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RSC, an Essential, Abundant Chromatin-Remodeling Complex

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Summary

A novel 15-subunit complex with the capacity to remodel the structure of chromatin, termed RSC, has been isolated from S. cerevisiae on the basis of homology to the SWI/SNF complex. At least three RSC subunits are related to SWI/SNF polypeptides: Sth1p, Rsc6p, and Rsc8p are significantly similar to Swi2/Snf2p, Swp73p, and Swi3p, respectively, and were identified by mass spectrometric and sequence analysis of peptide fragments. Like SWI/SNF, RSC exhibits a DNA-dependent ATPase activity stimulated by both free and nucleosomal DNA and a capacity to perturb nucleosome structure. RSC is, however, at least 10-fold more abundant than SWI/SNF complex and is essential for mitotic growth. Contrary to a report for SWI/SNF complex, no association of RSC (nor of SWI/SNF complex) with RNA polymerase II holoenzyme was detected.

Introduction

Nucleosomes, repeat units of chromatin structure, repress transcription (reviewed in Grunstein, 1990; Kornberg and Lorch, 1992). Remodeling of chromatin, possibly involving removal or repositioning of nucleosomes, accompanies transcriptional activation (Almer et al., 1986; Fascher et al., 1990; Hirschhorn et al., 1992). Recent studies suggest that the remodeling process is mediated by special multiprotein complexes that function at diverse promoters.

Components of one such complex, SWI/SNF, were revealed by genetic screens in yeast for mutants defective in mating-type switching (*swi* mutants) and for mutants defective in sucrose fermentation (*snf* mutants) (Neigeborn and Carlson, 1984; Stern et al., 1984; Breeden and Nasmyth, 1987). The five proteins revealed in this way—Swi1/Adr6p, Swi2/Snf2p, Swi3p, Snf5p, and Snf6p—all are required for proper transcriptional control of the same promoters (Estruch and Carlson, 1990; Laurent et al., 1990; Happel et al., 1991; Laurent et al., 1991; Peterson and Herskowitz, 1992; Treich et al., 1995). Suppressors of *swi* and *snf* mutations include mutations in genes encoding histones, suggesting that Swi and Snf proteins oppose repression by nucleosomes (Hirschhorn et al., 1992; Winston and Carlson, 1992; Kruger et al., 1995). Furthermore, *swi* and *snf* mutants are defective in the remodeling of chromatin at the *SUC2* promoter (Hirschhorn et al., 1992). Interaction of the five Swi and Snf proteins was suggested by their functional interdependence (Laurent et al., 1991), and was confirmed by their purification as a stable complex, including six additional polypeptides, termed Swp82p, Swp73p, Swp61p, Swp59p, Tfg3/Anc1p, and Snf11p. (Cairns et al., 1994; Côté et al., 1994; Peterson et al., 1994; Treich et al., 1995). It has been shown that mutations in *SWP73* confer Snf phenotypes, whereas mutations in *TFG3/ANC1* or *SNF11* do not (Treich et al., 1995; Cairns et al., 1996a, 1996b). The Swi2/Snf2p sequence includes a DNA-dependent ATPase motif, and both yeast SWI/SNF complex and recombinant Snf2/Swi2p possess such an activity (Laurent et al., 1993; Cairns et al., 1994; Côté et al., 1994). Purified yeast SWI/SNF complex perturbs nucleosome structure in an ATP-dependent manner, supporting conclusions from genetic studies for a role in the remodeling of chromatin (Côté et al., 1994).

A complex derived from human cells, hSWI/SNF, contains homologous subunits and displays biochemical properties similar to those of yeast SWI/SNF complex (Imbalzano et al., 1994; Kwon et al., 1994; Wang et al., 1996a, 1996b). Two related complexes have been identified in Drosophila: brm complex, which contains the Snf2/Swi2p homolog brm (Tamkun et al., 1992; Dingwall et al., 1995), and NURF complex, which consists of four proteins, including the Snf2/Swi2p homolog Iswi (Tsukiyama and Wu, 1995; Tsukiyama et al., 1995). NURF was detected by its capacity, with GAGA factor, to create a nuclease-hypersensitive site in an array of nucleosomes assembled in vitro (Tsukiyama et al., 1994; Tsukiyama and Wu, 1995).

Little is known about the number or identities of the genes regulated by either the human- or Drosophila-derived complexes. Two observations, however, suggest that yeast SWI/SNF acts on a limited set of targets: no characterized member of the complex is encoded by a gene essential for mitotic growth, and the remodeling of chromatin at certain promoters still occurs in *swi/snf* mutants. We report here the discovery of a novel yeast chromatin-remodeling complex that may play a wider role.

Results

Four Components of SWI/SNF Complex Have Single, Essential Homologs in S. cerevisiae

A homolog of the Snf2/Swi2p component of the SWI/SNF complex, termed Sth1/Nps1p, was isolated previously on the basis of its homology to Snf2/Swi2p (Laurent et al., 1992, Tsuchiya et al., 1992). The two

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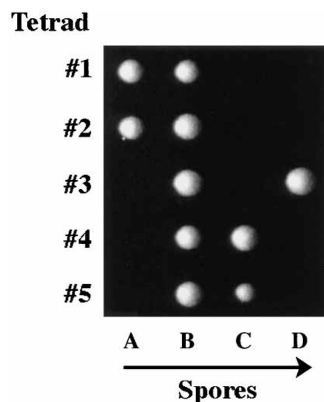


Figure 1. *RSC6/YCR052w* is Essential for Mitotic Growth

The heterozygous *ycr052w*Δ*::LEU2/YCR052w* diploid strain YBC310 (W303 genetic background) was sporulated, and dissections were performed on 16 tetrads. Five tetrads are shown, with the four spores (A–D) from each tetrad in a horizontal row.

proteins are signficantly similar along their entire length (basic local alignment search technique for protein sequences [BLASTP] value: <1 $\times$ 10^{-300}), and no other protein so closely related to either of them is found in the entire yeast genome. In contrast to the *SNF2/SWI2* gene, which is not essential, the *STH1/NPS1* gene is required for mitotic growth in both the S288C and the W303 genetic backgrounds (Laurent et al., 1992).

We recently identified YCR052w as the only open reading frame (ORF) in Saccharomyces cerevisiae similar to the Swp73p component of SWI/SNF complex (Cairns et al., 1996b). Swp73p and Ycr052p show extensive similarity, including many colinear regions (BLASTP value: 5.2×10^{-94}). To test the consequences of chromosomal deletion of *YCR052w*, we replaced the coding region of one allele of *YCR052w* with the promoter and coding region of the *LEU2* gene in the diploid strain W303. Following sporulation and tetrad dissection, viability segregated 2:2, and all viable spores from 16 tetrads analyzed were Leu$^-$. All inviable spores germinated and divided three or four times, showing that *YCR052w* is essential for mitotic growth (Figure 1). Swp73p and Ycr052p are not functionally redundant, because *swp73*Δ cells harboring *YCR052w* on a high copy (2μ origin) plasmid still display Ts$^-$ and Snf$^-$ phenotypes, and tetrads dissected from sporulated *ycr052w*Δ*::LEU2/YCR052w* heterozygous diploids harboring *SWP73* on a high copy (2μ origin) plasmid still show 2:2 segregation for viability.

Searches of the yeast database with the sequence of Swi3p disclosed the highly related ORF YFR037C. The deduced amino acid sequences of Swi3p and Yfr037p are 30% identical and 52% similar (BLASTP value: 3.8 $\times$ 10^{-56}). Others have shown that *YFR037c* is essential for mitotic growth (I. Treich and M. Carlson, personal communication). Searches of the yeast database with the sequence of Snf5p yielded the highly related ORF L8543.4, which we refer to as SFH1 (SNF5 homolog). The Sfh1p sequence shows greater similarity to the human Snf5p homolog Ini1 (BLASTP value: 3.6 $\times$ 10^{-31}) (Kalpana et al., 1994; Muchardt et al., 1995) and the Drosophila Snf5p homolog Snr1p (BLASTP value: 5.8 $\times$ 10^{-25}) (Dingwall et al., 1995) than it does to the yeast Snf5p itself (BLASTP value: 4.5 $\times$ 10^{-10}). The *SFH1* gene is also essential for mitotic growth (Y. Cao and B. L., unpublished data). Finally, we recently reported that searches of the yeast database with the sequence of Tfg3/Anc1p yielded the highly related ORF YOR50–3/SC33KB_3 (BLASTP value: 7.2 $\times$ 10^{-43}) (Cairns et al., 1996a). The phenotypes conferred by chromosomal deletion of *YOR50–3/SC33KB_3*, have not been determined. BLAST searches of the yeast database with the protein sequences of Adr6/Swi1p, Snf6p, and Snf11p failed to reveal significantly similar proteins. Putative homologs therefore exist for five of the eight previously characterized members of SWI/SNF complex, and at least four of these five homologs are essential for mitotic growth. Homologs of Snf2/Swi2, Swp73p, Swi3p, and Snf5p are components of mammalian SWI/SNF complexes (Khavari et al., 1993; Muchardt and Yaniv, 1993; Kwon et al., 1994; Wang et al., 1996a, 1996b). Together, these findings reveal counterparts of the four essential yeast SWI/SNF homologs in human cells and raise the possibility that all SWI/SNF-related complexes contain a conserved "core" of related proteins (see Discussion).

Cofractionation of Sth1 and Ycr052w/Rsc6 Proteins

The association of all previously described SWI/SNF proteins in a complex led us to inquire whether the Swi/Snfp homologs associate with one another as well. To this end, the fractionation of a yeast extract was monitored by immunoblot analyses with polyclonal anti-Sth1p and anti-Ycr052/Rsc6p antibodies. Proteins of apparent (expected) masses of 160 (157) and 64 (54) kDa were recognized by the anti-Sth1p and anti-Ycr052/Rsc6p antibodies, respectively (Figures 2B and 2D). These proteins cofractionated on Bio-Rex 70, DEAE-Sephacel, hydroxylapatite, Mono Q, TSK-heparin, Mono S, and gel filtration. Sth1p contains a region highly similar to known DNA-dependent ATPases, and such an ATPase activity coeluted with Sth1p and Ycr052/Rsc6p immunoreactivity (Figures 2A and 2C). SDS-PAGE of the Mono S eluate, followed by staining with Coomassie blue, revealed 14 polypeptides that cofractionate with the immunoreactive proteins (Figure 3A). A 15th polypeptide, with an apparent mass of 90 kDa, was nearly coincident, eluting on the leading side of the peak. When Mono S eluate with (fraction 38) or without (fraction 34) the 90 kDa polypeptide was subjected to gel filtration (at high ionic strength to minimize nonspecific interactions; see Experimental Procedures), all 14 (15 with fraction 38) polypeptides comigrated, indicative of their association in a complex (Figures 3B and 3C and data not shown).

To confirm further the stability of RSC complex, immunoprecipitations were performed with anti-Rsc6 antibodies and the peak Mono Q fraction. Immunoblot experiments revealed that the immune complexes formed could be washed extensively with a buffer containing 600 mM potassium acetate without loss of Sth1p (data not shown). Neither anti-Rsc6p nor anti-Sth1p antibodies were able to immunodeplete RSC, however, presumably owing to the small portions of each protein to which each antibody was raised and to the masking of their epitopes by other proteins in the complex. Comparison with molecular weight markers indicated that the mass

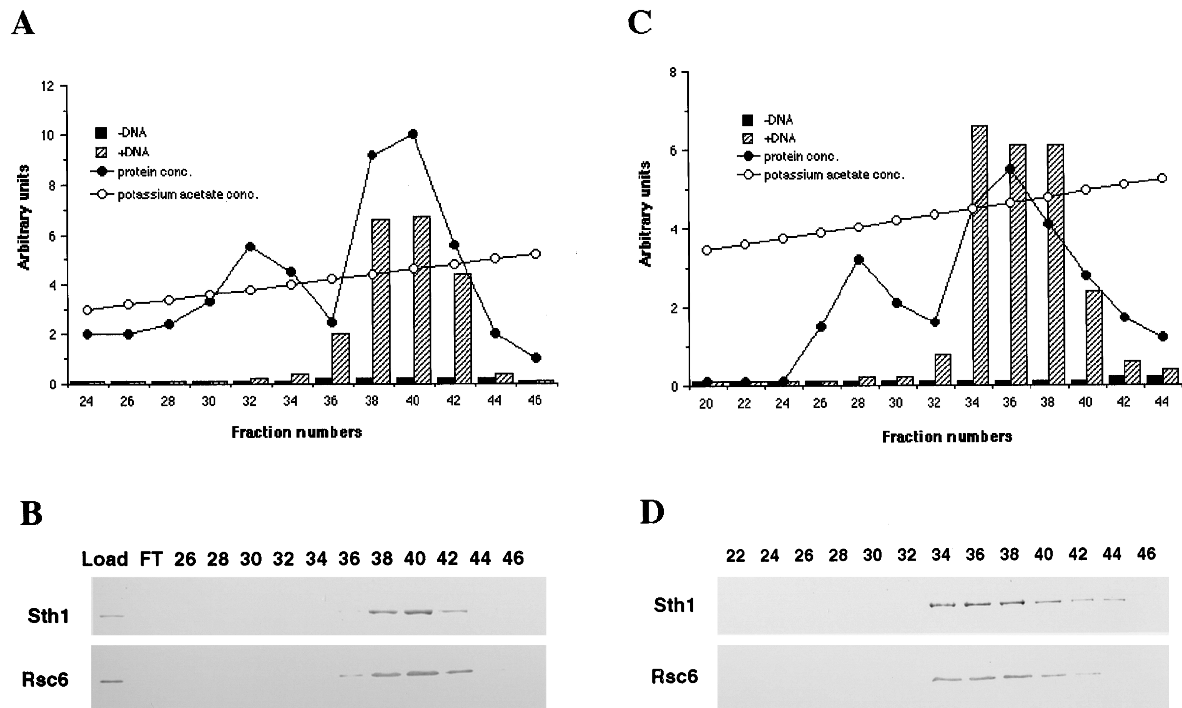


Figure 2. Anti-Sth1p and anti-Rsc6/Ycr052p Immunoreactivity Cofractionate with a Potent DNA-Dependent ATPase Activity

Yeast extracts were fractionated on Bio-Rex 70, DEAE Sephacel, hydroxylapatite, and Mono Q. Peak fractions were further resolved on TSK-heparin and Mono S. ATPase activity was assayed in the absence (closed bars) or presence (hatched bars) of 20 ng/μl double-stranded plasmid DNA. One arbitrary unit corresponds to the hydrolysis of 1 nmol of ATP per microliter of fraction per minute. Protein concentration is given in multiples of 0.1 mg/ml (closed circles), and potassium acetate concentration is given in multiples of 100 mM (open circles).

(A) DNA-dependent ATPase assays with TSK-heparin fractions. Peak fractions from Mono Q were further resolved on TSK-heparin. Adsorbed proteins were eluted in buffer B with a linear gradient of 200–800 mM potassium acetate.

(B) Sth1p and Ycr052/Rsc6p cofractionate on TSK-heparin. Fractions from TSK-heparin (2.5 μg per lane) were separated in an SDS–10% acrylamide gel and immunoblotted with anti-Sth1p or anti-Ycr052/Rsc6p antisera. FT, flow-through.

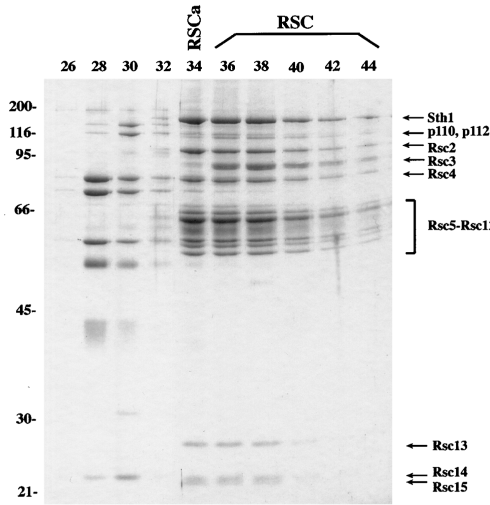
(C) DNA-dependent ATPase assays with Mono S fractions. Peak fractions from TSK-heparin were further resolved on Mono S. Adsorbed proteins were eluted in buffer B with a linear gradient of 100–800 mM potassium acetate.

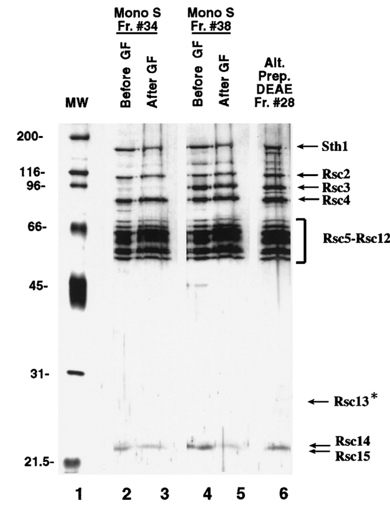
(D) Sth1p and Ycr052/Rsc6p cofractionate on Mono S. Fractions from Mono S (2.0 μg per lane) were separated in an SDS–10% acrylamide gel and immunoblotted with anti-Sth1p or anti-Ycr052/Rsc6p antisera.

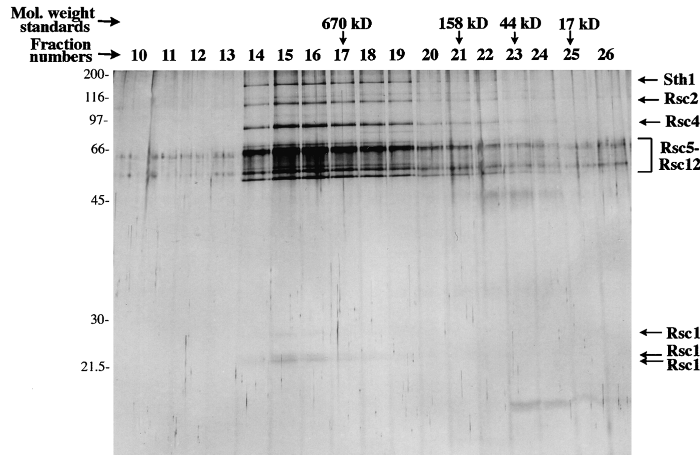
of the complex was ~1 MDa. Two substoichiometric polypeptides of 110 and 112 kDa may represent modified forms of other components of the complex or additional proteins stably associated in substoichiometric amounts. We refer to the complexes containing or lacking the 90 kDa component as RSC or RSCa, respectively, because (as shown below) they have the capacity to remodel the structure of chromatin. Several independent preparations have yielded an identical spectrum of major polypeptides (Figure 3C, lane 6), and the partial resolution of RSC from RSCa was always observed. Purification of RSC to homogeneity required 2,400-fold purification, whereas SWI/SNF has required >25,000-fold purification (Cairns et al., 1994; Côté et al., 1994) demonstrating that RSC (with RSCa) is at least 10-fold more abundant than SWI/SNF complex (Table 1). Starting after the largest component of the complex, Sth1p, the polypeptides are termed Rsc2p through Rsc15p, in order of decreasing mass. Superimposition of immunoblots with stained gels indicates that *YCR052w* encodes Rsc6p. The Rsc13p component stains poorly with silver (Figures 3B and 3C) but is easily detected with Coomassie blue (Figure 3A). The 90 kDa polypeptide that distinguishes RSC from RSCa is Rsc3p. Silver staining of an SDS–15% acrylamide gel clearly resolves Rsc14p and Rsc15p into two distinct protein bands (data not shown).

RSC Proteins Similar to Three SWI/SNF Components Identified by Peptide Sequencing and Mass Spectrometry

RSC proteins were identified by mass spectrometric and limited sequence analysis, with reference to the recently completed yeast genome sequence (Table 2). The proteins were resolved by SDS-PAGE, transferred to a membrane, and digested with trypsin. The resulting peptides were fractionated by microbore reversed-phase high performance liquid chromatography (RP HPLC) and analyzed by MALDI-TOF mass spectrometry and limited Edman sequencing. The identification of a protein in a band from SDS-PAGE has often been established by sequencing multiple tryptic peptides. Here, we identified RSC proteins by "mass fingerprinting": determining the experimental masses of multiple tryptic peptides and then querying the yeast SGD database with these masses and the algorithm PeptideSearch (M. Mann,







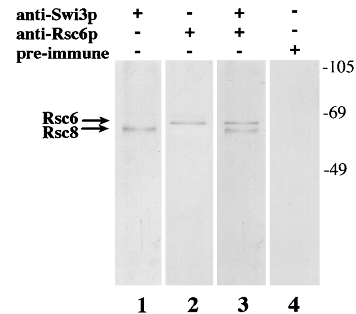


Figure 3. Mono S and Gel Filtration Chromatography Reveal Two Complexes Composed of 15 (RSC) or 14 (RSCa) Polypeptides

(A) Peak fractions from TSK-heparin were further resolved on Mono S. Fractions (5 μg) were separated on an SDS–12% acrylamide gel and revealed by staining with Coomassie blue. The fractions composed primarily of RSC (which contains Rsc3p) or RSCa (which lacks Rsc3p) are indicated at top. The positions and masses of molecular weight protein standards, run in another section of the gel, are indicated at left.

(B) All 14 polypeptides of RSCa comigrate during gel filtration chromatography. Mono S fraction 34 (11 μg) was applied to an 8 ml Bio-Sil gel filtration column equilibrated with a mobile phase containing 800 mM potassium acetate. Peak fractions eluted at an apparent size of 1 MDa, as estimated by the elution profile of molecular weight standards. Aliquots (50 μl, ~200 ng) of the eluate were concentrated by precipitation with 10% trichloroacetic acid and loaded on an SDS–10% acrylamide gel, and proteins were revealed by staining with silver. The positions and masses of the molecular weight protein standards, run in another section of the gel, are indicated at left.

(C) RSC contains and RSCa lacks the 90 kDa polypeptide Rsc3p, as shown by SDS-PAGE and silver stain analysis of the 1 MDa peak fractions from gel filtration of RSC or RSCa. Mono S fraction 38 (11 μg) was chromatographed on a Bio-Sil column as described in (B). Peak fractions eluted at an apparent size of 1 MDa, as estimated by the elution profile of molecular weight standards. Mono S fractions (fraction 34, 1 μg, lane 2; fraction 38, 1 μg, lane 4), the peak gel filtration fractions (~1 μg, lanes 3 and 5) concentrated by trichloroacetic acid precipitation, and the peak DEAE-Sephacel fraction from an independent preparation of RSC complex (1 μg, lane 6) were loaded on an SDS–10% acrylamide gel, and proteins were revealed by staining with silver. The masses of the molecular weight protein standards (lane 1) are indicated at left. Asterisk indicates the position of Rsc13p, which does not stain well with silver.

(D) Rsc8p cross-reacts with anti-Swi3p antibodies. Pure RSC (Mono S fraction 36, 1 μg) was separated in three lanes of an SDS–8% acrylamide gel and immunoblotted with anti-Swi3p (lanes 1 and 3), anti-Rsc6p (lanes 2 and 3), or serum aquired before immunization with recombinant Swi3p (preimmune, lane 4). The immunoblot used with anti-Swi3p (lane 1) was probed sequentially with anti-Rsc6p as well (lane 3) to allow a direct comparison of the migration of the immunoreactive proteins. The positions and masses of prestained molecular weight markers, run in another section of the gel, are indicated at right.

Table 1. Purification of RSC

Fraction	Protein (mg)	Total Activity[a] (mmol/hr)	Specific Activity (activity/mg protein)	Yield[b] (%)	Fold Purification[c]	
					Column	Total
Crude extract	30,000	ND	ND	(100)	—	—
Bio-Rex 70	3,600	ND	ND	80	6.7	6.7
DEAE-Sephacel	425	ND	ND	64	6.8	46
Hydroxylapatite	55	ND	ND	47	5.8	267
Mono Q	8.1	ND	ND	31	4.5	1,195
TSK-heparin	4.8	42.5	8.9	24	1.3	1,512
Mono S	1.44	19.8	14	11	1.6	2,387

Includes the contributions of RSC and RSCa.
[a] Activity was determined by DNA-dependent ATPase assays and is reported as millimoles of ATP hydrolyzed per hour.
[b] Yield of activity recovered is reported as percentage of total activity. Prior to TSK-heparin, yields are reported as the percentage immunoreactivity recovered. Yields from TSK-heparin and Mono S are reported as the percentage of total DNA-dependent ATPase activity recovered.
[c] The fold purification by column is the product of the percentage protein recovered and the yield from each column.
The total fold purification is the product of the fold purification from each step.
ND, not determined owing to the presence of other DNA-dependent ATPases in less-purified fractions.

EMBL), which identifies proteins with matching mass fingerprints. In each case, unique proteins were identified by mass fingerprinting alone and their identity then confirmed by Edman sequencing of one or two peptides (see Experimental Procedures). Mass fingerprinting with 10 of 12 selected peptides from the larger (~160 kDa) anti-Sth1p immunoreactive protein matched the fingerprint of only one protein in the SGD database, Sth1p (Table 2); this finding was confirmed by Edman sequencing of two peptides LIESETNRDDDDKAELDDDELNDTLAR and IFLDKIDKER, which correspond to residues 961–987 and 993–1002, respectively, of Sth1p. Likewise, the experimental masses of 6 of 8 peptides derived from the smaller (~64 kDa) anti-Ycr052/Rsc6p immunoreactive protein matched only Rsc6/Ycr052p, and the sequence of a single peptide, YQFFHELSLHPR, mapped to residues 416–427 of Rsc6/Ycr052p. We conclude that the immunoreactive proteins were indeed Sth1p and Rsc6p and refer to *YCR052w* hereafter as *RSC6*. Immunoblot analyses demonstrated that Rsc6p is not detected in purified SWI/SNF, nor is Swp73p detected in purified RSC (data not shown). Likewise, purified RSC lacks detectable Snf2/Swi2p, and purified SWI/SNF lacks detectable Sth1p.

Seven of 11 peptides from Rsc8p were perfectly positioned, by mass, in the protein encoded by the yeast ORF YFR037w, and one peptide from Rsc8p had the sequence NVYDSAQDFNALQDESR, corresponding to residues 233–249. The apparent mass of Rsc8p, 62 kDa, is consistent with the mass of 64 kDa expected for the *YFR037c* gene product. As mentioned above, BLAST database searches identified Rsc8p as the yeast protein most similar to Swi3p (BLASTP value: 3.8×10^{-56}). To confirm further that Rsc8p is encoded by *YFR037c*, we tested whether our anti-Swi3p antibodies would cross-react with Rsc8p. Immunoblot analysis with anti-Swi3p antibodies revealed a strong cross-reactivity with a 62 kDa protein in purified RSC that was not observed with preimmune serum (Figure 3D). We conclude that *YFR037c* encodes Rsc8p and refer to *YFR037c* hereafter as *RSC8*. In addition, the immunoreactive 62 kDa protein was not observed in purified SWI/SNF, nor was an immunoreactive protein of the apparent mass of Swi3p (115 kDa) found in purified RSC, suggesting that these components are not shared between the two related complexes. Swi3p (825 amino acids) is significantly larger than Rsc8p (557 amino acids), primarily because of an acidic amino-terminal region that is much larger in Swi3p than in Rsc8p. The similarity between Swi3p and Rsc8p also extends to the human Swi3p homologs Baf155p and Baf170p (BLASTP value: 1×10^{-66}) (Wang et al.,

Table 2. Independent Identification of RSC Proteins by Peptide Sequencing and Mass Spectrometric Analysis

RSC protein Mr (kDa)	Identification							Accuracy		
	Mass Spectrometry			Peptide Sequencing		Conclusions		Mass Spectrometry		Peptide Sequencing
	Peptides Determined[a]	Peptides Required[b]	Loci Identified	Peptides	Loci Identified	Locus	Predicted Mr (kDa)	Sequence Covered (%)[c]	ΔDa% Average[d]	Sequence Identity (Total aa's Matched)
160	12	10	1	2	1	STH1	157	11	0.013	37/37
64	8	6	1	1	1	RSC6	54	21	0.015	12/12
60	11	7	1	1	1	RSC8	64	27	0.021	17/17

RSC proteins were separated by SDS-PAGE, digested with trypsin, and peptides fractionated by microbore RP-HPLC. To identify RSC proteins by mass spectrometry, peptide masses were determined by MALDI-TOF mass spectrometry and used to search a nonredundant database with the PeptideSearch algorithm.
[a] The number of experimental peptide masses that were obtained.
[b] The number of matching masses that were required to identify a unique yeast protein with the PeptideSearch algorithm.
[c] Masses were fitted to peptides in the identified proteins. Shown is the percentage of the entire protein sequence that is covered by nonoverlapping fitted peptides.
[d] The difference (ΔDa) between the mean of the average isotopic masses of the theoretical peptides and the experimentally determined masses averaged for all peptides (ΔDa% average).
aa, amino acid.

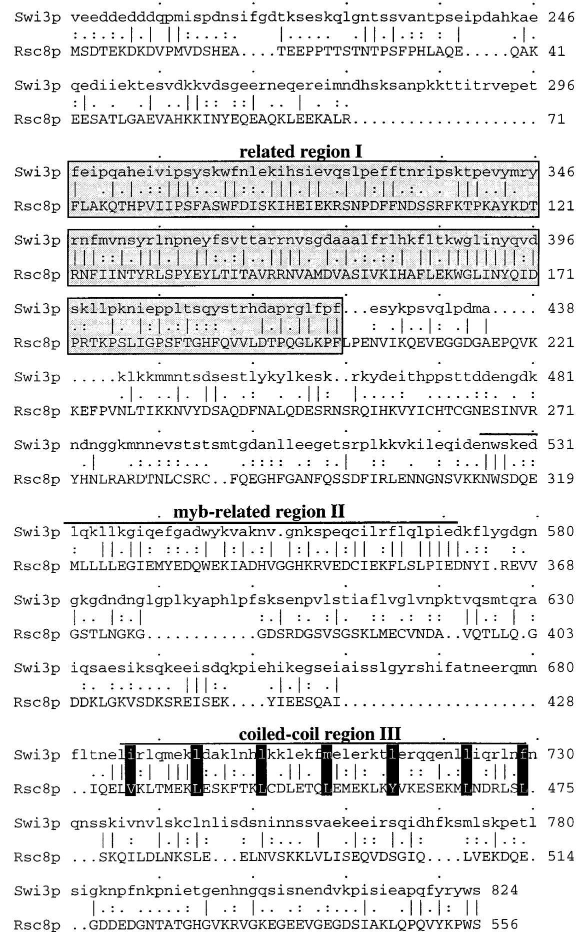


Figure 4. Rsc8p Is Similar to Swi3p and Contains a Possible Coiled-Coil Region and a Region Similar to the Mammalian Protein myb

Protein sequences of Swi3p and Rsc8p were aligned with the program BESTFIT. The nonconserved amino-terminal 196 amino acids of Swi3p are not shown. Three regions of significant similarity are highlighted: region I; a highly similar, uninterrupted section of 79 amino acids (shaded boxes); region II, a section of 47 amino acids that are similar to the proposed helix-turn-helix-turn-helix DNA-binding domain of the protein myb (Wang et al., 1996b); and region III, a possible coiled-coil region, with the putative interacting hydrophobic residues (separated by six amino acids) (shown in white on a black background) (Peterson and Herskowitz, 1992). The amino acid positions are shown at right.

1996b). Conserved regions between the two yeast proteins (Figure 4) include a highly related amino-terminal region (region I); a central domain with homology to the DNA-binding domain of the mammalian protein myb (region II) (Wang et al., 1996b); and a putative coiled-coil region (region III, which lacks prolines and glycines and is consistent with an α-helical structure) located near the carboxyl terminus (Peterson and Herskowitz, 1992). This myb-related domain may not confer a DNA-binding activity, however, because DNA cross-linking studies with yeast SWI/SNF complex did not reveal Swi3p (Quinn et al., 1996).

RSC-Associated DNA-Dependent ATPase Activity

The regions of homology between Sth1p and Snf2/Swi2p include a DNA-dependent ATPase domain, and

Table 3. Nucleic Acid and Nucleotide Specificities of the Nucleotide Triphosphatase

Cofactor	Percentage optimal activity: RSC	Percentage optimal activity: RSCa
Nucleic acid (20 ng/μl)		
None	<2	<2
Nucleosome cores	82	77
Plasmid (BSCR)	100	100
λ	109	105
ds oligo (44 bp)	48	56
poly dG·poly dC	118	127
poly dI·poly dC	98	95
ss M13mp18	83	79
ss oligo (17 nt)	2	2
ss oligo (26 nt)	10	12
ss oligo (35 nt)	32	35
ss oligo (44 nt)	42	51
Nucleotide (1 mM)		
ATP	100	100
dATP	117	105
GTP	12	7
dGTP	<2	<2
CTP	<2	<2
dCTP	<2	<2
UTP	12	11
dTTP	<2	<2

nt, nucleotides.

such an ATPase activity cofractionated with RSC on TSK-heparin, Mono S, and gel filtration columns (Figures 2A and 2C and data not shown). The DNA-dependent ATPase activities of purified RSC and RSCa (Mono S fractions 38 and 34, respectively) were virtually indistinguishable and exhibited the following notable characteristics (Table 3 and Figure 5): stimulation by both single-stranded and double-stranded DNA; no significant preference for nucleosomal DNA; requirement for DNA longer than ~25 bp; specificity for adenine nucleotides; optimal temperature of 37°C–40°C; inhibition by chloride ion at 100 mM (but not by acetate ion at up to 200 mM); and requirement for a divalent cation. The Michaelis constant (K_m) for ATP, from a double reciprocal plot, was 77 μM for RSC and 67 μM for RSCa. The K_m (in concentration of nucleotide) for plasmid DNA was 616 nM (RSC) and 542 nM (RSCa) and for single-stranded M13mp18 DNA was 310 nM (RSC) and 263 nM (RSCa). Assuming that RSC has a molecular weight of 945 kDa (the sum of the apparent masses of the polypeptides), the calculated turnover number under optimal conditions was 7.5 molecules of ATP per second.

RSC Perturbs Nucleosome Structure and Lacks Acetyltransferase Activity

Yeast SWI/SNF and related complexes isolated from higher eukaryotes perturb nucleosome structure, as shown for example by alteration of the pattern of digestion by DNase I (Côté et al., 1994; Imbalzano et al., 1994; Kwon et al., 1994; Wang et al., 1996a). RSC similarly caused a dramatic change in DNase I digestion of nucleosomal DNA (Figure 6); protection from nuclease attack, with the 10 bp periodicity of the DNA double helix (Figure 6, lane 3), was almost entirely lost, resulting in a digestion pattern closely resembling that of naked

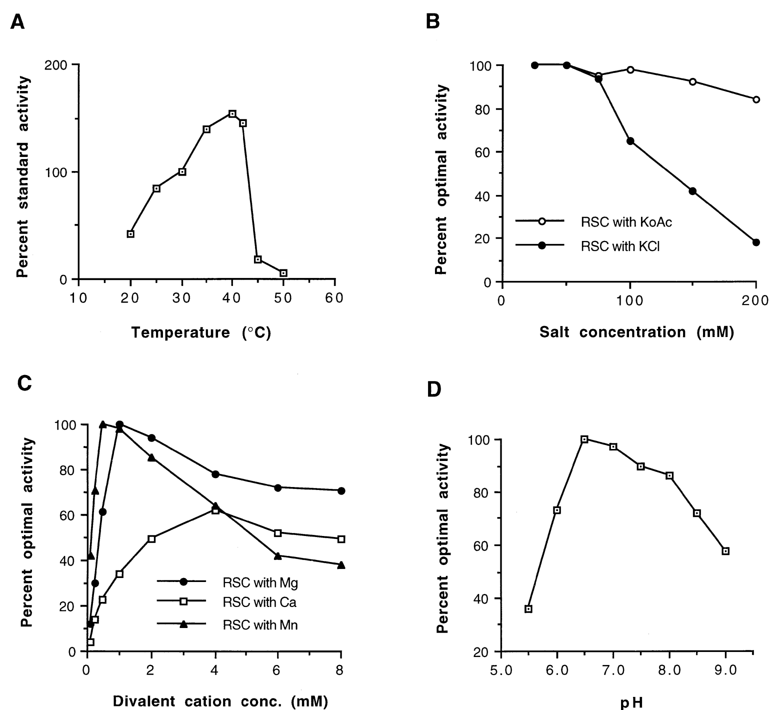


Figure 5. Properties of the RSC DNA-Dependent ATPase Activity

DNA-dependent ATPase assays were performed for 30 min at 30°C in the presence of 20 ng/μl plasmid DNA and 1 mM ATP (unless indicated otherwise). Activity was directly proportional to the amount of RSC added and linear for the duration of the assay and is reported relative to the standard assay. The properties of RSC were indistinguishable from those of RSCa (data not shown).

(A) Dependence of activity on temperature.

(B) Dependence of activity on acetate versus chloride salt.

(C) Dependence of activity on divalent cations. The counter-ion was chloride.

(D) Dependence of activity on pH.

DNA (Figure 6, lanes 5–8). This effect of RSC required an amount of RSC roughly equivalent to that of the nucleosome and was dependent on ATP (Figure 6, compare lane 4 to lane 7). The ATP analog ATPγ-S would not suffice (data not shown).

In case histone acetylation might contribute to the perturbation of nucleosome structure by RSC or SWI/SNF, the complexes were tested for acetyltransferase activities. Both purified RSC (DEAE fraction 28, Figure 3C) and SWI/SNF complex (40% pure) failed to acetylate histones in purified rat liver chromatin, whereas crude protein fractions from which the complexes were derived displayed significant histone acetyltransferase activity (data not shown). Because both RSC and SWI/SNF complexes are ATPases, an associated acetyltransferase activity might require ATPase activity. Identical results were obtained, however, when reactions contained Mg·ATP. Purified RNA polymerase II holoenzyme also failed to exhibit acetyltransferase activity.

Lack of Cofractionation of RSC and SWI/SNF Complex with RNA Polymerase II Holoenzyme

The purification of RSC reported here employs the initial chromatographic steps developed previously for resolution of RNA polymerase II transcription proteins from yeast (Sayre et al., 1992). Polymerase and general transcription factors are recovered in the same fractions from Bio-Rex 70 and DEAE-Sephacel step elutions and are then separated during gradient elution from hydroxylapatite. This procedure has been used for the isolation of polymerase II in a complex with the multiprotein mediator of transcriptional regulation, whose subunits include the products of *GAL11*, *SIN4*, *RGR1*, *SRB*, and *MED* genes (Kim et al., 1994; Koleske and Young, 1994; Li et al., 1995). Others have reported that SWI/SNF complex is a stable and stoichiometric component of RNA polymerase II holoenzyme (Wilson et al., 1996). We could find no evidence of a stable association. Rather, polymerase II holoenzyme and SWI/SNF complex formed distinct peaks on hydroxylapatite, eluting at 80–100 mM and 100–120 mM potassium phosphate, respectively (Figure 7). RSC was even better resolved from holoenzyme, eluting at 110–140 mM potassium phosphate. When fractions from hydroxylapatite containing both polymerase holoenzyme and SWI/SNF (~100 mM potassium phosphate) were fractionated on Mono Q, they again were resolved, further suggesting that these factors are not stably associated.

Discussion

We have purified and characterized RSC, an essential and abundant 15-protein complex that exhibits an ATP-dependent chromatin-remodeling activity and that includes at least three components related to members of SWI/SNF complex. Studies of the yeast SWI/SNF complex provided the first insight into the remodeling of chromatin structure in vivo and in vitro (Laurent et al., 1991; Peterson and Herskowitz, 1992; Cairns et al., 1994; Côté et al., 1994), and the isolation of a related complex from human cells points to the generality of the conclusions drawn (Imbalzano et al., 1994; Kwon et al., 1994; Wang et al., 1996a). At the same time, these studies revealed limitations on the scope of SWI/SNF action. SWI/SNF components are not required for mitotic growth; chromatin remodeling still occurs in *swi* and *snf* mutants; and SWI/SNF complex is comparatively rare, present at ~100–200 molecules per cell (based on the yield of SWI/SNF complex from our purifications and by comparison of the immunoreactivity of purified SWI/SNF with that of whole-cell extracts). RSC is free from these limitations: it includes proteins required for mitotic growth, and it is at least an order of magnitude more abundant, on the order of thousands

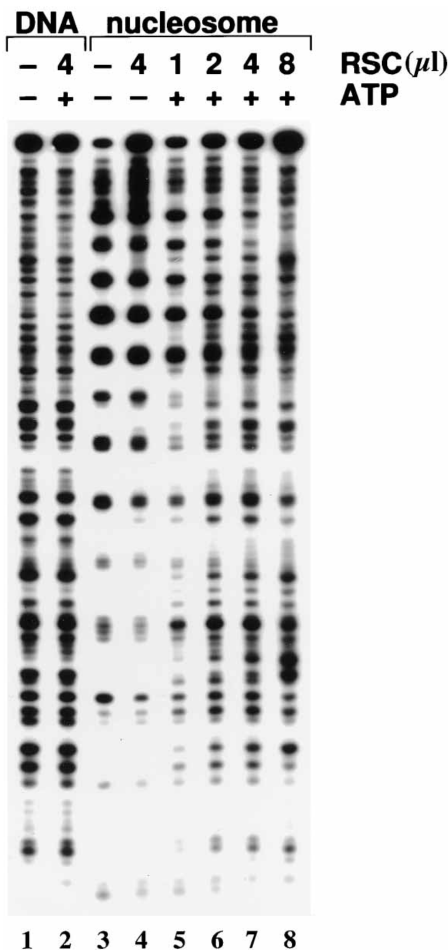


Figure 6. RSC Is an ATP-Dependent Nucleosome-Remodeling Complex

RSC uses ATP to convert the DNase protection pattern observed with a mononucleosome to one resembling naked DNA. Nucleosomes were formed on a 172 bp fragment of the sea urchin 5S RNA gene (Côté et al., 1994), labeled at the 3′-end by filling in the AvaI site. Reconstitution with rat liver histone octamers and purification by gradient centrifugation were performed as described (Lorch et al., 1992). Reaction mixtures (20 μl) contained 25 mM HEPES (pH 7.5), 5 mM $MgCl_2$, 50 mM potassium acetate, 0.125 mg/ml BSA, and 0.4 mg/ml reverse primer (17 bp oligodeoxyribonucleotide). Naked 5S DNA fragment (50 ng, lanes 1 and 2), an equivalent amount of nucleosomes (peak gradient fraction, 100 ng, lanes 3–8), purified RSC (Mono S fraction 38, diluted to 120 ng/μl, lanes 2–8), and ATP (1 mM, lanes 2, 4–8) were added. Following incubation for 15 min at 30°C, DNase I protection analysis was performed with 13 μg of DNase I for 6 s for naked DNA and 40 μg of DNase I for 40 s, followed by phenol extraction and gel electrophoresis as described (Lorch et al., 1992).

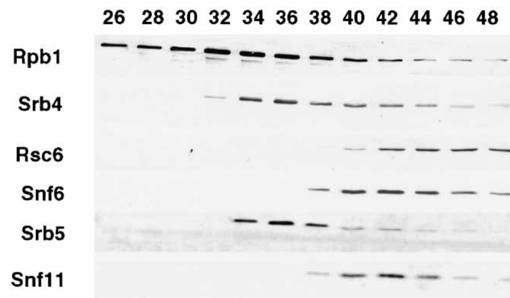


Figure 7. RSC, SWI/SNF, and RNA Polymerase II Holoenzyme Do Not Coelute

Immunoblot analysis of hydroxylapatite chromatography resolves SWI/SNF, RSC, core polymerase II, and polymerase II holoenzyme. SWI/SNF, RSC, core polymerase II (lacking mediator proteins), and polymerase II holoenzyme (containing mediator proteins) all are recovered in the same step elutions from Bio-Rex 70 and DEAE-Sephacel. The peak DEAE-Sephacel fractions were resolved further on hydroxylapatite. A shallow potassium phosphate gradient (~5 mM increase in phosphate per fraction) at 1.5 column volumes/hr was employed. Fractions (5 μg) were separated on an SDS–12% acrylamide gel, immunoblotted, and probed with antibodies to components of RNA pol II holoenzyme (Rpb1, Srb4, and Srb5), components of SWI/SNF (Snf6 and Snf11), or antibodies to Rsc6.

of molecules per cell. Although genetic experiments with viable *rsc* mutants are required to identify RSC targets, the abundance of the complex indicates that it may act at a great many yeast promoters or be involved in other cellular processes (such as DNA replication or recombination) that may require the disruption of large numbers of nucleosomes.

Despite these differences, RSC bears a striking resemblance to the SWI/SNF complex. At least three RSC subunits are homologous to SWI/SNF components. The catalytic activities of RSC and SWI/SNF are very alike and may be contrasted with those of NURF. RSC and SWI/SNF ATPase activities are stimulated by single-stranded, double-stranded, or nucleosomal DNA, whereas NURF ATPase activity is stimulated only by nucleosomal DNA (Tsukiyama and Wu, 1995). Other enzymatic properties of RSC ATPase activity detailed here are also very similar to those of SWI/SNF, except for turnover number, which is about seven times greater for RSC (7.5 s^{-1}) than for SWI/SNF (1.1 s^{-1}) under optimal conditions. By comparison, the turnover number of NURF is much lower (0.13 s^{-1}). These differences may be intrinsic, or they may reflect the percentage of active molecules recovered in the purified preparations.

RSC and SWI/SNF also appear to have similar effects on nucleosome structure, causing a marked change in the DNase I digestion pattern to very nearly that observed with naked DNA. For both RSC and SWI/SNF, such perturbation requires a 1:1 molar ratio of remodeling complex to nucleosomes (Côté et al., 1994; Kwon et al., 1994). NURF, at a level of 1 molecule per 18 nucleosomes, results in a DNase I protection pattern that resembles nucleosomal DNA much more than naked DNA but that clearly includes several distinct alterations (Tsukiyama and Wu, 1995). This cleavage pattern changes only slightly by the addition of NURF to stoichiometric levels. These differences in DNase I patterns could be due to differences in the histones or DNA of the nucleosomes used, but more likely they reflect differences in the physical state of nucleosomes perturbed by the remodeling complexes.

The functional distinction between SWI/SNF-related and NURF complexes corresponds with the classification of their ATPase components. The six chromatin-remodeling complexes so far described—yeast SWI/SNF, RSC, brm complex, NURF, and the two mammalian SWI/SNF complexes—contain the DNA-dependent ATPases Snf2/Swi2p, Sth1p, brm, Iswi, and mammalian

Brg1p (or hBrm protein), respectively (Laurent et al., 1991; Laurent et al., 1992; Tamkun et al., 1992; Khavari et al., 1993; Muchardt and Yaniv, 1993; Elfring et al., 1994; Dingwall et al., 1995; Tsukiyama et al., 1995; Wang et al., 1996a). All of these ATPases except Iswi are similar in their ATPase domains and in several additional regions, whereas their similarity to Iswi is limited to the ATPase domain alone (Eisen et al., 1995). One such region, present in the carboxyl termini of all of the ATPases except Iswi, constitutes a bromodomain (Tamkun et al., 1992) that is present in several proteins related to both chromatin and transcriptional regulation (Brownell et al., 1996). On the other hand, database searches have identified several proteins in S. cerevisiae, Caenorhabditis elegans, and human cells whose similarity to Iswi extends beyond the ATPase domain and includes two other colinear regions not found in Snf2/Swi2p (Tsukiyama et al., 1995). Thus, both functional and phylogenetic analyses have defined two subfamilies of chromatin-remodeling ATPases. These subfamilies may be further defined by the proteins that bind the nonconserved regions found outside their ATPase domains. For example, two regions found in Snf2/Swi2p (which are absent in Iswi) are involved in binding the Swi3p and Snf11p components of SWI/SNF complex (Treich et al., 1995).

Our studies, combined with those of others (Dingwall et al., 1995; Wang et al., 1996a, 1996b), suggest that all SWI/SNF-related complexes may contain a core of conserved members that includes proteins related to Snf2/Swi2p, Swp73p, Swi3p, and Snf5p. All four of these proteins are required for SWI/SNF function, and their RSC homologs Sth1p, Rsc6p, Rsc8p (I. Treich and M. Carlson, personal communication), and Sfh1p (Y. Cao and B. Laurent, unpublished data) all are essential for yeast mitotic growth. Homologs of these four components are also found in human SWI/SNF complex, suggesting that conservation of this core is important functionally (Wang et al., 1996a, 1996b), and BLAST and BESTFIT comparisons suggest that the core components of RSC are as similar or even more closely related to human SWI/SNF than to yeast SWI/SNF components. Although our peptide sequencing did not reveal Sfh1p, it may still prove to be a component of RSC.

Our studies also illustrate the value of mass spectrometry for identification of proteins, particularly when applied to biochemical studies in yeast. With the complete sequence of the yeast genome now available, mass fingerprinting enables the rapid identification of genes for proteins and eliminates the need for extensive chemical peptide sequencing. In addition, mass fingerprinting can rapidly establish whether a protein band from SDS-PAGE contains one or more polypeptides, a determination that would otherwise require extensive sequencing.

Our finding that yeast SWI/SNF complex fails to copurify with RNA polymerase II holoenzyme conflicts with the work of others (Wilson et al., 1996), and we show a lack of copurification of RSC with holoenzyme as well. The discrepancy is unexpected since we and the others used the same procedures, originally developed for fractionation of general transcription factors from yeast (Sayre et al., 1992). We suspect that the difference lies in the details of chromatography on hydroxylapatite. We found previously that resolution of transcription proteins on hydroxylapatite depended critically on the rate and slope of gradient elution, with better results obtained on slower, shallower elution (M. H. Sayre et al., unpublished data). It remains for further work to determine whether this or another explanation applies and to test definitively the possibility of SWI/SNF complex–RNA polymerase II holoenzyme interaction.

Many fundamental questions remain concerning the function of RSC and other SWI/SNF-related complexes, including their scope of action, their regulation, and the mechanisms underlying their remodeling activities. In contrast to SWI/SNF, RSC is readily purified in milligram quantities, a feature that should facilitate mechanistic analyses. Genetic studies, which have revealed many of the important functions of SWI/SNF complex, can easily be extended to RSC. Perhaps the most pressing issue to be addressed by genetic studies is the question of whether RSC plays a role in transcription or in other cellular processes, such as replication, recombination, and repair, which also require the remodeling of chromatin. Because RSC is both essential and abundant, it is likely to perform functions distinct from SWI/SNF. The two types of human SWI/SNF complex (containing either Brg1 or hBrm) are functionally distinguishable (Muchardt and Yaniv, 1993), and studies of RSC and SWI/SNF in yeast may illuminate such specialization of chromatin-remodeling complexes.

Experimental Procedures

Plasmids

The *RSC6* (*YCR052w*) gene was cloned from genomic DNA of the haploid yeast strain W303–1A by the polymerase chain reaction (PCR), using Taq polymerase and the following oligonucleotide primers: BC52w5 5′-CCCTCTAGAAGCCGATGAGGCCACTCTG CAACGCA-3′ and BC52w3 5′-CCCTCTAGAAGAGTGGCTTGGTC CACTTGTGGAATA-3′. The resulting 2.2 kbp amplified DNA product begins 427 bp upstream of the *RSC6* start codon and ends 397 bp downstream of the termination codon. This PCR product was digested with XbaI and ligated to XbaI-digested pRS316 (*URA3*, *CEN6*, and *ARS4*) (Sikorski and Hieter, 1989) to form pNCU-RSC6.

The plasmid pBCrsc6Δ::LEU2 replaces codons 31–435 (of 483) of the *RSC6* gene with the *LEU2* gene and promoter. The 3′ noncoding region of *RSC6* was isolated by cutting pNCU-RSC6 with PstI and XbaI and ligating the 496 bp fragment to XbaI- and PstI-digested pRS305 (*LEU2*) to created pKOL-3′RSC6. A DNA fragment comprising the 5′ noncoding region was generated by the polymerase chain reaction, using Taq polymerase, pNCU-RSC6, and the following oligonucleotide primers: BC52w5 (given above) and BCycrG 5′-CCCGAGCCGCGGCTGCCAGATTGGAGACCTTATCAT-3′. The resulting 520 bp DNA product was digested with XbaI and SacII and cloned into XbaI- and SacII-digested pKOL-3′RSC6 to create pBCrsc6Δ::LEU2. Plasmid pBCrsc6Δ::LEU2 was digested with BamHI to effect *RSC6* gene replacement by the γ-integration method (Sikorski and Hieter, 1989) in the diploid strain W303, creating the heterozygous diploid YBC310 (Sherman and Hicks, 1991).

A plasmid directing the expression of recombinant glutathione S-transferase fused to amino acids 381–483 of Rsc6p was prepared from pGEX-3X (Pharmacia, Inc.) and an amplified DNA product prepared with Taq polymerase, yeast genomic DNA, and the oligonucleotide primers BC1752w5B 5′-CCCCGGATCCTGGGAAGTACTC CAAAGGATAAGCC-3′ and BC1852w3 5′-CCCGAATTCTTATAGT CTTCCTTGGGAGTACAGTA-3′. The amplified DNA product was digested with BamHI and EcoRI and cloned into BamHI- and EcoRI-digested pGEX-3X to create pGEX-RSC6ΔN.

Antibodies and Immunblot Analyses

Recombinant Rsc6ΔN protein (amino acids 381–483) fused to glutathione S-transferase was overproduced in Escherichia coli XL1 cells

(Stratagene, Inc.) and purified from inclusion bodies as described previously for recombinant Snf6p (Cairns et al., 1994). Approximately 2 mg of GST-Rsc6ΔN protein was separated by SDS-PAGE and used to immunize rabbits. Anti-Sth1p antibodies were prepared (J. D. and B. L., unpublished data), and anti-Swi3 antibodies were a gift from Y.-J. Kim. Immunoblotting with alkaline phosphatase was performed as described previously (Cairns et al., 1996b).

Purification of RSC Complex

RSC complex was purified from yeast whole-cell extracts. Purification was monitored and quantified with immunoblot analyses from the first four columns and then additionally with DNA-dependent ATPase assays from subsequent columns. The first three chromatographic steps (Bio-Rex 70, DEAE-Sephacel, and hydroxylapatite) were performed as described previously (Sayre et al., 1992). Recovery of Sth1p and Rsc6p was quantified by immunoblot reactivity: ~80% was recovered from the Bio-Rex 70 300–600 mM potassium acetate elution step, ~80% from the DEAE-Sephacel 200–550 mM potassium acetate elution step, and ~75% from hydroxylapatite in the pool of fractions containing 110–140 mM sodium phosphate. For hydroxylapatite, a shallow potassium phosphate gradient (~5 mM increase in phosphate per fraction) at 1.5 column volumes/hr was employed. This hydroxylapatite pool was dialyzed against buffer A (20 mM Tris-acetate [pH 7.6], 20% glycerol, 1 mM dithiothreitol, 1 mM EDTA, 0.01% Nonidet P-40, a protease inhibitor cocktail [PIC] [2 μg/ml chymostatin, 2 μM pepstatin A, 0.6 μM leupeptin, 2 mM benzamidine, and 1 mM phenylmethylsulphonyl fluoride]) containing 100 mM potassium acetate and applied to Mono Q (8 ml, HQR). The column was washed with buffer A containing 400 mM potassium acetate and developed with a 120 ml linear gradient from 400 mM to 1.2 M potassium acetate in buffer A. Peak fractions from Mono Q (from 675 to 725 mM) were dialyzed against buffer B (20 mM HEPES-acetate [pH 7.6], 20% glycerol, 1 mM dithiothreitol, 1 mM EDTA, PIC, and 0.01% Nonidet P-40) containing 50 mM potassium acetate, loaded on a 3.3 ml TSK-heparin column, and eluted with a 40 ml linear gradient from 200 to 800 mM potassium acetate in buffer B. Peak fractions (from 450 to 525 mM potassium acetate) were pooled, dialyzed against buffer B containing 50 mM potassium acetate, applied to a 1 ml Mono S column, and eluted with a 20 ml linear gradient from 100 to 800 mM potassium acetate in buffer B. Gel filtration chromatography was performed with 11 μg of either Mono S fraction 34 (RSCa) or 38 (RSC) or molecular weight standards (20 μg, Bio-Rad, Inc.), run sequentially at 0.25 ml/min on an 8 ml Bio-Sil (Bio-Rad, Inc.) gel filtration column equilibrated with buffer C (20 mM Tris-acetate [pH 7.8], 20% glycerol, 5 mM β-mercaptoethanol, 5 mM EDTA, and PIC) containing 800 mM potassium acetate as the mobile phase.

Peptide Sequencing, Mass Spectrometric Analyses, and Sequence Comparisons

Approximately 200 μg RSC was separated in a single lane of a "step" gel containing an SDS–12% acrylamide gel on the lower half and an SDS–7.5% acrylamide gel on the upper half and then transferred at 4°C for 12 hr at 10 V/cm to a PVDF membrane (Trans Blot, Bio-Rad, Inc.). RSC proteins were revealed by Ponceau S staining, excised, digested with trypsin in Zwittergent 3–16 containing buffer (Lui et al., 1996), and peptides fractionated by RP HPLC using an 1.0 mm Reliasil C_{18} column (Elicone et al., 1994).

Aliquots (2%) from ten peak fractions of each digest were analyzed by matrix-assisted laser-desorption ionization time-of-flight mass spectrometry using a Voyager RP analyzer (Vestec/PerSeptive Biosystems) with a TDS 520 Tektronix oscilloscope and operated in the linear mode with two-peptide internal calibration (Geromanos et al., 1994; Erdjument-Bromage et al., 1994). Selected experimental masses (900 amu $\leq m/z \leq$ 4000 amu) were used to search a properly indexed, nonredundant database (European Bioinformatics Institute, UK) using the PeptideSearch algorithm (M. Mann, EMBL, Germany). Mass accuracy search restriction was arbitrarily set at <0.05%, and a maximum of two missed cleavage sites was allowed. The required number of matching masses for identification was incrementally reduced from the maximum number, one at a time, until a single yeast protein was retrieved.

Chemical sequencing was performed with a model 477A instrument from Applied Biosystems; stepwise-liberated phenylthiohydantoin amino acids were identified by on-line RP HPLC (Applied Biosystems 120A instrument equipped with an Applied Biosystems 2.1 mm phenylthiohydantoin C_{18} column). Instruments and procedures were optimized for femtomole-level phenylthiohydantoin amino acid analysis as described (Erdjument-Bromage et al., 1994; Tempst et al., 1994). Sequencing data were compared to entries in the S. cerevisiae Genome Database (Stanford University), using the National Center for Biotechnology Information BLAST program (Altschul et al., 1990). The accuracy of our identifications was determined by fitting the available masses to sequences from the identified proteins, allowing unrestricted m/z values, an unlimited number of missed cleavage sites, ≤0.1% ΔDa accuracy, and the presence of oxidized methionines and acrylamide-modifying cysteines. Sequence alignment was performed with the BESTFIT program (Genetics Computer Group, Madison, Wisconsin), and the parameters used to determine percentage identity and percentage similarity were as follows: gap weight, 2.0; gap length, 0.1; and scoring matrix, swgappep.cmp.

DNA-Dependent ATPase Assays

DNA-dependent ATPase activity was determined with a colorimetric assay. Formation of inorganic phosphate was quantified by the addition of an acidic malachite green–sodium molybdate solution to reaction mixtures as described previously (Cairns et al., 1994). Standard reaction mixtures (25 or 50 μl) contained 20 mM HEPES (pH 7.0), 5% glycerol, 50 mM potassium acetate, 5 mM $MgCl_2$, 0.1 mM dithiothreitol, 1 mM ATP, 0.1 mg/ml crystallized bovine serum albumin, and 20 ng/μl CsCl-purified supercoiled plasmid DNA (Bluescript KS, Stratagene, Inc.). Standard reactions were incubated at 30°C for 30 min. Activity is directly proportional to the amount of RSC added and is linear for the first 30 min. To determine the K_m for ATP, reactions were scaled up to 200 μl and were performed for only 5 min to avoid ATP depletion. All nucleotides were purchased from Pharmacia (ultrapure). The nucleosomes used were rat liver nucleosome core particles lacking histone H1. Calculations of activity and turnover number assumed 100% active RSC molecules and are therefore a lower limit.

Histone Acetyltransferase Assays

Histone acetytransferase activity was detemined with highly purified fractions of RSC or SWI/SNF, the cofactor [^{3}H] acetyl-coenzyme A, and purified rat liver chromatin lacking histone H1. Reactions (10–20 μl) were performed in buffer D (50 mM HEPES [pH 7.3], 1 mM ATP, 2.5 mM $MgCl_2$, 5 mM dithiothreitol, 2% glycerol, 1 mM sodium butyrate, and PIC) and contained 1 μg purified rat liver chromatin (lacking histone H1), [^{3}H]acetyl-coenzyme A (2.5 μl at 3.5 Ci/mmol), and protein fractions (1–4 μl). Reactions were incubated at 30°C for 1 hr and were terminated by the addition of an equal volume of 2× SDS loading buffer. Histones were resolved on an SDS–18% acrylamide gel and stained with Coomassie blue to ensure that equal levels of protein were loaded. Gels were impregnated with Amplify (Amersham, Inc.), dried, and exposed to film.

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