Direct interaction between Rsc6 and Rsc8/Swh3, two proteins that are conserved in SWI/SNF-related complexes

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ABSTRACT

The RSC complex of Saccharomyces cerevisiae is closely related to the SWI/SNF complex. Both complexes are involved in remodeling chromatin structure and they share conserved components. The RSC proteins Sth1, Rsc8/Swh3, Sfh1 and Rsc6 are homologs of the SWI/SNF proteins Swi2/Snf2, Swi3, Snf5 and Swp73 respectively. To investigate the RSC complex, we isolated a temperature-sensitive swh3-ts allele. A screen for multicopy suppressors yielded plasmids carrying the RSC6 and MAK31 loci. RSC6 also suppressed the formamide sensitivity of a strain with a C-terminal truncation of SWH3. We show that Swh3 and Rsc6 fusion proteins interact in the two-hybrid system and that the swh3-ts mutation impairs this interaction. Finally, bacterially produced Swh3 and Rsc6 fusion proteins interact in vitro, supporting the genetic evidence for direct interaction between Swh3 and Rsc6 in vivo. We have previously shown that Swh3 also interacts with Sth1. These findings, together with the conservation of these proteins in the SWI/SNF complex and in mammalian SWI/SNF-related complexes, strongly suggest that these proteins form a structural core for the complex.

INTRODUCTION

Transcription, replication, recombination and DNA repair in eukaryotes involve the interaction of proteins with DNA packaged into chromatin. The packaging of DNA in nucleosomes and higher order structures can represent an obstacle to such interactions. A convergence of genetic and biochemical studies has identified several ATP-dependent multiprotein complexes that may remodel chromatin during these processes (1,2). The SWI/SNF complex of Saccharomyces cerevisiae was the first complex shown to play such a role (for reviews see 2–4). The SNF genes and SWI genes were identified in genetic screens for mutants defective in expression of the HO and SUC2 genes and both genetic and biochemical evidence indicates that the SWI/SNF complex functions to overcome chromatin-mediated repression during transcription. The complex has 11 components, including Swi1/Adr6, Snf2/Swi2, Swi3, Snf5, Snf6, Snf11, Swp73/Snf12 and Tfg3/TAF30/Anc1 (5–11). Biochemical studies have shown that the Snf2 protein has a DNA-dependent ATPase activity (5,6,12) and that the SWI/SNF complex alters nucleosomal structure (13) and stimulates binding of Gal4 derivatives to nucleosomes in an ATP-dependent manner (6). The SWI/SNF complex also binds DNA (14) and catalyzes disruption of histones from Gal4-bound nucleosomes (15). In addition, the SWI/SNF complex has been found associated with RNA polymerase II holoenzyme (16).

Yeasts also contain a SWI/SNF-related complex known as RSC (remodels the structure of chromatin) (17). This 1 MDa complex was purified on the basis of homology to the SWI/SNF complex and contains at least four proteins with similarity to Swi/Snf proteins; Sth1/Nsp1 (18,19), Rsc8/Swh3 (17,20), Sfh5 (21) and Rsc6 (17) are homologous to Swi2, Swi3, Snf5 and Swp73 respectively (Table 1) and Sth1 is the closest Snf2 homolog in yeast. The RSC complex exhibits a DNA-dependent ATPase activity and alters chromatin structure (17). Although its physiological role is unknown, the RSC complex is 10-fold more abundant than the SWI/SNF complex and genes encoding four of its components are essential for mitotic growth (17,20,21). Moreover, lack of expression of Sth1 or shift of a conditional sfh1 mutant to non-permissive temperature induced a cell cycle arrest in G2/M (18,21).

A homolog of the SWI/SNF complex has also been identified in Drosophila. Brahma (brm) is the closest Snf2 homolog and was identified in a screen for suppressors of mutations in Polycomb, a repressor of homeotic genes that is thought to act by altering chromatin structure (22). brm is found together with snr1, a Snf5 homolog, in a complex similar in size to the yeast SWI/SNF complex (23). In addition, a more distantly related Snf2 homolog, ISWI, is a subunit of at least three different protein complexes: the nucleosome remodeling factor NURF (24–26), the chromatin accessibility complex CHRAC (27) and the ATP-utilizing chromatin assembly and remodeling factor ACF (28). Each of these complexes was identified using biochemical assays for chromatin remodeling. Homologs of the ISWI gene also exist in S.cerevisiae and humans, but the corresponding complexes have not yet been identified (29).

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Table 1. Conserved components of SWI/SNF-related complexes

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<tr>
<th>Saccharomyces cerevisiae</th>
<th>Drosophila</th>
<th>Human</th>
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<tr>
<td>SWI/SNF complex</td>
<td>RSC</td>
<td>BRM</td>
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<tr>
<td>complex</td>
<td>complex</td>
<td>BAF</td>
</tr>
<tr>
<td>Snf2/Swi2</td>
<td>Sth1</td>
<td>brm</td>
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<td></td>
<td></td>
<td>BRG1 or hbrm</td>
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<tr>
<td>Swi3</td>
<td>Rsc8/Swh3</td>
<td>?</td>
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<tr>
<td></td>
<td></td>
<td>BAF155 and BAF170</td>
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<tr>
<td>Snf5</td>
<td>Sfh1</td>
<td>snr1</td>
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<td></td>
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<td>Swp73</td>
<td>Rsc6</td>
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<td></td>
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<td>BAF60</td>
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Proteins that are conserved among multiple complexes are listed and alternative names are indicated. The human BAF complexes contain either BRG1 or hbrm; each type of complex contains both BAF155 and BAF170. A fifth component (BAF57) is conserved in all mammalian complexes purified so far, but does not have a yeast counterpart (49). See text for references.

Several putative functional homologs of the SWI/SNF complex have been identified in higher eukaryotes. Humans have at least two proteins closely related to Snf2, hbrm (hSNF2α) and BRG1 (hSNF2β) (30–32), which are assembled into distinct multiprotein complexes of 9–12 subunits containing either hbrm or BRG1 (33). Both types of complexes also contain homologs of Swi3 (BAF155 and BAF170; both proteins are present in the same complex), Snf5 (hSnf5/Ini1/BAF47) and Swp73 (BAF60) (33–36; Table 1). Like the yeast complex, the human SWI/SNF-related complexes show in vitro nucleosome remodeling activity and facilitate the binding of activators to nucleosomal DNA (33,36–38).

Thus SWI/SNF-related complexes are highly conserved and appear to have important roles in cellular processes. The complexes characterized thus far share a set of conserved proteins, homologs of the SWI/SNF components Snf2, Swi3, Snf5 and Swp73. The presence of these homologs may reflect a common structural core shared by these complexes.

In this study, we have taken a genetic approach that has provided insight into protein interactions within the RSC complex. We have focused on Rsc8/Swh3, which was identified by independent biochemical and genetic approaches. Rsc8 was isolated as the 60 kDa protein of the RSC complex (17) and Swh3 (Swi3 homolog) was identified as a protein that interacts in the two-hybrid system with a domain that is conserved in Snf2, Sth1, brm, BRG1 and hbrm (20). Swh3 has 30% identity and 52% similarity to Swi3. Both Swi3 and Swh3 contain a SANT domain and a leucine zipper motif located near the C-terminus and the Swh3 leucine zipper is involved in self-association (17,20,39). Despite their high similarity, Swh3 and Swi3 are functionally distinct and Swh3 is essential for cell viability (20).

In this work, we have isolated a temperature-sensitive swh3 allele and selected multicopy suppressors. We recovered the RSC6 gene, encoding a component of the RSC complex that is homologous to the Swp73 protein. We present genetic and biochemical evidence that Swh3 and Rsc6 interact directly in the RSC complex. These findings support a model in which these proteins and their homologs are components of the structural core of SWI/SNF-related complexes.

MATERIALS AND METHODS

Strains and genetic methods

Saccharomyces cerevisiae strains are listed in Table 2. Standard genetic methods were followed. Media used were yeast extract–peptone (YPD) or selective synthetic complete (SC) medium containing 2% glucose (40), except where otherwise noted. SC + 5-FOA is SC containing 50 mg/l uracil and 1 g/l 5-fluoroorotic acid. The Escherichia coli strain was XL1-Blue (Stratagene), except where otherwise noted.

Plasmids

pRS426-HA-SWH3, pRS315-HA-SWH3, pGAD-SWH3, pGAD-SWH3Δ and pLexA-SWH3 have been described previously (20). Plasmid pIT399 was recovered in the screen for multicopy suppressors of MCY3888 and carries the SWH3 gene. Plasmids pIT432 and pIT433 were recovered in the screen for multicopy suppressors of MCY3890. Plasmid pIT432 contains a 5 kb genomic DNA fragment of chromosome III, starting at position 157 236, and contains HSP30, MAK31, PET18 and MAK32. PCR reactions were done with Taq polymerase (Perkin Elmer Cetus) and the primers listed in Table 3. To construct pGAD-RSC6, pLexA-RSC6 and pTRX-RSC6, a BamHI–XhoI PCR fragment was generated by amplification of pIT433 using OL125 and OL126 and cloned in

Table 2. List of S.cerevisiae strains

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aMCY strains have the S288C genetic background except MCY3890, which is a derivative of W303-1A.
bConstructed by R.Sternglanz.
pACTII (41), pEG202 (a gift from E.Golemis and R.Brent) and pET32c (Novagen) respectively. To construct pLexA-SWH3-ts16, a BamHI–SalI PCR fragment amplified on MCY3890 genomic DNA using OL93 and OL94 was cloned in pEG202. pGAD-SWH3-ts16 contains the same fragment cloned in the BamHI and XhoI sites of pACTII. To construct pGST-SWH3, a PCR fragment was generated using W303-1A genomic DNA as template and primers OL93 and OL127 and cloned in pGEX-3X (Pharmacia). For each construct, clones from two independent PCR reactions were tested in functional assays.

**Screen for swh3-ts mutations**

Plasmid pRS315-HA–SWH3, which carries the SWH3 gene on a CEN, LEU2 plasmid, was subjected to hydroxylamine mutagenesis (42). Mutagenized DNA was used to transform strain MCY3874, which carries a swh3Δ chromosomal deletion that is complemented by SWH3 on the URA3. 2μ plasmid pRS426-HA–SWH3. A total of 8000 transformants obtained at 23°C and replica plated at 37°C. Transformants that grew on these plates were replica plated again on two SC–Leu+5-FOA plates; one was incubated at 23 and the other at 37°C. Putative temperature-sensitive transformants were colony purified and retested. Plasmid DNAs from 21 transformants were isolated in E.coli and used to retransform MCY3874. Of these clones, 11 produced temperature-sensitive cells again after plasmid shuffling.

**Introduction of swh3 mutant alleles into the chromosome**

The EcoRI–SalI fragment carrying the mutant swh3-ts16 and swh3-ts21 alleles was subcloned from the original plasmid into the integrative URA3 plasmid pRS306. The resulting plasmid was linearized with MluI and used to transform the wild-type strains MCY3647 and W303-1A. Transformants were patched first on SC–Ura and then SC+5-FOA and incubated at 23°C. Cells from the plate were plated for single colonies on SC+5-FOA and then SC+Leu+5-FOA and incubated at 23°C. Transformants were colony purified and used to retransform MCY3874. Of these clones, 11 produced temperature-sensitive cells again after plasmid shuffling.

**Isolation of multicopy suppressor plasmids**

Strain MCY3890 was transformed with a genomic library in the multicopy vector Yep24 (43). Approximately 75 000 Ura+ transformants were recovered at 23°C and replica plated at 37°C. Plasmid DNA from 134 temperature-resistant transformants was isolated and used to transform E.coli. PCR reactions with OL91 and OL92 carried out on plasmid DNA showed that 61 of them carried the SWH3 gene. Restriction digests of the remaining clones and sequence analysis of 11 of them showed that 17 contained RSC6 and 52 mapped to one locus that encompassed the PET18, MAK31 and HSP30 genes.

**β-Galactosidase assay**

β-Galactosidase activity was assayed either quantitatively in permeabilized cells (40) or using filter lifts from cells patched on plates as described (44). For quantitative assays, transformants were grown to mid log phase in SC–His–Leu medium to select for plasmids. β-Galactosidase activity is expressed in Miller units.

**Protein expression and in vitro binding assays**

Protein expression and in vitro binding assays were performed as described previously (45). Escherichia coli XL1-Blue was used for expression of glutathione S-transferase (GST) fusions and BL21(DE3)pLysS (Novagen) was used for expression of thioredoxin (TRX) fusions. Expression of the fusion proteins was induced by addition of isopropyl-β-D-thiogalactoside (0.5 mM final concentration) to the culture. Bacterial pellets were resuspended in buffer ST (50 mM Tris–HCl, pH 8.0, 150 mM NaCl) with 1 mg/ml lysozyme and were sonicated briefly on ice. Extracts were cleared by centrifugation at 12 000 r.p.m. for 15 min. Triton X-100 was added to 1% final concentration. Extracts containing GST–SwH3 or GST proteins were incubated with glutathione–Sepharose 4B beads (Pharmacia) at 4°C for 30 min. After being washed, 8 µg GST–SwH3 or GST bound to the beads were added to extracts (600 µg) expressing TRX–Rsc6 or TRX proteins and allowed to bind at 4°C for 2 h in buffer ST+1% Triton X-100. After being washed with 2 ml buffer ST+1% Triton X-100 and with 5 ml buffer ST, beads were boiled in SDS sample buffer and loaded on a 10% SDS–polyacrylamide gel. Bound proteins were detected by western analysis with a polyclonal antibody against TRX (Santa Cruz Biotechnology) and enhanced chemoluminescence (ECL) with ECL reagents (Amersham).

**RESULTS**

Isolation of conditional alleles of SWH3

To isolate temperature-sensitive swh3 alleles, we used a plasmid shuffling technique, because SWH3 is an essential gene (see Materials and Methods). We mutagenized a centromeric LEU2-marked plasmid carrying the SWH3 gene with hydroxylamine and transformed a swh3Δ mutant strain (MCY3874) carrying the wild-type SWH3 on a URA3-marked plasmid. After plasmid shuffling, 11 plasmids carrying a temperature-sensitive

Figure 1. Temperature sensitivity of swh3-ts16 and swh3-ts21 mutants. Strains were a swh3Δ deletion mutant carrying a wild-type copy of SWH3 on a centromeric plasmid (MCY3881) or mutated versions of this plasmid carrying swh3-ts16 and swh3-ts21. Ten-fold serial dilutions were spotted on SC–Leu plates, incubated at 23 and 36°C and photographed.

Figure 2. Maps of multicopy suppressors of swh3-ts16. Only the yeast DNA segment of each plasmid is shown. pIT432, pIT433 and pIT434 were recovered in the multicopy suppressor screen. Arrows show the open reading frames present in the clones. MCY3890 was transformed with the indicated plasmids and tested for growth on SC–Ura in the presence of 3% formamide or at 37°C. The ability of each plasmid to suppress growth defects caused by swh3-ts16 is indicated. (A) MAK31 locus and (B) RSC6 locus. Restriction sites: H, HindIII; B, BglII; S, SpeI; C, ClaI; K, KpnI.

allele of swh3 were isolated. Two mutant alleles, swh3-ts16 and swh3-ts21, conferred sufficiently strong temperature-sensitive phenotypes for use in selection of suppressors (Fig. 1).

These two alleles were introduced into the chromosome of W303-1A and MCY3647 respectively and the resulting mutant strains were temperature sensitive. We also tested if the mutations conferred sensitivity to high concentrations of formamide or ethanol at 30°C. The swh3-ts21 mutant (MCY3890) did not grow on 3% formamide or 6% ethanol while the swh3-ts16 mutant (MCY3890) did not grow on 3% formamide but was insensitive to 6% ethanol (data not shown; Fig. 3).

RSC6 is a multicopy suppressor of swh3-ts16

To identify genes that in multicopy suppress the temperature-sensitive phenotype of the swh3-ts16 allele, we transformed MCY3890 with a yeast genomic library in a multicopy vector (see Materials and Methods). In addition to SWH3, plasmids carrying DNA from two different loci were recovered. Fifty-two clones contained the MAK31 and HSP30 genes (Fig. 2A). Plasmids carrying these genes also suppressed the formamide sensitivity of the swh3-ts16 mutant (Fig. 3). To identify the gene responsible for suppression, we constructed subclones in a multicopy vector and tested their ability to suppress the formamide-sensitive phenotype (Fig. 2A). The results show that MAK31 or a previously unidentified ORF of 79 codons is the relevant gene.

MAK31 is required for maintenance of the killer phenotype (46); its sequence is similar to a mammalian component of a small nuclear ribonucleoprotein, snRNP E (47).

The 17 remaining clones contained RSC6, encoding a 483 amino acid subunit of the RSC complex (17) that is 28% identical to Swp73 (Fig. 2B). Plasmids carrying RSC6 also suppressed the formamide-sensitive phenotype of swh3-ts16 (Fig. 3).

In another multicopy suppressor screen carried out in a different strain background with the swh3-ts21 allele (MCY3888), plasmids carrying the SWH3 gene were the only ones recovered. Consistent with this result, neither RSC6 nor the MAK31 locus suppressed the formamide-sensitive phenotype of MCY3890 (Fig. 3).

Multicopy RSC6 suppresses conditional phenotypes caused by C-terminal truncation of SWH3

To determine whether multicopy suppression by RSC6 is specific to the swh3-ts16 allele, we tested for suppression of swh3ΔC, a deletion of the C-terminal coding sequence (codons 505–558) that confers conditional phenotypes. Previously, we had constructed a Gal4 activation domain (GAD) fusion to Swh3 truncated at the C-terminus after amino acid 505 (GAD–Swh3ΔC), which
complements swh3Δ as effectively as GAD–Swh3 at 30°C (20). However, at 37°C GAD–Swh3 still complements swh3Δ, but GAD–Swh3ΔC does not. We therefore introduced the swh3ΔC mutation into the chromosomal locus; the resulting mutant, MCY4016, failed to grow at 37°C. This strain also did not grow on 2.5% formamide, but was insensitive to 6% ethanol at 30°C. In multicycopy both RSC6 and MAK31 weakly suppressed the formamide sensitivity of MCY4016 (Fig. 3); only RSC6 conferred suppression on 3% formamide (data not shown). Thus, suppression by multicycopy RSC6 and MAK31 is not specific to the swh3-ts16 allele.

**Swh3 and Rsc6 interact in the two-hybrid system**

Suppression of the mutant phenotypes of the swh3-ts16 or swh3ΔC alleles by overexpression of Rsc6 suggested that the Swh3 and Rsc6 proteins interact. To obtain evidence for interaction between Swh3 and Rsc6, we first used the two-hybrid system. Strains expressing both LexA–Swh3 and GAD–Rsc6 or LexA–Rsc6 and GAD–Swh3 activated β-galactosidase expression from a lexAop–GAL1–lacZ reporter gene (Fig. 4A). Activation depends on the presence of both Rsc6 and Swh3, because no activation was detected when the fusion proteins were expressed in combination with LexA or GAD (Fig. 4A and data not shown).

To test if the mutation swh3-ts16 decreases the affinity of Swh3 for Rsc6, we examined the interaction of the mutant protein with Rsc6 in the two-hybrid system. Interaction between LexA–Rsc6 and GAD–Swh3Δs16 was decreased 27-fold compared with GAD–Swh3 (Fig. 4A). We also observed a 6.5-fold decrease in the interaction between GAD–Rsc6 and LexA–Swh3Δs16 compared with LexA–Swh3. Western blot analysis confirmed that the Swh3Δs16 and Swh3 fusions were expressed at comparable levels (Fig. 4B). These results suggest that the affinity of Swh3Δs16 for Rsc6 is decreased compared with that of the wild-type Swh3.

**Swh3 binds to Rsc6 in vitro**

To obtain biochemical evidence for direct interaction between Swh3 and Rsc6, we tested bacterially produced proteins in an in vitro binding assay. Swh3 was expressed as a fusion protein to GST (GST–Swh3) and RSC6 as a fusion to TRX (TRX–Rsc6). GST–Swh3 protein was immobilized on glutathione–agarose beads and incubated with an E.coli extract expressing TRX–Rsc6. After extensive washing, bound proteins were analyzed by western blotting using an antibody against TRX. TRX–Rsc6 after extensive washing, bound proteins were analyzed by western blotting using an antibody against TRX. TRX–Rsc6 was not detected by anti-LexA and GAD fusions with anti-HA (GAD fusions made in pACTII contain an HA epitope in-frame with GAD). Size markers are in kDa.

**DISCUSSION**

We present genetic and biochemical evidence for a direct interaction between Rsc6 and Swh3, two proteins of the RSC complex that are conserved in the yeast SWI/SNF and human BAF complexes. First, we identified the RSC6 gene as a multicopy suppressor of the temperature sensitivity of the swh3-ts16 allele and we showed that multicopy RSC6 also suppresses the conditional phenotype of the C-terminal truncation swh3ΔC. Furthermore, we showed that Rsc6 and Swh3 interact in the two-hybrid system and that the ts16 mutation impairs this interaction. Finally, bacterially produced Swh3 and Rsc6 protein interact in vitro, supporting the genetic evidence for direct interaction between Swh3 and Rsc6 in vivo.

Previous studies have identified other interactions between proteins of the RSC complex. We reported two-hybrid evidence that the C-terminus of Swh3 interacts with a region of Sh1, termed domain 2, that is conserved in Drosophila and human Snf2 homologs (22,30,31) and that Swh3 has at least one additional interaction with Sh1 (20). The conditional phenotype caused by C-terminal truncation of Swh3 may reflect loss of an interaction and overexpression of RSC6 may suppress this defect by stabilizing the association of Swh3ΔC with the complex; Rsc6 interacts with Swh3ΔC in the two-hybrid system (I.Treich, unpublished results). We have also presented evidence that Swh3 self-associates and that the leucine zipper is required (20). These findings suggest that the RSC complex contains two Sh3 proteins, which is consistent with evidence that human SWI/SNF-related complexes each contain two different Sh3 homologs (BAF155 and BAF170) (36). Finally, both genetic and biochemical studies indicate that Sh1 also interacts with Sh1, the Snf5 homolog of the RSC complex (21). These four proteins, which are known to interact with one another in the RSC complex, are...
all conserved in other SWI/SNF-related complexes (Table 1). Evidence suggests that protein interactions are also conserved. Swi3 interacts with Sfn2 of the SWI/SNF complex (8) and domain 2 of the Drosophila brm protein is required for assembly or stability of the BRM complex, suggesting that domain 2 interacts with another subunit (48).

Thus, the SWI/SNF-related complexes contain a set of conserved components, including homologs of the SWI/SNF proteins Sfn2, Swi3, Sfn5 and Swp73, and members of this set of conserved proteins interact with one another. We propose that this set of proteins constitutes a structural core for the SWI/SNF-related complexes (Fig. 6). Other components may also prove to be common to all complexes and part of this core. However, at least some proteins are specific to certain complexes; for example, no homologs of the Swi/Snf proteins Swi1, Sfn6 and Sfn11 are present in the yeast genome. Such proteins may be associated with the complex by interactions with components of the core; for example, in the SWI/SNF complex, Sfn11 interacts directly with a region in the N-terminus of Sfn2 that is conserved in brm, hbrm and BRG1 but not in Sth1 (8). Thus, the core may serve as a scaffold for the assembly of distinct SWI/SNF-related complexes.

ACKNOWLEDGEMENT

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REFERENCES


Figure 5. Binding of Rsc6 to Swh3 in vitro. GST (lanes 1 and 2) or GST–Swh3 (lanes 3 and 4) (8 µg) was immobilized on glutathione–Sepharose 4B beads (Pharmacia) and incubated with protein extracts (600 µg) from E.coli expressing TRX–Rsc6 (lanes 2 and 4) or TRX (lanes 1 and 3). After washing, beads were boiled in sample buffer. Half of each sample of bound proteins was electrophoresed on a 10% SDS–polyacrylamide gel and analyzed by western blotting with an antibody against TRX (Santa Cruz Biotechnology Inc.). Extracts (18 µg) used for binding experiments (Input) were loaded in lanes 5 and 6. The filled and open arrows mark positions of TRX–Rsc6 and TRX respectively. In lane 6, the protein detected at the position of TRX is probably a degradation product of TRX–RSC6. Protein markers are in kDa.

Figure 6. A model for the structural core of RSC complexes. Represented here is a summary of the observed interactions (black boxes) between proteins from the RSC complex. The two Swh3 proteins may make different contacts with Sth1; one of the interactions involves the Swh3 C-terminus. All the indicated interactions have been shown in the two-hybrid system. Interactions between Sth1 and Sfh1 and between Swh3 and Rsc6 have been confirmed in vitro. Finally, we have shown here that RSC6 is a multicopy suppressor of conditional swh3 alleles.


