

# Fission yeast SWI/SNF and RSC complexes show compositional and functional differences from budding yeast

Brendon J Monahan<sup>1</sup>, Judit Villén<sup>2</sup>, Samuel Marguerat<sup>3</sup>, Jürg Bähler<sup>3</sup>, Steven P Gygi<sup>2</sup> & Fred Winston<sup>1</sup>

SWI/SNF chromatin-remodeling complexes have crucial roles in transcription and other chromatin-related processes. The analysis of the two members of this class in *Saccharomyces cerevisiae*, SWI/SNF and RSC, has heavily contributed to our understanding of these complexes. To understand the *in vivo* functions of SWI/SNF and RSC in an evolutionarily distant organism, we have characterized these complexes in *Schizosaccharomyces pombe*. Although core components are conserved between the two yeasts, the compositions of *S. pombe* SWI/SNF and RSC differ from their *S. cerevisiae* counterparts and in some ways are more similar to metazoan complexes. Furthermore, several of the conserved proteins, including actin-like proteins, are markedly different between the two yeasts with respect to their requirement for viability. Finally, phenotypic and microarray analyses identified widespread requirements for SWI/SNF and RSC on transcription including strong evidence that SWI/SNF directly represses iron-transport genes.

Changes in chromatin structure are required to facilitate fundamental nuclear processes including transcription, DNA replication, DNA repair, recombination and chromosome segregation. The restructuring of chromatin is often accomplished by ATP-dependent chromatin remodelers, which alter DNA-histone contacts to mediate nucleosomal structural alterations, removal and movement<sup>1</sup>. There are four different classes of ATP-dependent chromatin-remodeling complexes, SWI/SNF, ISWI, Ino80 and Mi-2, and each of these has at its catalytic core an ATPase subunit that belongs to the Snf2-ATPase superfamily<sup>2</sup>. The most widely studied ATP-dependent chromatin-remodeling complexes are in the SWI/SNF class, broadly conserved among eukaryotes<sup>2</sup>.

The two SWI/SNF-class remodelling complexes of *S. cerevisiae*, SWI/SNF, the founding member of this class, and RSC, have been shown to have vital roles *in vivo*<sup>3</sup>. SWI/SNF is involved in transcriptional activation<sup>4,5</sup>, telomeric and rDNA silencing<sup>6</sup> and DNA repair<sup>7</sup>. RSC is ten-fold more abundant than SWI/SNF and, in contrast to SWI/SNF, is essential for cell viability<sup>8,9</sup>. RSC regulates transcription by RNA polymerases II and III<sup>10–14</sup>, and possibly by RNA polymerase I<sup>14,15</sup>. Furthermore, RSC has important roles throughout the cell cycle<sup>16</sup>, including functions in kinetochore function<sup>17</sup>, sister chromatid cohesion<sup>18</sup> and DNA repair<sup>7,19</sup>. Thus, SWI/SNF and RSC function in most chromatin-related processes in *S. cerevisiae*.

*Saccharomyces cerevisiae* SWI/SNF and RSC have been extensively analyzed biochemically. Although both complexes have ATP-dependent nucleosome-remodeling activity<sup>3</sup>, they have distinct compositions. SWI/SNF contains 12 different proteins, whereas RSC

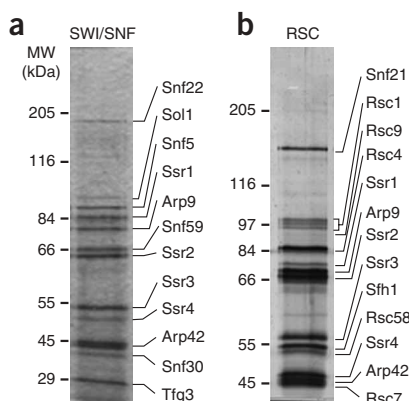
contains 17 different subunits<sup>2</sup>. Snf2, the ATPase subunit of SWI/SNF, is a paralog of Sth1 of RSC. In addition, the two complexes contain four other paralogs (Snf5, Swi3, Snf12/Swp73 and Swp82 of SWI/SNF, and Sfh1, Rsc8, Rsc6 and Rsc7 of RSC, respectively). Three other components are shared between the complexes, two of which, the actin-related proteins Arp7 and Arp9, are crucial for growth<sup>8,20</sup>. Many components of SWI/SNF and RSC contain motifs involved in different activities, including DNA binding, protein-protein interaction and recognition of acetylated histones<sup>2</sup>.

The SWI/SNF class of chromatin-remodeling complexes is evolutionarily conserved throughout eukaryotes, and they are of substantial importance *in vivo*<sup>3,21</sup>. The two mammalian SWI/SNF subclasses, BAF and PBAF (corresponding to SWI/SNF and RSC, respectively), are important in several cellular processes (reviewed in refs. 2,21). These complexes contain the core ATPase subunit, Brg1 (also known as Smarca4) or Brm (also known as Smarca2), plus seven or more noncatalytic subunits that are largely shared between BAF and PBAF<sup>22,23</sup>, with the precise composition of the complex dependent on cell type and the cell cycle. Mammalian SWI/SNF can function both as a positive and negative regulator of transcription, depending on association with other proteins<sup>21,24</sup>. The importance of SWI/SNF in mammalian cells is emphasized by the finding that several mammalian SWI/SNF subunits are defined as tumor suppressors<sup>25</sup>.

*Saccharomyces cerevisiae* has been a valuable system for understanding the *in vivo* mechanisms and roles of SWI/SNF complexes. However, *S. cerevisiae* has major differences in chromatin structure

<sup>1</sup>Department of Genetics and <sup>2</sup>Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115, USA. <sup>3</sup>Wellcome Trust Sanger Institute, Cambridge CB10 1HH, UK. Correspondence should be addressed to F.W. (winston@genetics.med.harvard.edu).

Received 5 March; accepted 27 May; published online 11 July 2008; doi:10.1038/nsmb.1452



**Figure 1** Purification of the fission yeast SWI/SNF and RSC complexes. Representative silver-stained 10% SDS-PAGE gels are shown for the SWI/SNF (a) and RSC (b) purifications. For SWI/SNF an Snf5-TAP (FWP220) purification is shown, and for RSC a Snf21-TAP (FWP218) purification is shown. Molecular weight (MW) marker positions are shown on the left. Components of each complex were identified by MS (Table 1), and the predicted assignment of a protein band to a subunit is shown.

compared to other eukaryotes. Therefore, we have embarked on studies of SWI/SNF and RSC in the distantly related yeast, *S. pombe*. Study of these complexes in *S. pombe* provides an opportunity to gain insight into their *in vivo* functions in an organism whose chromatin more closely resembles that of mammalian cells<sup>26</sup>. In this work, we present the purification and characterization of the *S. pombe* SWI/SNF and RSC complexes, thereby establishing *S. pombe* as a new model system for the study of chromatin-remodeling complexes and providing the resources for such analysis. We show that the two *S. pombe* complexes differ greatly in composition from their *S. cerevisiae* counterparts and in some ways are more similar to those of metazoans. Furthermore, the two actin-related proteins shared between the *S. pombe* SWI/SNF and RSC complexes are functionally distinct from those in the *S. cerevisiae* complexes as they are not required for growth in *S. pombe*, whereas they are essential or critical for growth in *S. cerevisiae*, depending on the strain background. Finally, microarray analyses and other phenotypic characterizations of *S. pombe* SWI/SNF and RSC mutants show that these complexes have widespread roles in transcription and strongly suggest a direct role for SWI/SNF in transcriptional repression.

**RESULTS**

**Purification of *S. pombe* SWI/SNF and RSC complexes**

To initiate study of *S. pombe* SWI/SNF and RSC, we identified putative *S. pombe* homologs of *S. cerevisiae* SWI/SNF and RSC components by sequence homology (Supplementary Table 1 online). Among the candidates identified were the putative homologs of the *S. cerevisiae* ATPases Snf2 of SWI/SNF and Sth1 of RSC, previously named Snf21 and Snf22 (ref. 27). Although sequence homology could not clearly distinguish which *S. pombe* gene corresponded to which *S. cerevisiae* homolog, previous analysis showed that *snf22*<sup>+</sup> is not essential for growth and *snf21*<sup>+</sup> is essential<sup>27</sup>, suggesting that *snf22*<sup>+</sup> is the *SNF2* ortholog and *snf21*<sup>+</sup> is the *STH1* ortholog. Among the other genes identified were SPAC2F7.08c and SPCC16A11.14, putative *S. pombe* orthologs to *S. cerevisiae* *SNF5* and *SFH1*, respectively. To purify the putative *S. pombe* SWI/SNF and RSC complexes, a tandem-affinity purification (TAP) sequence was fused to the 3' end of each of these four *S. pombe* genes. Then, each TAP-tagged protein was purified, and the associated proteins were analyzed by SDS-PAGE and MS.

For the putative *S. pombe* SWI/SNF complex, purification of Snf22-TAP and Snf5-TAP yielded the identical 12-protein complex (Fig. 1a, Table 1 and data not shown). This complex also contained homologs of other *S. cerevisiae* SWI/SNF proteins, including Sol1 (switch one-like, a homolog of *S. cerevisiae* Swi1) and Tfg3 (a homolog of *S. cerevisiae* Taf14), strongly suggesting that this is the *S. pombe* SWI/SNF complex. The complex that we have identified is probably the same as an uncharacterized complex that was shown to contain Sol1 (ref. 28). Our MS analysis also identified a previously uncharacterized protein of approximately 30 kDa, which we called Snf30, and a highly divergent Swp82 homolog, called Snf59 (Table 1 and data not shown). To confirm that Snf30 and Snf59 are components of SWI/SNF, each was TAP-tagged and purified. Both SDS-PAGE and MS gave results identical to those seen for the Snf22-TAP and Snf5-TAP purifications (Table 1 and data not shown), demonstrating that Snf30 and Snf59 are both components of *S. pombe* SWI/SNF. In summary, the composition of the 12-subunit *S. pombe* SWI/SNF complex has both substantial similarities and differences with the *S. cerevisiae* SWI/SNF complex (Table 2).

To determine the composition of *S. pombe* RSC, we purified Snf21-TAP and Sfh1-TAP. These purifications yielded an identical 13-member complex (Fig. 1b, Table 1 and data not shown). To confirm this composition, two additional putative members of the complex, Rsc1 and Rsc7, were TAP-tagged and used to purify the complex. These purifications gave identical results to the Snf21 and Sfh1 purifications (Table 1). *S. pombe* RSC contains orthologs to many of the proteins in

**Table 1** The *S. pombe* SWI/SNF and RSC chromatin-remodeling complexes

Protein	Systematic ID	Size <sup>a</sup>	Sequence coverage (%) <sup>b</sup>			
<b>SWI/SNF complex</b>			<b>Snf22<sup>c</sup></b>	<b>Snf5</b>	<b>Snf59</b>	<b>Snf30</b>
Snf22	SPCC1620.14c	1680	44	44	41	39
Sol1	SPBC30B4.04c	865	43	49	43	31
Snf5	SPAC2F7.08c	632	37	38	42	38
Ssr1	SPAC17G6.10	527	58	55	53	44
Arp9	SPAC1071.06	523	37	37	30	29
Snf59	SPBC26H8.09c	515	57	62	64	50
Ssr2	SPAC23H3.10	503	33	38	34	23
Arp42	SPAC23D3.09	430	47	47	45	45
Ssr3	SPAC23G3.10c	425	73	71	59	67
Ssr4	SPBP23A10.05	395	36	39	35	32
Snf30	SPAC23G3.07c	274	37	42	33	32
Tfg3	SPAC22H12.02	241	34	37	39	30
<b>RSC complex</b>			<b>Snf21</b>	<b>Sfh1</b>	<b>Rsc7</b>	<b>Rsc1</b>
Snf21	SPAC1250.01	1199	58	48	58	64
Rsc1	SPBC4B4.03	803	57	47	53	51
Rsc9	SPBC1703.02	780	41	34	42	45
Rsc4	SPBC1734.15	542	64	64	61	61
Ssr1	SPAC17G6.10	527	62	54	51	55
Arp9	SPAC1071.06	523	43	40	30	34
Ssr2	SPAC23H3.10	503	33	32	35	38
Arp42	SPAC23D3.09	430	64	59	54	62
Ssr3	SPAC23G3.10c	425	81	75	67	76
Sfh1	SPCC16A11.14	418	42	41	36	28
Rsc58	SPAC1F3.07c	403	52	50	55	54
Ssr4	SPBP23A10.05	395	42	37	35	42
Rsc7	SPCC1281.05	390	54	55	61	59

Mass-spectrometry results showing the identification SWI/SNF and RSC components for each TAP-tagged subunit preparation.

<sup>a</sup>Protein size in amino acids. <sup>b</sup>Sequence coverage (percentage of amino acids) for the respective protein. <sup>c</sup>Protein name indicates the TAP fusion used for purification.



**Table 2** Composition of *S. pombe* SWI/SNF and RSC complexes compared to those of *S. cerevisiae* and human

<i>S. pombe</i>		<i>S. cerevisiae</i>		Human	
SWI/SNF <sup>a</sup>	RSC	SWI/SNF	RSC	BAF	PBAF
Snf22	Snf21	Snf2	Sth1	BRG1 or BRM	BRG1
Sol1		Swi1		BAF250	
Snf5	Sfh1	Snf5	Sfh1	SNF5	SNF5
Ssr1, Ssr2 <sup>b</sup>	Ssr1, Ssr2	Swi3	Rsc8	BAF170, BAF155	BAF170, BAF155
Ssr3	Ssr3	Snf12	Rsc6	BAF60a	BAF60a or BAF60b
Ssr4	Ssr4				
Arp42	Arp42			BAF53	BAF53
Arp9	Arp9	Arp9	Arp9		
		Arp7	Arp7		
				Actin	Actin
Tfg3		Taf14			
	Rsc1		Rsc1 or Rsc2		BAF180
	Rsc4		Rsc4		
	Rsc9		Rsc9		
	Rsc58		Rsc58		
Snf59	Rsc7	Swp82	Rsc7		
Snf30				BAF57	BAF57
		Rtt102	Rtt102		
		Snf11			
		Snf6			
			Rsc3		
			Rsc30		
			Ldb7		
			Htl1		

<sup>a</sup>Subunits of the respective complexes are listed in columns, with orthologous proteins grouped horizontally. <sup>b</sup>Paralogs in the same complex are grouped in the same row of the column.

its *S. cerevisiae* counterpart, including the bromodomain proteins Rsc1, Rsc4 and Rsc58 (Table 2). However, *S. pombe* RSC also has notable differences from *S. cerevisiae* RSC. First, *S. pombe* RSC does not contain five subunits that are found in *S. cerevisiae* RSC but that are not conserved in metazoans, including Rsc3, which is essential for growth in *S. cerevisiae*<sup>10,29</sup>. In addition, *S. cerevisiae* RSC exists in at least two forms owing to the mutually exclusive presence of the bromodomain proteins Rsc1 or Rsc2 (ref. 30). However, *S. pombe* RSC has only a single Rsc1 and Rsc2 homolog, suggesting that *S. pombe* RSC exists in fewer forms than in *S. cerevisiae*.

### *Shizosaccharomyces pombe* SWI/SNF and RSC share six components

A notable feature of the *S. pombe* SWI/SNF and RSC complexes is that they share six proteins, a much greater degree of overlap than the three subunits shared between the *S. cerevisiae* complexes (Table 2). Analysis of these shared proteins highlights similarities between the two *S. pombe* complexes and their metazoan counterparts (Table 2). The presence of Ssr1 and Ssr2 in both *S. pombe* complexes is similar to human BAF and PBAF, which share both BAF150 and BAF170 (ref. 23); however, this contrasts with complexes from *S. cerevisiae*, where Swi3 and Rsc8 are specific to SWI/SNF and RSC, respectively. Ssr3 is a homolog of *S. cerevisiae* Snf12 (in SWI/SNF) and Rsc6 (in RSC) and, similarly to its metazoan ortholog, BAF60a, is shared between SWI/SNF and RSC. Ssr4 is a member of a previously uncharacterized protein family (Pfam family PF08549) that has no apparent *Saccharomyces* homologs. Finally, the shared actin-related proteins, Arp42 and Arp9, differ from the Arp7–Arp9 module in

*S. cerevisiae* SWI/SNF and RSC (Supplementary Fig. 1 online). Notably, this is the first example in which an Arp4-like protein is associated with Arp9 and not nuclear actin<sup>31</sup>. In summary, *S. pombe* SWI/SNF and RSC components overlap extensively, a situation distinct from *S. cerevisiae* and similar to the mammalian BAF and PBAF complexes.

### Deletion analysis of SWI/SNF and RSC genes

To analyze the roles of *S. pombe* SWI/SNF and RSC *in vivo*, we deleted each of the genes that encodes a subunit of the complexes (summarized in Supplementary Table 2 online). Our results show that none of the six genes encoding SWI/SNF-specific proteins are essential for viability (Fig. 2a). Thus, as in *S. cerevisiae*, SWI/SNF is not essential for growth. In contrast, four of the seven RSC-specific genes (*snf21*<sup>+</sup>, *sfh1*<sup>+</sup>, *rsc9*<sup>+</sup> and *rsc7*<sup>+</sup>), as well as four of the six genes encoding shared proteins (*ssr1*<sup>+</sup>–*ssr4*<sup>+</sup>) are essential for growth, demonstrating that *S. pombe* RSC is required for cell viability. One unexpected discovery from the deletion analysis was that *S. pombe* *arp42Δ*, *arp9Δ* and *arp42Δ arp9Δ* mutants are viable and grow well on rich medium at 32 °C (Fig. 2a). This result contrasts with results for *S. cerevisiae* ARP7 and ARP9, which are essential or critical for growth depending on the genetic background<sup>20,32</sup>. The requirement for viability for other components also differed

between *S. pombe* and *S. cerevisiae* (Supplementary Table 2). These notable differences between the *S. cerevisiae* and *S. pombe* complex members indicate that they mediate distinct roles in each organism.

To gain more information about the roles of SWI/SNF and RSC, we tested the viable deletion mutants for growth defects under different conditions (Fig. 2a and Supplementary Fig. 2 online). For SWI/SNF, a subset of mutants (*snf22Δ*, *snf5Δ*, *sol1Δ* and *tfg3Δ*) showed tight cold sensitivity at 16 °C (Fig. 2a), a phenotype not seen for their *S. cerevisiae* counterparts. Some mutants also showed modest sensitivity to benomyl or hydroxyurea (Fig. 2a). Notably, *S. pombe* SWI/SNF mutants do not show certain phenotypes characteristic of *S. cerevisiae* SWI/SNF mutants<sup>33</sup> (Supplementary Fig. 2). For RSC, two of the three viable mutants that are RSC specific, *rsc58Δ* and *rsc1Δ*, showed pleiotropic phenotypes, indicating roles for RSC in several cellular processes (Fig. 2a, Supplementary Fig. 2 and data not shown). In contrast, the *rsc4Δ* mutant showed near-wild-type growth. Microscopy of the *rsc58Δ* and *rsc1Δ rsc4Δ* mutants revealed elongated cells and apparent chromosomal segregation defects (Fig. 2b). Finally, the phenotypes observed for *arp42Δ*, *arp9Δ* and *arp42Δ arp9Δ* encompass those seen for the SWI/SNF and RSC mutants (for example, temperature sensitivity and caffeine sensitivity), consistent with Arp42 and Arp9 being important for both SWI/SNF and RSC functions (Fig. 2).

### Arp proteins are not required for complex assembly

Given the marked difference between *S. cerevisiae* and *S. pombe* with respect to the requirement for Arp proteins in SWI/SNF and RSC, we determined the composition of the SWI/SNF and RSC complexes that









