 to the HSP and HSC family of ATPases suggests the possibility that chromatin remodeling by SWI/SNF may involve chaperone-like activities.**

The Actin-related protein (ARP)[1] family is a branch of the larger actin superfamily of proteins that includes conventional actins, heat shock protein 70 (Hsp70), heat shock cognate 70 (Hsc70), sugar kinases, glycerol kinase, and other ATP-binding proteins from prokaryotic and eukaryotic sources (for reviews, see Refs. 1 and 2). The ARP family in the yeast *Saccharomyces cerevisiae* is composed of 10 different ARPs (ARP1–10) that share 17–45% overall identity (38–69% similarity) to conventional actins (3). These similarities span the length of actin, including 13 blocks of homology that encompass sequences that are known to be important for actin structure or function. Furthermore, even the most distantly related ARP, ARP10, contains 41% similarity to the ATPase domain of the yeast Hsp/Hsc70, SSA1, indicating that the ATP binding pocket is well conserved among all ARP family members.

Phylogenetic tree analyses (3) suggest that the 10 yeast ARPs may each define a separate class of ARP that has a distinct function or cellular localization. In yeast, the majority of ARPs were identified based on sequence analysis, and a genetic analysis has only been applied to ARP1–4. Although not an essential gene, ARP1 encodes a component of the dynactin complex that is required for orientation of the mitotic spindle and nuclear migration; ARP2 and ARP3, on the other hand, are essential genes in yeast that are believed to function together and with the actin cytoskeleton (2). ARP4 is also an essential protein, but it is localized in the nucleus, and the phenotype of a *arp4* mutant suggests a role in chromatin structure or function (4, 5).

The SWI/SNF complex is an enormous, 2000-kDa protein complex that appears to be highly conserved in all eukaryotes (for review, see Ref. 6). The yeast SWI/SNF complex is composed of 11 different polypeptide subunits, and it is required *in vivo* for the transcriptional induction of a subset of yeast genes and for the functioning of a variety of sequence-specific transcriptional activators. Genetic studies in yeast indicate that the role of the SWI/SNF complex in transcriptional regulation is to antagonize chromatin-mediated transcriptional repression. Likewise, a mammalian counterpart of SWI/SNF, the BRG1 complex, is associated with hormone-bound glucocorticoid receptor and is required for glucocorticoid receptor-dependent changes in chromatin structure *in vivo* (7). *In vitro* the purified yeast or mammalian SWI/SNF complex can use the energy of ATP hydrolysis to disrupt nucleosome structure, facilitating the binding of transcription factors or restriction enzymes to mononucleosomes (8–10) or to nucleosome arrays (11, 12).

The SWI2/SNF2 subunit of the yeast SWI/SNF complex is believed to be the catalytic subunit, because SWI2/SNF2 contains sequence motifs found in DNA-stimulated ATPases. Consistent with this view, the bacterially expressed SWI2/SNF2 subunit, as well as the intact SWI/SNF complex, exhibit potent DNA-stimulated ATPase activity (8, 13). Furthermore, a single amino acid change in the putative ATP binding site of SWI2/SNF2 greatly reduces ATPase activity and nucleosome remodeling activities *in vitro* and eliminates SWI/SNF function *in vivo* (8, 13, 14). Previous sequence analyses of the other seven cloned SWI/SNF subunit genes has failed to reveal sequence motifs that might shed light on the catalytic mechanism of SWI/SNF remodeling activity.

Here we describe the use of MALDI-TOF mass spectrometry to identify the genes that encode the SWP59/p47 and SWP61/p50 subunits of yeast SWI/SNF (8, 15). We find that each of these subunits is encoded by members of the ARP family; *ARP7* encodes SWP61/p50, and *ARP9* encodes SWP59/p47. Strains harboring deletions of either *ARP7* or *ARP9* exhibit typical *swi/snf* mutant phenotypes, indicating that these ARP subunits play crucial roles in SWI/SNF activity *in vivo*. These

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[1] The abbreviations used are: ARP, actin-related protein; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction.

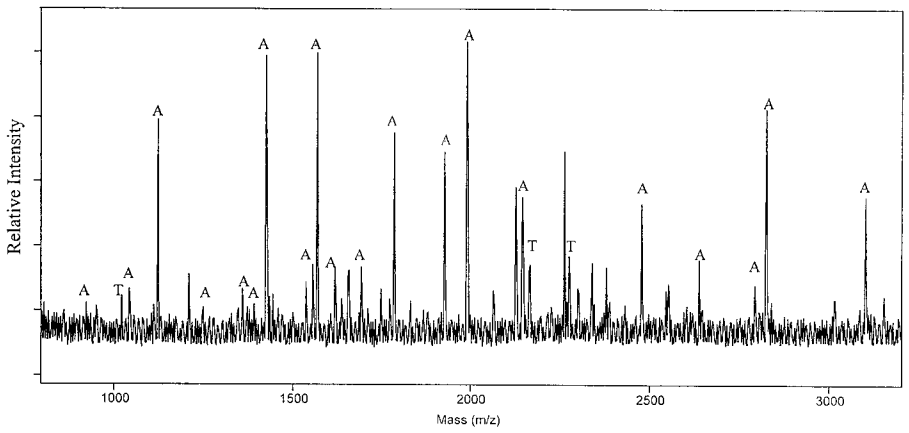


FIG. 1. **Identification of protein ARP7 by MALDI-TOF mass spectrometry.** Mass spectrum of tryptic peptides obtained from protein band SWP61/p50. A data base search using the measured molecular masses of the tryptic peptides yields unambiguous identification of the protein ARP7. Peaks marked "A" designate tryptic peptides from protein ARP7 and peaks marked"T" designate autolysis products from trypsin.

results have major implications for the role of ATP in SWI/SNF-dependent chromatin remodeling.

## EXPERIMENTAL PROCEDURES

*MALDI-TOF Mass Spectrometry Analysis*—The SWI/SNF complex was purified from a 20-liter culture of yeast strain CY396 as described previously (16). The final Superose 6 pool was precipitated with 15% final concentration of trichloroacetic acid, the protein pellet was washed with −20 °C acetone, dried, and resuspended in 20 μl of SDS sample buffer. The protein sample was separated by 10% SDS-PAGE, and SWI/SNF subunits were visualized by copper staining (Bio-Rad). The bands of interest were excised, subjected to in-gel digestion with trypsin, and the resulting peptide mixtures extracted as described previously (17). Peptide mixtures were analyzed with a MALDI-TOF mass spectrometry using delayed ion extraction and ion mirror (Voyager-DE STR, Perseptive Biosystems, Inc., Framingham, MA). The accurately measured masses of the tryptic peptides were used to search for protein candidates in SWISS-PROT protein sequence data base with the program "ProFound" (18) (http://prowl/PROWL/prot-id-main.html).

*Strains and Media*—All strains are congenic to S288C and are isogenic derivatives of strain yPH274 (19). Strains isogenic to CY114 contain a chromosomal *HO-lac*Z fusion gene integrated at the *HO* locus. Cultures were grown at 30 °C in YEP medium (2% yeast extract, 1% bacto-peptone) containing 2% final concentration of either glucose, galactose, glycerol, or sucrose. Media containing galactose or sucrose also contained 1 μg/ml of antimycin. Minimal medium contained 6.7 g/liter yeast nitrogen base without amino acids (Difco), supplemented with amino acids as described (20).

*ARP7 and ARP9 Deletion Alleles*—Precise replacements of the *ARP7* or *ARP9* coding regions with the *HIS3* gene were constructed by a PCR approach. 80-Mer oligonucleotides (Genosys) contained 61 base pairs of homology to either the 5′ or 3′ ends of the *ARP7* or *ARP9* coding region and 19 base pairs of homology to either the 5′ or 3′ ends of the HIS3 locus. PCR primers had the following sequences: ARP7 5′ (5′-3′), CAGAGTAGTAGGCTATTAGCAAAAAGCGCGAGAATTACTACATTATAAAGGATCTGTCAAGGGCCTCCTCTAGTACACTC; ARP7 3′ (5′-3′), GGGTCGAGATCTCATCTCCTTCTAGCCGCCTACAATCCCTTTTGGATATTTGCGCCTTACCGCGCGCCTCGTTCAGAATG; ARP9 5′ (5′-3′), AATTAGAAAAAATGTACCACGCGAAGTTAAGTGTCTTGAACAACTACTATATGCATTGAATGGCCTCCTCTAGTACACTC; ARP9 3′ (5′-3′), AAACTAAACAGCACATATTCACACGGATTTTGGATACACGAAGCATCTATCACGTATTCTTGCGCGCCTCGTTCAGAATG. Each pair of primers was used to amplify the *HIS3* locus from plasmid pRS403 (19). PCR products from one 100-μl reaction were used to transform yeast strain CY114. $His^+$ transformants were screened by PCR to confirm the presence of the *arp7Δ:HIS3* or *arp9Δ:HIS3* deletion alleles.

*Gel Filtration and Western Analyses*—Crude whole cell extracts were prepared and analyzed by gel filtration on a Superose 6 gel filtration column as described previously (21). Superose 6 fractions (0.5 ml) were trichloroacetic acid-precipitated, resuspended in SDS sample buffer, separated on 10% SDS-PAGE gels, and transferred to nitrocellulose. Immunoblots were probed with either the 12CA5 monoclonal antibody (Babco, Emeryville, CA) to detect SWI2 (21) or with a rabbit polyclonal antibody to RPD3; blots were developed with a chemiluminescent substrate as described (22).

## RESULTS AND DISCUSSION

*MALDI-TOF Mass Spectrometry Analysis*—Purification of the yeast SWI/SNF complex through four chromatographic steps yields a preparation that is >50% pure and is primarily composed of 10 polypeptides (8, 16). A small 11th subunit, SNF11, is not visualized by silver staining (23). Previous studies have identified the genes that encode 8 of the 11 SWI/SNF subunits (8, 23–25). The genes that encode the SWP82/p78, SWP61/p50, and SWP59/p47 subunits have not been identified to date. To identify genes encoding the SWP59/p47 and SWP61/p50 subunits, a SWI/SNF preparation was separated by SDS-PAGE and the protein bands corresponding to SWP73 (a positive control), SWP61/p50, and SWP59/p47 were each excised and digested *in situ* with trypsin. The molecular masses of the tryptic peptides obtained from each protein band were measured by MALDI-TOF mass spectrometry. The resulting peptide masses were used in a search of the yeast data base to identify proteins in the band of interest (26, 27).

Fig. 1 shows an example of mass spectrometric peptide mapping for the SWP61/p50 subunit. The measured molecular masses of the peptides were used in a search of the *S. cerevisiae* yeast data base. Twenty peptides were found to match the calculated molecular masses of theoretical peptides from the ARP7 gene product (GenBank™ accession number 809599) with mass accuracy better than 0.2 dalton (accounting for 52% of the protein sequence (18)). Likewise, MALDI-TOF analysis of peptides from the SWP73 subunit uniquely identified the SWP73 gene. The predicted ARP7 protein has a molecular mass of 53.81 kDa, which is consistent with the size of the SWP61/p50 subunit. The protein identifications were further verified by fragmentation of the tryptic peptides in a liquid chromatography tandem mass spectrometry experiment using a LCQ mass spectrometer (Finnigan Corp., San Jose, CA) and the search routine PepFrag (28) (data not shown). Thus, the SWP61/p50 subunit appears to be encoded by the ARP7 gene, which encodes an actin-related protein (3).

In contrast to the unique identification of the gene encoding the SWP61/p50 subunit, MALDI-TOF analysis of the SWP59/p47 subunit yielded two different genes. Sixteen tryptic peptides from the SWP59/p47 band matched the calculated molecular masses of predicted peptides from the RPD3 gene product (accounting for 53% of the protein sequence), whereas 22 tryptic peptides matched the predicted peptides from the ARP9 gene product (accounting for 60% of the protein sequence; GenBank™ accession number 798959). Both sets of identifications were accurate to better than 0.2 dalton. The predicted molecular masses of ARP9 (53.074 kDa) and RPD3 (48.904 kDa) are both consistent with the size of the SWP59/p47 subunit. The ARP9 gene, like ARP7, encodes an actin-related protein (3), whereas the RPD3 gene encodes a histone deacetylase (29, 30).

*RPD3 Is a Subunit of a Distinct 2000-kDa Complex*—Strains that harbor mutations in SWI/SNF subunit genes exhibit a battery of characteristic phenotypes; *swi/snf* mutants grow

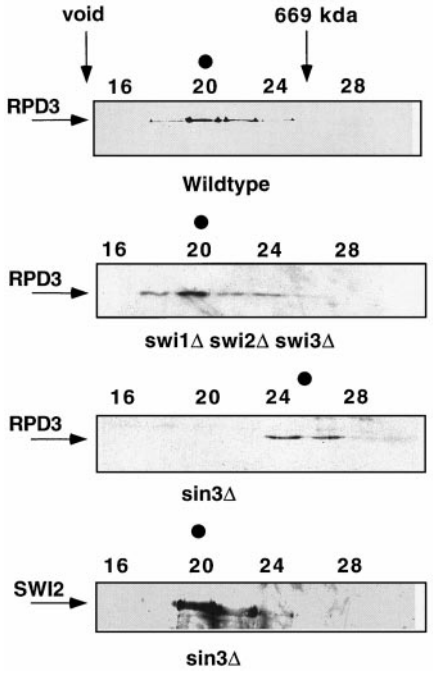


FIG. 2. **RPD3 is a subunit of a large complex distinct from SWI/SNF.** Whole cell extracts from wild-type (CY114), *sin3*Δ (CY35), or *swi1*Δ *swi2*Δ *swi3*Δ (CY93) strains were fractionated on a fast protein liquid chromatography Superose 6 gel filtration column, and fractions were assayed for SWI2 or RPD3 by immunoblots. Peak elution of SWI2 or RPD3 is marked with a *dot* above the corresponding fraction. *Arrows* denote the elution of the calibration protein, thyroglobulin (669 kDa), and position of the void volume (~7000 kDa).

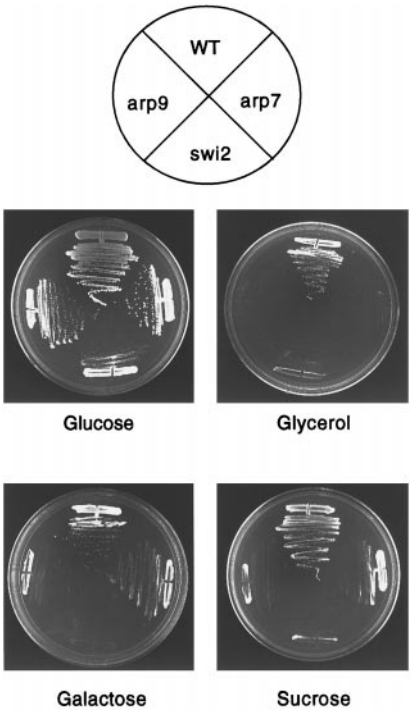


FIG. 3. **Deletions of *ARP7* or *ARP9* cause growth defects similar to *swi/snf* mutants.** Isogenic strains harboring deletions of either *ARP7* (Cy745), *ARP9* (Cy744), or *SWI2/SNF2* (CY407) were streaked on plates containing the indicated carbon sources and incubated for 3–5 days at 30 °C.

slowly on media that contain glucose, and they cannot grow on media that contain galactose, glycerol, or sucrose as sole carbon sources. Furthermore, *swi/snf* mutants show defects in transcription of several genes (*e.g. HO*), and several activators, such as GAL4, require SWI/SNF function for full activity. A deletion of the *RPD3* gene does not result in a defect in *HO* expression or in the loss of transcriptional enhancement by GAL4. In fact, a deletion of RPD3 causes a large *increase* in *HO* expression (31), and a *rpd3* deletion also suppresses the defect in *HO* transcription due to inactivation of SWI/SNF (32). Strains containing *rpd3* mutations also do not show the growth defects that are characteristic of mutations in SWI/SNF subunit genes. These genetic studies suggest that it is unlikely that RPD3 encodes a subunit of the SWI/SNF complex. Previous studies, however, have shown that RPD3 is a subunit of a 2000-kDa protein complex that also contains the SIN3 protein (33). One possibility is that this RPD3 complex is equivalent to the 2000-kDa SWI/SNF complex. Alternatively, the RPD3 complex may be distinct from SWI/SNF, but both complexes may co-purify in our purification scheme. To test these possibilities, we used a gel filtration assay to determine whether disassembly of one complex altered the integrity of the second complex.

Extracts were prepared from wild-type, *sin3*Δ, or *swi1*Δ *swi2*Δ *swi3*Δ strains and fractionated by gel filtration. Fractions were assayed by immunoblotting using antibodies to RPD3 or the SWI2/SNF2 subunit of SWI/SNF. When extracts were prepared from a wild-type strain, both RPD3 and SWI2 elute at an apparent molecular mass of 2000 kDa (Fig. 2 and data not shown (21); peak elution in fraction 20). However, when extracts were prepared from a *sin3*Δ strain, the elution of RPD3 shifted to a much smaller apparent molecular mass (~670 kDa; fraction 25), whereas the elution of SWI2/SNF2 remained unchanged (Fig. 2). In contrast, the elution of RPD3 was not changed when extracts were prepared from the *swi1 swi2 swi3* triple mutant (Fig. 2), which causes disassembly of SWI/SNF (Ref. 21 and data not shown). These data indicate that RPD3 is not a subunit of SWI/SNF, but that the large RPD3 complex is a distinct complex that co-purifies with SWI/SNF. Consistent with this view, the RPD3 complex binds to DNA cellulose and to $Ni^{2+}$ resins even in the absence of a hexahistidine tag,[2] and the SWI/SNF complex purified from a *sin3*Δ strain lacks detectable RPD3 (data not shown).

*ARP7 and ARP9 Are Required for SWI/SNF Function*—To address the functional role of ARP7 and ARP9 in SWI/SNF function, strains harboring deletion alleles of *ARP7* or *ARP9* were constructed and analyzed for growth and transcriptional defects. Like other *swi/snf* mutants, *arp7* and *arp9* mutants grow slowly on glucose media, and they are unable to form single colonies on media that contains glycerol or sucrose as the sole carbon source (Fig. 3). The *arp9* mutant was also unable to grow on galactose media, whereas the *arp7* mutant had a slow growth phenotype on galactose (Fig. 3). The strong growth defect on sucrose media is consistent with a defect in expression of the SWI/SNF-dependent gene, *SUC2*.

In addition to the growth properties of *arp7* and *arp9* mutants, we analyzed expression of a SWI/SNF-dependent gene, *HO*, and the functioning of the GAL4 activator. Disruption of the SWI/SNF complex leads to a 10–100-fold decrease in expression of a chromosomal *HO-lacZ* fusion gene (22) and an 8–15-fold reduction in the ability of the GAL4 activator to enhance transcription from two low affinity, nucleosomal GAL4 binding sites (22). Deletion of *ARP7* or *ARP9* results in a 7–10-fold decrease in expression of the *HO-lacZ* fusion (Fig. 4A). Likewise, deletion of *ARP7* or *ARP9* led to a 5–11-fold reduction in expression from the GAL4-dependent reporter gene (Fig. 4*B*). Thus, the phenotypes of *arp7* and *arp9* mutants are very similar to those of known *swi/snf* mutants, and the genetics are fully consistent with the MALDI-TOF identification of ARP7 and ARP9 encoding SWP61/p50 and SWP59/p47,

[2] A. Carmen and M. Grunstein, personal communication.

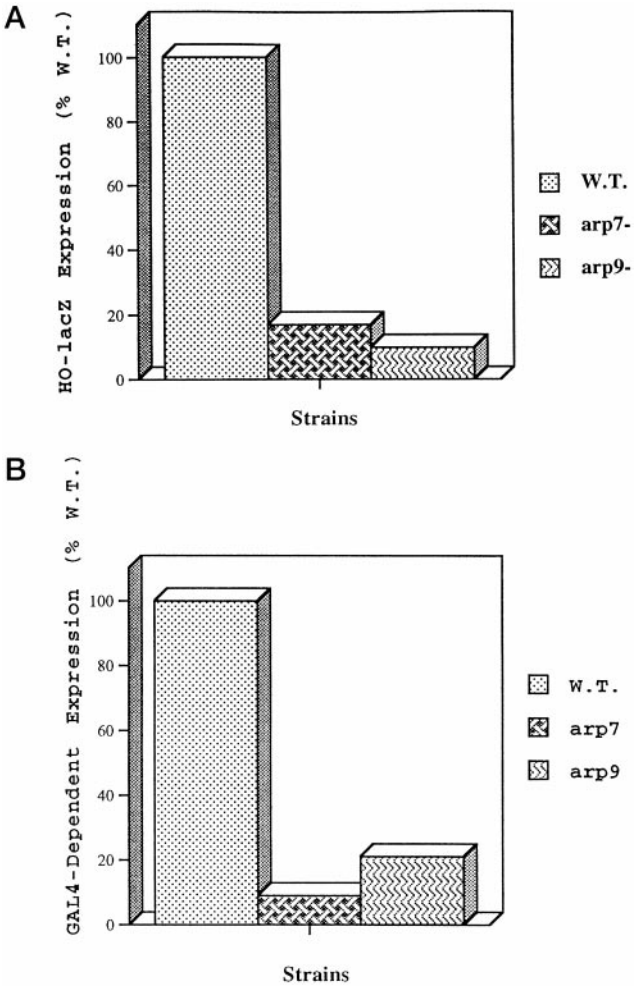


FIG. 4. **ARP7 and ARP9 are required for expression of an *HO-lacZ* fusion gene and for full functioning of the GAL4 activator.** *A*, *HO-lacZ* expression. Isogenic wild-type (CY114), *arp7*Δ (Cy745), or *arp9*Δ (Cy744) strains were grown to mid-log phase in YEP medium containing 2% glucose and then analyzed for β-galactosidase activity. Miller units were normalized to percentages of the wild-type levels. Analyses were performed in triplicate, and the values were averaged; values varied by <20%. *B*, GAL4 reporter. A 2-μ GAL4 reporter plasmid that contains two low affinity, nucleosomal GAL4 binding sites upstream of a *GAL1-lacZ* fusion gene (p121Δ10; Ref. 22) was introduced into the isogenic wild-type (CY114), *arp7*Δ (Cy745), and *arp9*Δ (Cy744) strains. Three independent transformants were grown in minimal medium containing 2% galactose and 0.5% sucrose; β-galactosidase assays were performed as in *A*.

respectively. Furthermore, the defects in growth and transcription indicate that ARP7 and ARP9 play nonredundant, essential roles in SWI/SNF function *in vivo*.

*ARP7 and ARP9 Are Members of the Actin-related Protein Family*—The ARP7 and ARP9 genes were previously identified by their sequence similarity to conventional cytoplasmic actins (3). ARP7 and ARP9 show 17 and 21.9% identity (40.2 and 43.6% similarity), respectively, to the compiled cytoplasmic actin family (3) and 24–26% identity (48–51% similarity) to yeast actin (2). Based on these sequence similarities, ARP7 and ARP9 are ARPs that have conserved the overall actin fold, which, of course, includes an ATPase domain. The presence of actin-related proteins in the SWI/SNF complex is not unique to the yeast complex, as the *Drosophila* counterpart to SWI/SNF, the brm complex, also contains one ARP subunit as well as a structurally related, hsc (heat shock cognate) subunit.[3] Thus, members of the actin superfamily have been maintained during evolution as subunits of the SWI/SNF complex.

The actin ATPase domain is structurally identical to the ATPase domain of the HSP70 family of chaperones, which includes HSC70 and bacterial DnaK. A hallmark of this family of ATPases is that binding of ATP and the subsequent, slow hydrolysis of ATP (0.02 $min^{-1}$ for DnaK (34)) are linked to large protein conformational changes (discussed in Ref. 35). The presence of two actin-related proteins in the yeast SWI/SNF complex suggests that the complex may contain a total of three ATP binding subunits (SWI2/SNF2, ARP7, ARP9); each is required for SWI/SNF function *in vivo*. What roles do each of these ATP binding proteins play in SWI/SNF function? SWI2/SNF2 is likely to generate most of the mechanical energy needed for chromatin remodeling, because it can hydrolyze about 1000 ATP molecules/min in the presence of nucleosomal DNA (8). In contrast, ARP7 and ARP9 may undergo only a few rounds of ATP binding and hydrolysis that control structural rearrangements within the SWI/SNF complex. Changes in SWI/SNF conformations may be required for interactions with nucleosomal components (*i.e.* the histones) or for chromatin remodeling activity. Purification of mutant SWI/SNF complexes that contain ATPase-defective versions of either ARP7 or ARP9 will be invaluable for defining their roles in ATP-dependent nucleosome remodeling.

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