The RSC Chromatin Remodeling Complex Bears an Essential Fungal-Specific Protein Module with Broad Functional Roles

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Running Head: A Fungal-Specific RSC module

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ABSTRACT

RSC is an essential and abundant ATP-dependent chromatin remodeling complex from *Saccharomyces cerevisiae*. Here we show that the RSC components Rsc7/Npl6 and Rsc14/Ldb7 interact physically and/or functionally with Rsc3, Rsc30, and Htl1 to form a module important for a broad range of RSC functions. A strain lacking Rsc7 fails to properly assemble RSC, which confers sensitivity to temperature and to agents that cause DNA damage, microtubule depolymerization, or cell wall stress (likely via transcriptional misregulation). Cells lacking Rsc14 display sensitivity to cell wall stress and are deficient in the assembly of Rsc3 and Rsc30. Interestingly, certain *rsc7Δ* and *rsc14Δ* phenotypes are suppressed by an increased dosage of Rsc3, an essential RSC member with roles in cell wall integrity and spindle checkpoint pathways. Thus, Rsc7 and Rsc14 have different roles in the module as well as sharing physical and functional connections to Rsc3. Using a genetic array of non-essential null mutations (SGA), we identified mutations that are sick/lethal in combination with the *rsc7Δ* mutation which revealed connections to a surprisingly large number of chromatin remodeling complexes and cellular processes. Taken together, we define a protein module on the RSC complex with links to a broad spectrum of cellular functions.
INTRODUCTION

The eukaryotic genome is packaged into chromatin which can inhibit the accessibility of certain DNA binding factors to their cognate sites in vivo. However, the structure of chromatin can be altered in a regulated manner to allow factor binding (Vignali et al. 2000). Dynamic chromatin alterations help regulate processes such as transcription, replication, DNA damage repair, and recombination. A topic of considerable current interest is the characterization of factors that regulate these dynamic chromatin transitions (Becker and Horz 2002; Jenuwein and Allis 2001).

Two general classes of enzymes exist that help regulate DNA accessibility in a chromatin environment: histone modifiers and ATP-dependent chromatin remodeling complexes (remodelers) (Narlikar et al. 2002). This work focuses on remodelers, all of which contain an ATPase subunit that is essential for the remodeling mechanism. The ATPase subunit has been proposed to function as a DNA-translocating enzyme that utilizes the energy of ATP-hydrolysis to mobilize nucleosomes (Saha et al. 2002; Whitehouse et al. 2003). Remodeler complexes can be divided into families with unique biochemical properties and subunit compositions. The SWI/SNF family includes the human BRM/BAF and BRG/PBAF complexes, the Drosophila BAP and PBAP complexes, and the Saccharomyces cerevisiae RSC and SWI/SNF complexes (Martens and Winston 2003; Mohrmann and Verrijzer 2005).

Remodeling complexes in the SWI/SNF family bear 8 to 15 subunits which work in cooperation with the ATPase to efficiently regulate chromatin structure. Catalytic ATPase subunits in the SWI/SNF family include: Swi2/Snf2 (yeast SWI/SNF), Sth1 (yeast RSC), Brm (human BRM/BAF) and Brg1 (human BRG/PBAF or BAF) (Martens and Winston 2003; Mohrmann and Verrijzer 2005; Tsukiyama 2002). The catalytic subunit is bound to a set of
‘core’ subunits that are highly conserved in all SWI/SNF family remodelers. Studies on human BRM and BRG core subunits, BAF170 and BAF155 (Swi3 and Rsc8 orthologs), and INI1 (a Snf5 and Sfh1 ortholog), show that core subunits contribute to the efficiency of chromatin remodeling in vitro, and together with the catalytic subunit enable remodeling within two-fold of wild type activity (PHELAN et al. 1999). Remodeling complexes are targeted to genes/nucleosomes by several different modes including specific interactions with transcriptional activators/repressors, by binding to modified histone tails, or (possibly) through DNA recognition. For example, the transcriptional activator Gcn4 binds RSC and SWI/SNF and is required for remodeler recruitment to a gene required for amino acid metabolism (ARG1) (SWANSON et al. 2003). Furthermore, conserved protein domains that bind histone tails or DNA are present in yeast remodelers. For example, the Rsc3 subunit of RSC contains a zinc-cluster DNA binding domain and Rsc4 contains bromodomains which bind acetylated histone tails (ANGUS-HILL et al. 2001; KASTEN et al. 2004). Certain members also serve as structural components important for assembly (PETERSON et al. 1994). Thus, ATP-dependent chromatin remodeling complexes require the cooperative action of the catalytic, core, targeting and structural components. Although roles have been ascribed to certain SWI/SNF and RSC subunits, many subunits still remain to be characterized.

RSC is a large (15 subunit), essential, and abundant remodeling complex with many cellular functions (CAIRNS et al. 1996). In RSC, the catalytic ATPase is essential for viability (DU et al. 1998) and is also sufficient for remodeling in vitro (SAHA et al. 2002). Mutations in other RSC members also confer lethality or conditional phenotypes suggesting important in vivo functions for these attendant subunits (BUNGARD et al. 2004; CAIRNS et al. 1998; CAIRNS et al. 1999; CAO et al. 1997; DAMELIN et al. 2002; SAHA et al. 2002; TANEDA and KIKUCHI 2004;
Mutations in certain RSC subunits (Sth1, Htl1, Rsc3 and Rsc4) confer cell wall defects (osmotically remedial temperature sensitivity) and link RSC to the cell wall integrity pathway likely through proper transcriptional regulation of cell wall components/regulators (Angus-Hill et al. 2001; Chai et al. 2002; Kasten et al. 2004; Romeo et al. 2002). The cell wall integrity pathway is a kinase cascade which proceeds through a central kinase, Pkc1, and helps regulate changes in cell wall composition. Mutations in components of this pathway confer cell wall defects and render cells sensitive to osmotic changes (Heinisch et al. 1999; Levin 2005). Rsc3 is an essential member of RSC with genetic links to the cell wall integrity pathway; a pkc1Δ rsc3 double mutant is nearly lethal, and certain rsc3 phenotypes can be suppressed by an increased dosage of Pkc1 (Angus-Hill et al. 2001).

Interestingly, Rsc3 forms a stable heterodimer with Rsc30, a non-essential protein with functional roles distinguishable from Rsc3 (Angus-Hill et al. 2001). As mutations in other RSC components also have cell wall defects, Rsc3 likely cooperates with other RSC subunits to regulate genes involved in cell wall biogenesis (Kasten et al. 2004; Romeo et al. 2002). However, the contribution of individual subunits to the cell wall integrity pathway or other cellular processes is not well understood.

To gain further insight into the roles of individual RSC subunits, we characterized two additional RSC members, Rsc7 and Rsc14, and examined the scope of RSC function through a large-scale genetic analysis. We have shown that Rsc7 and Rsc14 have structural roles in the RSC complex, and help define a fungal-specific module in RSC that includes Rsc3, Rsc30, Htl1, Rsc7, and Rsc14. Rsc7 and Rsc14 display strong genetic interactions with Rsc3 and help mediate the association of Rsc3 with the RSC complex. In addition, Rsc7 helps maintain the structural integrity of the entire RSC complex, and a strain lacking Rsc7 provided us with a tool to examine
the scope of RSC function in vivo. Here, we crossed the \textit{rsc7}Δ strain to a library of non-essential deletion mutants and identified a broad spectrum of null mutations that conferred growth defects in combination with the \textit{rsc7}Δ. This study provides insight into RSC assembly, strengthens the connection between RSC and the cell wall integrity pathway, and identifies novel links to other signaling pathways and cellular processes.

**MATERIALS AND METHODS**

**Yeast strains and genetic methods:** All \textit{S. cerevisiae} strains are S288C derivatives. Standard procedures were used for mating, sporulation, transformation, and tetrad dissection. All media was prepared as described previously (ROSE \textit{et al.} 1990). The null mutations for \textit{rsc7}, \textit{swp82}, and \textit{rsc14} were constructed using standard methods (BAUDIN \textit{et al.} 1993; LORENZ \textit{et al.} 1995) and verified by PCR analysis. All primer sequences for PCR reactions are available upon request. Alleles of \textit{rsc3}, \textit{htl1}, \textit{rsc9}, \textit{rsc30}, \textit{rsc1}, and \textit{rsc2} have been described previously (ANGUS-HILL \textit{et al.} 2001; CAIRNS \textit{et al.} 1999; DAMELIN \textit{et al.} 2002; ROMEO \textit{et al.} 2002). Genes encoding tagged derivatives of Rsc7 (including tagged truncation derivatives for Rsc7), Rsc14, and Swp82 were constructed using a one-step PCR mediated gene tagging procedure and verified by PCR and western analysis (LONGTINE \textit{et al.} 1998). A \textit{rsc7}Δ strain was modified for use in synthetic genetic array analysis. Briefly, a \textit{MFA1pr-HIS3} cassette was integrated into the genomic \textit{CAN1} locus of YBC1968 (using standard procedures, BAUDIN \textit{et al.} 1993; LORENZ \textit{et al.} 1995) and confirmed by PCR analysis. The mating-type locus of this strain was then converted from \textit{MATa} to \textit{MATα} to generate YBC2039. All other null mutations (including mutations from the non-essential haploid deletion library) were obtained through Research Genetics (Invitrogen) and are isogenic to either BY4741 or BY4742 (BRACHMANN \textit{et al.} 1998).
**Plasmids:** Plasmids p137 and p136 (gifts from Pam Silver) harbor a 2.6 kb SnaBI fragment of the genomic RSC7 locus sub-cloned into YCP50 or YEP352, respectively. Plasmid p1310 was constructed using a Xho1-Spe1 fragment bearing RSC3 that was isolated from p906 (pRS314.RSC3; ANGUS-HILL *et al.* 2001) and sub-cloned into pRS426. Plasmids p1401 and p1403 were constructed by cloning a 900 bp PCR product (using genomic DNA as template) containing RSC14 into pRS315 and pRS425, respectively. Plasmid p1204 (pRS426.HTL1.3×HA) was described previously (ROMEO *et al.* 2002). Plasmids p185 (pRS426.Rsc8.3×HA) and p186 (pRS315.Rsc8.3×HA), gifts from Marian Carlson were described previously (TREICH and CARLSON 1997). Plasmids p935 (*MET25pr.rsc7* 248-435.13×Myc. TRP1) and p936 (*MET25pr.rsc7* 1-435.13×Myc. TRP1) were constructed by cloning RSC7 fragments generated by PCR (using genomic DNA from YBC834) into the FB1521 plasmid (FUNK *et al.* 2002) using Xho1 and Xba1 or Spe1 and BamH1, respectively.

**Extract preparation and immunoprecipitation analysis:** Preparation of whole cell extracts was as described previously (CAIRNS *et al.* 1999), except cells were subjected to bead beating for two minutes (three pulses) and five minutes of cooling on ice. Anti-Myc (0.5 mg/ml) or –HA (7 µg/ml) antibodies were prebound to protein G-agarose or magnetic beads, respectively. To immunoprecipitate tagged derivatives of Rsc7, Swp82, Rsc8, and Rsc14, anti-Myc beads (40 µls of a 50% slurry) or Anti-HA beads (50 µls of slurry) were incubated with 200 µg of the indicated protein extract in immunoprecipitation buffer (50 mM Tris-HCl [pH 7.5], 1 mM EDTA, 10% glycerol, 100 mM NaCl, 0.05% Tween-20) (total volume was 140 µls) at 4° for 3.5-7.5 hours. When checking RSC stability in *rsc14Δ* extracts, the immunoprecipitation buffer contained 250 mM NaCl (total immunoprecipitation volume was 400 µls). When checking RSC stability in *rsc7Δ* extracts, the total immunoprecipitation volume was 400 µls. Precipitates were
recovered by centrifugation (10,600×g for 2 min.), or using a magnetic bead stand, and washed three times with immunoprecipitation buffer (250 mM NaCl). Precipitates were eluted with SDS and separated on a 7.5% acrylamide-SDS gel (with supernatants and input) and transferred to polyvinylidene difluoride (PVDF) membrane. Standard immunoblotting and chemiluminescence detection methods were used.

**Isolation and identification of Rsc7, Swp82, and Rsc14:** The purification of RSC and SWI/SNF to homogeneity was performed as described previously (CAIRNS et al. 1994; CAIRNS et al. 1996), respectively. Rsc7, Rsc14, and Swp82 peptides were isolated, sequenced, and analyzed by mass spectrometry as previously described for other RSC and SWI/SNF subunits.

**FACS Analysis:** Methods used for FACS analysis were described previously (ANGUS-HILL et al. 2001).

**Synthetic Genetic Array Screen:** The modified rsc7Δ strain (YBC2039) was mated with the yeast haploid deletion set (BY4741) from Research Genetics (Invitrogen) (catalog #95401.H2) on rich solid media. Diploids were selected on synthetic complete (SC) medium containing G-418 and lacking leucine. These diploids were sporulated on solid medium and meiotic haploid MATa double mutant progeny containing the plasmid-borne RSC7+ were isolated on SC medium containing canavanine and G-418 while lacking uracil, histidine, leucine, and arginine. Selection was based on haploid selection markers (can1Δ::MFA1pr-HIS3), double mutant selection markers (KanMX and LEU2), and a plasmid selection marker (URA3). To monitor growth of double mutants after loss of the URA3-marked RSC7+ plasmid, haploids were replica plated to haploid selection media with or without 5-fluoroorotic acid (5-FOA). Double mutants were scored as sick/lethal by comparing growth on medium lacking 5-FOA (enabling RSC7+ retention) with medium containing 5-FOA (enforcing RSC7+ loss).
RESULTS

Rsc7 and Swp82 are paralogs in RSC and SWI/SNF: Our studies began with the identification of two paralogs in the RSC and SWI/SNF complexes, Rsc7 and Swp82. These complexes were previously purified to homogeneity from yeast cellular extracts (Figure 1B; CAIRNS et al. 1994; CAIRNS et al. 1996). The 50 kD RSC subunit, Rsc7 (also designated Npl6; BOSSIE et al. 1992) was isolated and analyzed by MALDI-TOF mass spectrometry, mass fingerprinting, and limited Edman sequencing which uniquely identified the open reading frame YMR091c/NPL6.

To verify the interaction between Rsc7 and RSC (as opposed to fortuitous co-purification), we immunoprecipitated an epitope tagged Rsc7 derivative (Rsc7.13×Myc) and tested for association with known RSC members. Rsc7.13×Myc efficiently co-precipitated other members of the RSC complex, including Sth1, Rsc3, Rsc30, Arp7, and Arp9 (Figure 1C). These interactions were stable under stringent wash conditions and confirm Rsc7 as a stable component of the RSC complex. Our findings support and extend proteomic and biochemical approaches showing Rsc7 associated with RSC members (BAETZ et al. 2004; GAVIN et al. 2002; GRAUMANN et al. 2004; SANDERS et al. 2002; UETZ et al. 2000) and the interaction between Rsc7 and Rsc8 (a core RSC subunit) observed in genome-wide two-hybrid studies (UETZ et al. 2000).

A proteomic strategy similar to that used for Rsc7 identification was used to identify the ∼80 kD subunit of SWI/SNF as YFL049w/Swp82 (data not shown). An epitope tagged Swp82 (Swp82.13×Myc) efficiently co-precipitates with Swi3 and Swp73 and remains associated even under stringent wash conditions (Figure 1D). This data supports and extends proteomic
approaches by others who observed Swp82 co-purifying with SWI/SNF subunits (AASLAND and STEWART 1995; GAVIN et al. 2002; GRAUMANN et al. 2004; LEE et al. 2004).

**Rsc7 and Swp82 define a new family of proteins conserved in fungi:** Using the algorithm BLAST (ALTSCHUL et al. 1990), we searched for proteins related to Rsc7. Orthologs were identified in closely related yeast of the hemiascomycete family including *Candida glabrata*, *Ashbya gossypii*, and *Kluyveromyces lactis*. In addition, orthologs were identified in distantly related fungal species such as *Schizosaccharomyces pombe* and *Neurospora crassa*; however, orthologs were not identified in higher eukaryotes. Importantly, a paralogous protein was identified in *S. cerevisiae*, Swp82 (BLAST P-value 1.36 X 10^{-4}). Above, we verified Swp82 as a novel member of the SWI/SNF chromatin remodeling complex. Thus, Rsc7 and Swp82 are paralogous components of the RSC and SWI/SNF complexes, respectively.

Based on alignments with orthologs, we have separated Rsc7 into three regions: (1) an acidic amino terminus (Figure 1A; black box), (2) a conserved region (described below) (Figure 1A; shaded box), and (3) a C-terminal region (Figure 1A; hatched box). First, the amino acids 1-84 of Rsc7 are rich in aspartic and glutamic acid, but lack any significant homology with other proteins. Second, the conserved region (amino acids 85-247) is present in Rsc7 orthologs in all fungi and is thus the defining feature of all Rsc7 family members. Searches with this conserved region revealed two orthologs in the closely related hemiascomycete yeast *C. glabrata*, CAG60715 (BLAST P-value 4.68 × 10^{-4}) and CAG61742 (BLAST P-value 3.65 × 10^{-3}). Two proteins bearing the conserved region can also be identified in more distantly related fungi such as the filamentous yeast, *S. pombe*, and the red bread mold, *N. crassa*. Third, the C-terminus of Rsc7 (amino acids 248-435) is not significantly conserved between Rsc7 and Swp82. However, a search for proteins with homology to this region of Rsc7 revealed *C. glabrata* CAG60715.
(BLAST P-value $1.71 \times 10^{-16}$), *S. pombe* SPCC1281.05 (BLAST P-value $2.02 \times 10^{-2}$), and putative Rsc7 counterparts in other fungal species. Thus, we have identified a new family of proteins in fungi related to Rsc7.

A search for proteins related to Swp82 revealed Rsc7 (BLAST P-value $2.27 \times 10^{-4}$) in *S. cerevisiae*, proteins in hemiascomycete yeast, and proteins in more distantly related fungal species such as *S. pombe* and *N. crassa*. Similar to Rsc7, searches using the conserved region of Swp82 (Figure 1A; shaded box) revealed orthologs in *C. glabrata* CAG61742 (BLAST P-value $2.81 \times 10^{-13}$) and CAG60715 (BLAST P-value $6.74 \times 10^{-3}$) and identified orthologs in other fungal species. Furthermore, searches using the C-terminal non-conserved region of Swp82 helped to identify Swp82 counterparts in closely related hemiascomycete yeast, such as CAG61742 (BLAST P-value $1.10 \times 10^{-34}$) in *C. glabrata*. However, unlike Rsc7 the C-terminal region of Swp82 was not conserved in more distantly related fungal species such as *S. pombe* and *N. crassa*. Taken together, Swp82 has homologues in fungi and has identifiable counterparts in closely related yeast species.

**Rsc14 is a RSC component conserved in yeast:** Among the subunits identified in the initial purification of RSC to homogeneity were two species of approximately 15 kD, previously designated Rsc14 and Rsc15 (Figure 1B; Cairns *et al.* 1996). Mass fingerprinting and peptide sequencing identify both Rsc14 and Rsc15 as proteins encoded by the open reading frame YBL006c (also named *LDB7*; Corbacho *et al.* 2004). We suspect the faster migrating form of Rsc14 is a degradation product as the tagged Rsc14 is present only as a single species (data not shown). Therefore, the species previously designated as Rsc14 and Rsc15 will hereafter be called Rsc14.
To verify the interaction between Rsc14 and RSC, we tagged the C-terminus of Rsc14 with thirteen copies of the Myc epitope (Rsc14.13×Myc). Co-immunoprecipitation experiments confirmed association between Rsc14 and other members of RSC. Indeed, Rsc14 co-precipitates with Sth1 and Arp9 and remains stably associated under stringent wash conditions (Figure 1E). Our results support recent proteomic studies revealing that Rsc14 co-purifies with RSC complex members (GRAUMANN et al. 2004).

The algorithm BLAST identified Rsc14 orthologs in closely related hemiascomycete yeast but not other higher eukaryotes or more distantly related fungal species. In C. glabrata, Rsc14 is conserved with CAG58728 (BLAST P-value $6.07 \times 10^{-9}$) and also has counterparts in A. gossypii and K. lactis. Thus, we have verified Rsc14 as a RSC component, and have revealed its conservation in yeast.

**Phenotypic analysis of the rsc7Δ, swp82Δ, and rsc14Δ strains:** To address the in vivo functions of Rsc7, Swp82, and Rsc14 we isolated null alleles and examined their growth characteristics. The rsc7Δ haploid spores grew slowly at 28°C and were inviable at 37°C. Furthermore, the rsc7Δ strain displayed severe growth defects when exposed to any of an assortment of cellular stresses: NaCl (0.5 M), congo red (800 µg/ml), formamide (2%), caffeine (15 mM), ultraviolet radiation (UV; 8000 µJ/cm²), methyl methanesulfonate (MMS; 0.03%), hydroxyurea (HU; 150 mM), and benomyl (12.5%) (Figure 2A). Mutant phenotypes were complemented largely or fully by a plasmid-borne copy of RSC7+. Somewhat surprisingly, the rsc7Δ mutation did not confer a narrow range of rsc phenotypes (expected of a specific targeting subunit), but rather conferred a broad range of phenotypes representing the full spectrum of all known rsc mutations. This suggested a broad and/or general role for Rsc7 in the RSC complex.
To better characterize the domains of Rsc7, we made genomic truncations of the C-terminus of Rsc7 by integrating (via homologous recombination) a cassette encoding a 13×Myc tag (see Materials and Methods). A Rsc7 derivative lacking its C-terminus failed to assemble into the RSC complex, and failed to complement rsc7Δ phenotypes, but was produced in the cell. (Supplemental Table 1). To determine the extent to which Rsc7 function depends upon this region we expressed a Rsc7 derivative bearing only the C-terminal region (amino acids 248-435). Surprisingly, the C-terminal region of Rsc7 fully complemented all known rsc7Δ phenotypes, showing that the conserved homology region is apparently not required for the large spectrum of Rsc7 functions examined (Supplemental Table 1).

The swp82Δ strain grew well under all conditions tested. Our tests included growth at 16°, 35° and 38°, growth on glycerol (2%), galactose (2%), raffinose (2%), sucrose (2%) and ethanol (6%) (common phenotypes conferred by other swi/snf mutations), growth on media containing lower/higher than normal levels of phosphate, growth on medium lacking amino acids (except those required for auxotrophic markers), growth on rapamycin (15 nM) or growth on increased concentrations of LiCl (0.25 M), ZnSO₄ (5 mM), NaCl (1.2 M), MnCl₂ (6 mM), CaCl₂ (200 mM), and CoCl₂ (1.5 mM). Furthermore, we tested for sensitivity to ultra-violet radiation (100 μJ/cm²×100), 4-nitroquinoline 1-oxide (0.5 μg/ml), hydroxyurea (100 mM) and camptothecin (20 μg/ml) (DNA damaging agents), sensitivity to formamide (2%) and caffeine (15 mM) (cellular stress), sensitivity to sulfometuron methyl (3 μg/ml) (amino acid metabolism), sensitivity to tunicamycin (4 μg/ml), dithiothreitol (100 mM), brefeldin A (30 μg/ml), low pH media (secretory pathway), sensitivity to 6-azauracil (35 μg/ml) (transcription elongation) and sensitivity to cycloheximide (1 μg/ml) (protein synthesis). The swp82Δ strain displayed no obvious sporulation or mating defects, and genes displaying altered expression in swi/snf mutants
(including SER3) were not misregulated in a *swp82Δ* strain (data not shown). Finally, the *swp82Δ rsc7Δ* double mutant displayed phenotypes indistinguishable from *rsc7Δ* alone (data not shown). Therefore, the role of Swp82 in SWI/SNF remains elusive.

The haploid *rsc14Δ* strain was generally healthy, but showed clear sensitivity to NaCl (1.2 M) and caffeine (15 mM) at normal temperatures (30°) and formamide (2%) and congo red (800 µg/ml) at elevated temperatures (38°). This sensitivity could be complemented by a plasmid-borne *RSC14*+ (Figure 3). Thus, we have identified *rsc14Δ* phenotypes which connect Rsc14 to the cell wall integrity pathway and certain functions shared by Rsc3 and Rsc7.

**rsc7Δ and rsc14Δ mutations are lethal in combination with other rsc mutations:** To help identify functional relationships between RSC subunits and Rsc7 we attempted to cross the *rsc7Δ* strain to other *rsc* mutants. However, we found that strains lacking Rsc7 rapidly acquired mutations that conferred spore inviability (revealed in subsequent mating/sporulation tests, data not shown). To circumvent this problem, *rsc7Δ::HIS3/RSC7*+ heterozygous diploids bearing *RSC7*+ on a *URA3*-marked plasmid were sporulated and dissected to generate a haploid *rsc7Δ* strain covered by *RSC7*+. This strain was crossed to other *rsc* mutants and growth defects were determined by the ability of the haploid double mutants to lose the *URA3*-marked plasmid bearing *RSC7*+. Surprisingly, in all cases tested *rsc7Δ* double mutant combinations showed lethality (Table 2). These results support a broad and/or general role for Rsc7 in promoting RSC complex function.

To test for functional relationships between Rsc14 and other members of RSC, we crossed the *rsc14Δ* null with other *rsc* mutants. Similar to the *rsc7Δ* strain, *rsc14Δ* mutants acquired mutations that conferred low spore viability. To circumvent this problem, *rsc14Δ::KanMX/RSC14*+ heterozygous diploids bearing *RSC14*+ on a *URA3*-marked plasmid
were sporulated and dissected to generate a haploid *rsc14Δ* strain covered by plasmid-borne *RSC14*+. This strain was crossed to other *rsc* mutants and growth defects were determined by the ability of the haploid double mutants to lose the *URA3*-marked plasmid bearing *RSC14*+. Similar to any observations with *rsc7Δ, rsc14Δ* combinations with other *rsc* mutations were inviable (Table 3). These results are consistent with Rsc14 conducting an essential RSC function in combination with other members of RSC.

**RSC structural integrity requires Rsc7 and Rsc14:** The breadth of phenotypes conferred by *rsc7Δ* raised the possibility that RSC complexes lacking Rsc7 might fail to assemble properly, impairing all RSC functions. To test this, we performed co-immunoprecipitation studies with a strain expressing an HA-tagged Rsc8 derivative (Rsc8.3×HA). Rsc8 is an essential subunit of RSC that interacts with several other RSC components (Rsc6, Sth1, and Htl1) and likely forms a structural scaffold for the RSC complex (Lu et al. 2003; Treich and Carlson 1997; Treich et al. 1998). We found Rsc8 protein levels greatly reduced in a *rsc7Δ* strain confirming that Rsc7 contributes to the general assembly of the RSC complex. Importantly, Rsc3 association with Rsc8.3×HA was severely reduced in a *rsc7Δ* strain (Figure 4A). Taken together, these results confirm Rsc7 is a structural component required for full assembly of the RSC complex, providing a basis for the broad range of phenotypes displayed by the *rsc7Δ* strain.

The *rsc14Δ* strain also exhibits sensitivity to NaCl, caffeine, and formamide (at elevated temperatures), all phenotypes shared by *rsc7* and *rsc3* mutants. These results suggested that Rsc14 might be physically and/or functionally linked to Rsc7 and Rsc3. A notion supported by double mutant phenotypes; *rsc7Δ rsc14Δ* or *rsc3 rsc14Δ* combinations were inviable (Table 2 and 3). To test whether Rsc14 has a structural role in linking Rsc3 to RSC, we performed co-
immunoprecipitation studies with a strain expressing an HA-tagged Rsc8 derivative (Rsc8.3×HA). In a rsc14Δ strain, Rsc8.3×HA co-precipitates with Sth1 normally whereas its association with Rsc3 and Rsc30 is severely diminished (Figure 4B). Previous work has established that Rsc3 and Rsc30 form a stable heterodimer (ANGUS-HILL et al. 2001) which would predict their co-loss. Together, our data supports a model where Rsc7 and Rsc14 help the Rsc3/30 heterodimer assemble into RSC.

We next tested whether Rsc7 also promotes Rsc14 association with RSC. Here, we immunoprecipitated an HA-tagged Rsc14 derivative (Rsc14.3×HA) in a rsc7Δ strain, which showed full association with Sth1 (Figure 4C). Taken together, we show that Rsc7 and Rsc14 promote Rsc3 association with RSC, whereas Rsc7 is not required for Rsc14 to assemble into RSC.

**Rsc7 and Rsc14 are physically and functionally linked to Rsc3:** We further tested for functional and/or physical relationships among Rsc7, Rsc14, and Rsc3 by testing for dosage suppression. First, we tested whether an increased dosage of Rsc3 could suppress rsc7Δ phenotypes. We found that plasmid-borne RSC3 in high copy partially suppressed rsc7Δ sensitivity to temperature, osmotic stress, and formamide whereas increased dosage of other members of RSC (including Htl1 and Rsc30) did not suppress (Figure 2B and data not shown). Furthermore, we found that phenotypes conferred by other rsc mutations (rsc1Δ rsc2, arp7, arp9, rsc2Δ rsc1Δ rsc8) could not be suppressed by an increased dosage of Rsc3 (A. Schlichter and B. R. Cairns, unpublished and Figure 5). These results support our biochemical work suggesting that Rsc7 promotes the interaction of the Rsc3/30 heterodimer with RSC.

Rsc3 has strong genetic interactions with components of the cell wall integrity pathway; rsc3 mutants are sick in combination with pkc1Δ and display cell wall defects which can be
suppressed by an increased dosage of Pkc1 (ANGUS-HILL et al. 2001). These results prompted us to test whether the rsc7Δ strain displayed an osmotically remedial temperature sensitive phenotype. Indeed, we found that the temperature sensitivity of the rsc7Δ strain can be rescued by the addition of sorbitol (a cell wall stabilizer), CaCl2, and MgCl2 to the media (Figure 2C and data not shown) whereas other rsc mutants (such as rsc1Δ rsc2 Ts- mutants) cannot be rescued by the addition of cell wall stabilizers (A. Schlichter and B. R. Cairns, unpublished). These results are consistent with a role for Rsc7 in the cell wall integrity pathway.

If Rsc14 also promotes Rsc3 association with the RSC complex, then increasing the levels of the Rsc3 protein might likewise suppress rsc14Δ phenotypes. Indeed, we found that rsc14Δ phenotypes are suppressed by an increased dosage of Rsc3 but not by an increased dosage of Rsc7 (Figure 3 and data not shown). This data is consistent with Rsc14 being physically linked to Rsc3, and rsc14Δ phenotypes being conferred by a decreased association of Rsc3 with the RSC complex. Thus, Rsc7 and Rsc14 are physically/functionally linked to Rsc3 and suggest an important function for the module in the maintenance of cell wall integrity.

*rsc8 Ts- mutations are suppressed by an increased dosage of Rsc7:* Our biochemical results suggest that Rsc7 associates with and stabilizes Rsc8. In support of this notion, genome-wide two-hybrid experiments revealed an interaction between Rsc8 and Rsc7 (UETZ et al. 2000). To test for a functional connection, we utilized rsc8 temperature sensitive mutants (J. Lenkart and B. R. Cairns, unpublished), and found that increased dosage of Rsc7 suppressed rsc8 temperature sensitivity (Figure 5). In contrast, increased dosage of Rsc3 did not suppress rsc8 Ts- mutations, suggesting that this affect with Rsc7 is specific (Figure 5). This data supports a model where Rsc7 associates with and stabilizes the core subunit Rsc8 and also helps facilitate Rsc3 association.
**Rsc7 and Rsc14 are components of a fungal-specific module in RSC:** Our data provides evidence for an essential fungal-specific module in RSC containing Rsc7, Rsc14, Htl1, Rsc30, and Rsc3. Indeed, these subunits are restricted to fungi and most (including Rsc14, Htl1, Rsc30, and Rsc3) are present only in yeast-species. We show that Rsc7 and Rsc14 are linked genetically to Htl1, Rsc3, and Rsc30 and share mutant phenotypes, suggesting they cooperate in vivo to perform specific RSC functions. In addition, we show that Rsc7 and Rsc14 have roles in mediating the assembly of module components (including Rsc3), but do not affect the assembly of other RSC subunits (including Sth1). Taken together, our results suggest that Rsc7 and Rsc14 help link Rsc3, Rsc30, and Htl1 to RSC, and together form a fungal-specific module in the RSC complex (Figure 7).

**Isolation of null mutations conferring growth defects in combination with rsc7Δ:**
Rsc7 is important for general RSC function (via Rsc8 stability) and also for module functions. As rsc7Δ strains show moderate phenotypes that encompass all known RSC functions, the rsc7Δ allele represented a moderate hypomorph ideal for examining the full scope of RSC function. Therefore, we utilized the rsc7Δ strain to probe the scope of RSC function in vivo using a synthetic genetic array (SGA). This approach identified null mutations that are sick or lethal in combination with the rsc7Δ null allele. A rsc7Δ strain was modified to enable crossing to each strain of a haploid deletion library, composed of ~4700 strains each bearing a deletion in a non-essential gene and double mutant phenotypes were determined. Remarkably, the rsc7Δ mutation displayed a strong double mutant phenotype in combination with 125 deletion mutations, which together represented a broad range of cellular processes. We further characterized 111 of these double mutants, as 14 of these genes were linked to RSC7 or CAN1 (a haploid selection marker) and thus are likely false positives. Of these 111 we selected 67 double mutants for verification,
as this subset included members of each of the many protein complexes and signaling pathways identified. We tested 67 double mutant combinations by random spore analysis and/or tetrad dissection and determined that 45 interactions were genuine and 22 were false positives (Table 4). A comparable rate of false positives has been reported with this array by others (TONG et al. 2001).

The 45 confirmed combinations were categorized into four broad classes: (1) Chromatin/Transcription; (2) Chromosome metabolism; (3) Cell wall integrity; (4) Transport. The chromatin/transcription class includes members of many transcription initiation and elongation complexes, including SAGA, SET1, SWR1, INO80, elongator, and mediator. Additionally, certain RNA processing mutants were identified which we have grouped into the transcription class. The second class involved chromosome metabolism and included asf1Δ (chromatin assembly), ctf4Δ and ctf8Δ (cohesion), bub1Δ and mad1Δ (spindle checkpoint), cbf1Δ (kinetochore), msc1Δ (recombination), and rtt103Δ, rtt101Δ, and rtt109Δ (Ty transposition). The third class had roles in cell wall organization and biogenesis and includes bck1Δ (cell wall integrity pathway), gas1Δ (cell wall organization), and mid1Δ (calcium transporter). Finally, the fourth class of deletion mutants identified had roles in transport: nup84Δ and sxm1Δ (nuclear transport); apg17Δ (autophagy); bre5Δ, ric1Δ, and rgp1Δ (intracellular transport) (Table 4). Taken together, this data reveals the broad scope of RSC function by identifying non-essential deletion mutants that are sick/lethal in combination with the rsc7Δ strain. These results strengthen the connection between RSC and the cell wall integrity and spindle checkpoint pathways, two pathways with which RSC was previously linked. Furthermore, we link RSC to several chromatin/transcription related processes, suggesting partial redundancy of function with other chromatin complexes in vivo.
**Rsc7 is linked to the spindle checkpoint pathway:** Recent data suggests that RSC has roles in chromosome metabolism and the spindle checkpoint pathway (Baetz et al. 2004; Hsu et al. 2003; Huang et al. 2004; Tsuchiya et al. 1998). The spindle checkpoint pathway helps regulate progression from metaphase to anaphase during mitosis. Improperly assembled spindles promote checkpoint activation through the Mad proteins and arrest cells in metaphase until the spindle is assembled properly (Lew and Burke 2003; Musacchio and Hardwick 2002). Mutations in several RSC components, sth1, rsc3, rsc9 and htl1, all confer an arrest or delay in the G2/M phase of the cell cycle (Damelin et al. 2002; Hsu et al. 2003; Romeo et al. 2002). The G2/M arrest observed with certain rsc mutants (rsc3 and sth1) can be relieved by the removal of spindle checkpoint components (Mad proteins) (Angus-Hill et al. 2001; Hsu et al. 2003; Tsuchiya et al. 1998). To test for Rsc7 involvement in the spindle checkpoint pathway we performed FACS analysis on the rsc7Δ, which revealed a slight G2/M bias (Figure 6B). This data suggests that the spindle checkpoint pathway has become partially activated in the rsc7Δ strain. Furthermore, although the removal of key checkpoint components can relieve the G2/M growth arrest, these double mutants often display synergistic growth defects due to loss of viability (Hsu et al. 2003; Tsuchiya et al. 1998). Consistent with this notion, we find that combining spindle checkpoint pathway mutations (bub1Δ and mad1Δ) with rsc7Δ confers very slow growth (Figure 6A and Table 4). These results link Rsc7 to the spindle checkpoint pathway and strengthen the genetic connections between RSC and this pathway.

**DISCUSSION**

Chromatin remodeling complexes regulate the dynamic properties of chromatin and allow accessibility to the DNA template. In the SWI/SNF family of remodelers, the catalytic
subunit possesses coupled ATPase and remodeling activities, while the role of other subunits is not well understood. In this study, we examined the roles of two non-catalytic RSC subunits, Rsc7 and Rsc14, and one SWI/SNF subunit, Swp82. Rsc7 and Swp82 define a new family of proteins related to Rsc7 in fungal-specific chromatin remodeling complexes. Rsc7 and Rsc14 interact with Rsc3/30 and Htl1 to define a fungal-specific functional module. We identified structural/assembly roles for Rsc7 and Rsc14, and utilized strains lacking these subunits to examine in vivo RSC functions. Importantly, we have probed the scope of RSC function in vivo through the use of SGA analysis and found novel links to chromatin/transcription, chromosome metabolism, cell wall, and transport processes.

The Rsc7 family is defined by a conserved region: Here, we identify a novel family of proteins related to Rsc7, which may represent orthologs in fungal chromatin remodeling complexes. Family members exist in closely related hemiascomycetes yeast and in more distantly related fungal species. Like Rsc7 and Swp82, family members in other fungal species probably have roles in remodeling complexes. However, the function of the conserved domain remains elusive as we were not able to observe any phenotypes with the swp82Δ strain or with a rsc7 derivative lacking the conserved region. Therefore, our efforts have focused on Rsc7, as this member has clear phenotypes in RSC, and has helped define a fungal-specific protein module.

An essential fungal-specific protein module in RSC: Here, we provide evidence for an essential protein module in RSC containing Rsc7, Rsc14, Htl1, Rsc30 and Rsc3. While catalytic/core subunits of RSC are conserved throughout eukaryotes, this module appears restricted to fungi, with most module members present only in yeast species. Interestingly, these subunits are linked genetically and share mutant phenotypes suggesting a cooperative function. In addition, Rsc7 and Rsc14 mediate the assembly of Rsc3/30 into RSC (Figure 4A and 4B).
Thus, we propose that Rsc3/30 are required for an essential cellular process that does not require Rsc3/30 orthologs in higher eukaryotes. Rsc7, Rsc14, and Htl1 help stabilize the association of Rsc3/30 with the RSC complex and form a functional module that is conserved in yeast.

Rsc3 is a putative targeting molecule in RSC containing a zinc cluster DNA binding domain, which cooperates with module subunits functionally. We show that an increased dosage of Rsc3 can suppress phenotypes conferred by the $rsc7\Delta$, $rsc14\Delta$, and $htl1\Delta$ mutations (Figure 2B, Figure 3, and ROMEO et al. 2002), while overexpression of Rsc7, Rsc14, or Htl1 will not suppress phenotypes conferred by $rsc3$ mutations (data not shown). $RSC3$ is essential, whereas, $RSC7$, $RSC14$, and $HTL1$ are not. However, combining $rsc7\Delta$ with either $rsc14\Delta$ or $htl1\Delta$ results in lethality, supporting the notion that the module is essential. Taken together, Rsc3 has a central and essential role and is connected to RSC by other module members.

We propose that this module has an essential role in regulating gene expression in fungi. We speculate that RSC is targeted to certain genes by the putative DNA-binding functions of Rsc3/30, which are themselves found only in yeast species. Additional module subunits might have evolved to help regulate Rsc3/30 and to mediate their assembly into RSC. Additionally, the Rsc7, Rsc14, and Htl1 subunits may serve as an interface between specific signaling pathways (such as the Pkc1 pathway) and the RSC complex, thus helping to regulate RSC recruitment in response to cellular signals. Future studies will test these hypotheses to arrive at a mechanistic understanding of the module’s function.

**The scope of RSC function:** Strains lacking Rsc7 are deficient in RSC assembly yet remain viable. As Rsc7 affects both core functions (via Rsc8), and module functions (via Rsc3), the $rsc7\Delta$ strain can be utilized to examine the full scope of RSC function. We note that our work is the first in which a $rsc$ mutant was utilized to interrogate the entire SGA array. We identified
45 non-essential null mutations that conferred increased sickness when combined with the \textit{rsc7}\textDelta mutation and identified links between Rsc7 and chromatin/transcription, chromosome metabolism, cell wall, and transport. A significant challenge for future studies will be to determine whether the genetic interactions revealed via SGA involve a direct role for RSC in these processes, or an indirect role via transcriptional misregulation.

\textit{Chromatin/transcription:} The SGA screen identified synthetic sick interactions with subunits from 13 different complexes with various roles in transcription and chromatin regulation (Table 4). As transcriptional regulation requires the cooperative effort of multiple regulatory complexes at each gene, we suggest that impaired RSC function renders the cell totally reliant on the full function of other transcription-related complexes.

\textit{Chromosome metabolism and the spindle checkpoint pathway:} The \textit{rsc7}\textDelta mutation confers a G2/M bias (Figure 6), as do mutations in other RSC members such as, Rsc3, Htl1, Rsc9, Sth1, and Sfh1 (\textsc{ANGUS-HILL} \textit{et al.} 2001; \textsc{BAETZ} \textit{et al.} 2004; \textsc{CAO} \textit{et al.} 1997; \textsc{CHAI} \textit{et al.} 2002; \textsc{DAMELIN} \textit{et al.} 2002; \textsc{HSU} \textit{et al.} 2003; \textsc{ROMEO} \textit{et al.} 2002), and suggests that Rsc7 has a role in chromosome transmission. Furthermore, Rsc7 has genetic interactions with other components involved in chromosome maintenance and severely reduced growth ability on media containing benomyl (Figure 2 and Table 4). Surprisingly, the \textit{rsc7}\textDelta mutant does not display significant chromosome missegregation defects, in contrast to many other \textit{rsc} mutations (\textsc{BAETZ} \textit{et al.} 2004; \textsc{HSU} \textit{et al.} 2003; \textsc{HUANG} \textit{et al.} 2004; \textsc{LANZUOLO} \textit{et al.} 2001; \textsc{TSUCHIYA} \textit{et al.} 1998). Therefore, we propose that the low level of RSC assembly in \textit{rsc7}\textDelta mutants confer mild defects that are exacerbated in combination with additional perturbations to the chromosome segregation process (such as the addition of benomyl).
We further show that Rsc7 is linked to both cohesin and centromere functions. Previous experiments by others have shown that RSC has at least two roles in chromosome metabolism: (1) cohesin loading on chromosome arms and (2) centromere function. First, RSC physically and genetically interacts with cohesin subunits, and occupies cohesin binding sites on chromosome arms. Furthermore, mutations in Sth1 and Rsc2 prevent cohesin loading to chromosome arms (Baetz et al. 2004; Huang et al. 2004). Second, RSC affects centromeric chromatin structure independent of cohesin function (Hsu et al. 2003; Tsuchiya et al. 1998). Our work identified genetic interactions between Rsc7 and components required for cohesin function (Ctf4 and Ctf8) and a component required for centromere function (Cbf1), thus strengthening the connection between RSC and both of these processes. Furthermore, we identify genetic interactions with components involved in other areas of chromosome metabolism that may lead to new RSC functions (Table 4).

**Cell wall integrity:** Previous experiments have linked RSC to cell wall function (Angus-Hill et al. 2001; Chai et al. 2002; Corbacho et al. 2004; Hosotani et al. 2001; Jones et al. 2003; Kasten et al. 2004; Romeo et al. 2002). Here, we show that rsc7Δ and rsc14Δ mutants have clear cell wall defects. More importantly, we identify cell wall regulators that interact genetically with Rsc7. Among these are Gas1 (which regulates cross-linking of cell wall glucans), and the Fyv1 and Fyv5 proteins (which influence cell wall glucan levels). Interestingly, mutations in all components of the yeast-specific RSC module, and not other subunits (unpublished data), confer cell wall defects, suggesting an important module function in regulating cell wall integrity.

**DNA damage response:** Our work supports and extends links between RSC and the DNA double-strand break repair (Koyama et al. 2002). Rsc8 and Rsc30 were identified in a screen for
novel non-homologous end joining (NHEJ) factors, and mutations in either of these RSC components lead to an impairment in NHEJ. Furthermore, RSC is recruited to double-strand breaks and interacts with other NHEJ factors such as Mre11 and Ku80 (Shim et al. 2005). Very recently, RSC was shown to have roles in double-strand break repair via homologous recombination (Chai et al. 2005). Indeed, our analysis of the rsc7Δ mutation strengthens this link as the rsc7Δ mutation confers sensitivity to an assortment of DNA damaging agents. Interestingly, we find that cells lacking Rsc7 are compromised for growth in cells lacking Mec3 (part of a putative sliding clamp loading complex required for double strand break repair) (Table 4) and Rad9 (a DNA repair checkpoint protein) (Figure 6).

**Conclusion:** The RSC complex is now emerging as a mosaic of modules with specialized functions. Understanding how these modules and their individual subunits function is of critical importance to understanding overall complex regulation. Here, we have characterized two components in the remodeler RSC, Rsc7 and Rsc14, which have led to the identification of a fungal-specific functional module. Importantly, Rsc7 and Rsc14 help mediate the association between the essential Rsc3 component and RSC, and the rsc7Δ and rsc14Δ mutations have given insight into the in vivo functions of the module. Thus, our studies on Rsc7 and Rsc14 have broadened our understanding of RSC assembly, led to the identification of several subunits which cooperate physically/functionally in RSC, and strengthened links between RSC and many important cellular processes.

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FIGURE 1.— Rsc7 and Swp82 are paralogs found in RSC and SWI/SNF, whereas Rsc14 is unique in RSC. (A) The three regions of Rsc7: (1) a non-conserved acidic amino terminus, (2) a region conserved in Swp82 and other fungi, and (3) a C-terminal region conserved in other fungi with low/no homology to Swp82. (B) Purified SWI/SNF and RSC. SWI/SNF was purified to homogeneity and stained with silver (CAIRNS et al. 1994), and RSC was purified to homogeneity and stained with Coomassie (CAIRNS et al. 1996). For SWI/SNF, the small components (Tfg3 and Snf11) are not shown. (C) Verification of Rsc7 association with RSC components. A Rsc7.13×Myc derivative was immunoprecipitated (from an extract derived from YBC834) with protein G-agarose beads coupled to the α-Myc antibody (labeled α-Myc), or beads alone (labeled control). Beads were washed with a wash buffer containing 250mM NaCl, and proteins were eluted with SDS. Eluates were separated on a 7.5% acrylamide-SDS gel (input 25%, supernatant 25%, eluate/pellet 100%), transferred to a PVDF membrane, and immunoblotted with the polyclonal antibodies anti-Sth1 and anti-Arp9. (D) Verification of Swp82 association with SWI/SNF components. Association of Swp82 with SWI/SNF was determined by the methods used in part c except with a Swp82.13×Myc derivative (from an extract derived from YBC818) and probing with antibodies raised against Swi3 and Swp73 (input 50%, supernatant 19%, Eluate/pellet 50%). (E) Verification of Rsc14 association with RSC components. Association of Rsc14 with RSC was determined by the methods used in part c except with a Rsc14.13×Myc derivative (from an extract derived from YBC1946) and probing with antibodies raised against Sth1 and Arp9 (input 25%, supernatant 25%, pellet/eluate 100%).
FIGURE 2.—Growth ability of a \textit{rsc7}Δ strain and dosage suppression by \textit{RSC3} or sorbitol. (A) Growth ability of \textit{rsc7}Δ strains. Wild type (YBC62) and \textit{rsc7}Δ (YBC1332) strains were grown in liquid medium to log phase, subjected to ten-fold serial dilution (four spots per row) or five-fold serial dilution (six spots per row), and spotted on solid medium containing the compounds indicated. \textit{rsc7}Δ phenotypes were complemented by a plasmid-borne \textit{RSC7}+ (p137). We note that the incomplete complementation of the benomyl phenotype by a low-copy plasmid-borne \textit{RSC7}+ is likely due to lower levels of Rsc7 expression as this construct lacks the full promoter/upstream region. (B) An increased dosage of \textit{RSC3}+ (p1310) partially suppresses certain \textit{rsc7}Δ phenotypes. (C) Sorbitol suppresses the \textit{rsc7}Δ \textit{Ts−} phenotype.

FIGURE 3.—Growth ability of a \textit{rsc14}Δ strain and dosage suppression by \textit{RSC3}. Wild type (YBC1895) and \textit{rsc14}Δ (YBC1926) strains were grown in liquid medium to log phase, subjected to five-fold serial dilution, and spotted on media containing the compounds indicated. \textit{rsc14}Δ phenotypes were complemented by plasmid-borne \textit{RSC14}+ (p1401, CEN; p1403 2\(\mu\)), and suppressed by a plasmid-borne \textit{RSC3}+ (p1310). We note that the incomplete complementation by a low-copy plasmid-borne \textit{RSC14}+ is likely due to lower levels of Rsc14 expression as this construct lacks the full promoter/upstream region. Indeed, a high-copy plasmid-borne \textit{RSC14}+ fully complements the \textit{rsc14}Δ phenotypes.

FIGURE 4.—Rsc7 and Rsc14 are structural components of RSC. (A) Loss of Rsc7 leads to Rsc8 instability and failure of Rsc8 to associate with other RSC components. Extracts were derived from wild type (YBC62) and \textit{rsc7}Δ (YBC1332) strains harboring the plasmid p186 (encoding Rsc8.3×HA). Rsc8.3×HA protein was immunoprecipitated using an HA antibody (\(\alpha\)-HA)
coupled to magnetic beads or beads alone (control). Beads were washed and eluates were separated on a 7.5% acrylamide-SDS gel, immunoblotted to PVDF and probed with anti-Rsc3, -Sth1 or -HA antibodies (input 40%, eluate/pellet 100%). (B) Loss of Rsc14 leads to failure of Rsc8 to associate with Rsc3 and Rsc30. Extracts were derived from wild type (YBC1894) and rsc14Δ (YBC1928) strains harboring the plasmid p186 (encoded Rsc8.3×HA). Rsc8.3×HA protein was immunoprecipitated using an HA antibody (α-HA) coupled to magnetic beads, or beads alone (control). Precipitated proteins were analyzed by the methods used in part a (input 25%, eluate/pellet 100%). (C) Rsc14 associates with Sth1 in a rsc7Δ strain. Extracts were derived from wild type (YBC2297) and rsc7Δ (YBC2281) strains. Rsc14.3×HA protein was immunoprecipitated using an HA antibody coupled to protein G agarose. Precipitated proteins were analyzed by the methods used in part a and b (input 10%, beads 100%).

FIGURE 5. —Growth defects conferred by rsc8 are suppressed through increased dosage of RSC7 or HTL1. A rsc8 mutant strain (J. Lenkart and B. R. Cairns, unpublished) harboring high-copy plasmids, p136 (RSC7+), p1310 (RSC3+), p1204 (HTL1+) p185 (RSC8+), or pRS426 (empty vector), were grown and spotted as in Figure 2.

FIGURE 6.—Links between Rsc7 and the spindle checkpoint pathway. (A) The rsc7Δ mad1Δ double mutation confers severe growth defects. Wild type (YBC62), rsc7Δ (YBC1332), mad1Δ (YBC936), rad9Δ (YBC939), rsc7Δ mad1Δ (YBC1470), and rsc7Δ rad9Δ (YBC1944) strains were grown to log phase, subjected to a five-fold serial dilution, and spotted onto rich media. (B) The rsc7Δ mutation confers a G2/M bias. Wild type (YBC62) and rsc7Δ (YBC1332) strains were grown to log phase in rich medium and analyzed by FACS.
FIGURE 7.—A yeast-specific module containing Rsc7, Rsc14, Htl1, Rsc30, and Rsc3. The physical and functional connections among members are indicated: pink boxes represent IP relationships, red boxes indicate a two-hybrid interaction, light blue arrows indicate dosage suppression.
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lys2Δ0/LYS2 ura3Δ0/ura3Δ0 rsc14Δ::KanMX/RSC14

YBC1926  MATα his3Δ1 leu2Δ0 ura3Δ0 rsc14Δ::KanMX  This study
[p1402;pRS316.RSC14]

YBC1927  MATα his3Δ1 leu2Δ0 ura3Δ0 rsc14Δ::KanMX  This study
[p1402;pRS316.RSC14]

YBC1887  MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 rsc14Δ::KanMX  Research Genetics

YBC2297  MATα his3 leu2Δ ura3 trp1Δ63 lys2
Rsc14.13×MYC.His3MX6

YBC2281  MATα his3 leu2Δ ura3 trp1Δ63 lys2 rsc7Δ::LEU2  This study
Rsc14.13×MYC.His3MX6

YBC939  MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 rad9Δ::Kanmx  Research Genetics

YBC936  MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 mad1Δ::Kanmx  Research Genetics

YBC1470  MATα his3 leu2Δ0 lys2 ura3 rsc7Δ::HIS3  This study
mad1Δ::KanMX [p137;YCP50.RSC7]

YBC1944  MATα his3 leu2Δ0 lys2 ura3 rsc7Δ::HIS3  This study
rad9Δ::KanMX

YBC1918  MATα his3Δ1 leu2Δ0 ura3Δ0 rsc14Δ::KanMX  This study
rsc7Δ::HIS3 [p137;YCP50.RSC7]

YBC1349  MATα his3Δ200 leu2Δ1 lys2-128∂ trp1Δ63 ura3-52
htlΔ::TRP1[p1127;pRS316.HTL1]  (ROMEO et al. 2002)

YBC1984  MATα lys2-128∂ leu2Δ1 ura3-52 trp1Δ63 his3Δ200  This study
htl1Δ::LEU2 [p1127;pRS316.HTL1]

YBC1429 MATa his3Δ200 leu2Δ1 lys2-128Δ trp1Δ63 ura3-52 rsc7Δ::HIS3 htl1Δ::TRP1 [p1127;pRS316.HTL1]

This study

YBC842 MATa his3Δ200 leu2Δ1 lys2-128Δ trp1Δ63 ura3-52 [p746;pRS315.rsc3-2]

(ANGUS-HILL et al. 2001)

YBC1393 MATa his3Δ200 leu2Δ1 lys2-128Δ trp1Δ63 ura3-52 rsc7Δ::HIS3 rsc3Δ::HIS3 [p137;YCP50.RSC7]

[p746;pRS315.rsc3-2]

YBC906 MATa his3Δ200 leu2Δ1 lys2-128Δ trp1Δ63 ura3-52 rsc3Δ::HIS3 [p817;pRS315.rsc3-3]

(ANGUS-HILL et al. 2001)

YBC1430 MATa his3Δ200 leu2Δ1 lys2-128Δ trp1Δ63 ura3-52 rsc7Δ::HIS3 rsc3Δ::HIS3 [p137;YCP50.RSC7]

[p817;pRS315.rsc3-3]

YBC828 MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 ade2Δ::hisG rsc30Δ::LEU2 [p731;pRS316.RSC30]

(ANGUS-HILL et al. 2001)

YBC1386 MATa his3Δ200 leu2 lys2 trp1Δ63 ura3 ade2Δ::hisG rsc7Δ::HIS3 rsc30Δ::LEU2 [p137;YCP50.RSC7]

This study

YBC1156 MATa his3 leu2Δ1 trp1Δ63 ura3-52 rsc9-1

(DAMELIN et al. 2002)

YBC1381 MATa his3 leu2Δ1 lys2-128Δ trp1Δ63 ura3-52 rsc7Δ::HIS3 rsc9-1[p137;YCP50.RSC7]

This study

YBC849 MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 ade2Δ::hisG rsc1Δ::LEU2

(CAIRNS et al. 1999)
YBC1505  MATa his3Δ200 leu2Δ0 lys2 trp1Δ63 ura3 rsc7Δ::HIS3  This study
          rsc1Δ::LEU2 [p137;YCP50.RSC7]
YBC79   MATa his3Δ200 leu2Δ1 lys2-128Δ trp1Δ63 ura3-52          (CAIRNS et al.
          rsc2Δ::LEU2    1999)
YBC1507  MATa his3Δ200 leu2Δ1 lys2-128Δ trp1Δ63 ura3-52  This study
          rsc2Δ::LEU2    rsc7Δ::HIS3 [p137;YCP50.RSC7]
YBC2006  MATa his3 leu2 trp1Δ63 ura3 htl1Δ::TRP1    This study
          rsc14Δ::KanMX [p1127;pRS316.HTL1]
YBC2028  MATa lys2 leu2 ura3 trp1Δ63 his3 rsc3Δ::HIS3  This study
          [p746]    rsc14Δ::KanMX [p746;pRS315.rsc3-2]
YBC2028  MATa lys2 leu2 ura3 trp1Δ63 his3 rsc3Δ::HIS3  This study
          [p817-3A] rsc14Δ::KanMX [p817.pRS315.rsc3-3]
YBC1968  MATa lys2-128Δ leu2Δ1 ura3-52 trp1Δ63 his3Δ200  This study
          rsc7Δ::LEU2 [p137;YCP50.RSC7]
YBC2039  MATa lys2-128Δ leu2Δ1 ura3-52 trp1Δ63 his3Δ200  This study
          rsc7Δ::LEU2 can1Δ::MFA1pr-HIS3 [p137;YCP50.RSC7]
YBC2014  MATa lys2-128Δ leu2Δ1 ura3-52 trp1Δ63 his3Δ200  This study
          rsc71-224.13Xmyc.TRP1 [p137;YCP50.RSC7]
YBC2048  MATa lys2-128Δ leu2Δ1 ura3-52 trp1Δ63 his3Δ200  This study
          rsc71-370.13XMYC.TRP1 [p137;YCP50.RSC7]
YBC2017  MATa lys2-128Δ leu2Δ1 ura3-52 trp1Δ63 his3Δ200  This study
          rsc71-414.13×MYC.TRP1 [p137;YCP50.RSC7]
TABLE 2
*rsc7Δ rsc* double mutants are inviable

<table>
<thead>
<tr>
<th>RSC7 allele</th>
<th>rsc allele</th>
<th>Growth at 28˚C on 5-FOA</th>
<th>Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>WT</td>
<td>++++</td>
<td>YBC62</td>
</tr>
<tr>
<td>rsc7Δ</td>
<td>WT</td>
<td>+++</td>
<td>YBC1332</td>
</tr>
<tr>
<td>WT</td>
<td>rsc14Δ</td>
<td>++++</td>
<td>YBC1927</td>
</tr>
<tr>
<td>rsc7Δ</td>
<td>rsc14Δ</td>
<td>-</td>
<td>YBC1918</td>
</tr>
<tr>
<td>WT</td>
<td>htl1Δ</td>
<td>++++</td>
<td>YBC1349</td>
</tr>
<tr>
<td>rsc7Δ</td>
<td>htl1Δ</td>
<td>-</td>
<td>YBC1429</td>
</tr>
<tr>
<td>WT</td>
<td>rsc3-2</td>
<td>++++</td>
<td>YBC842</td>
</tr>
<tr>
<td>rsc7Δ</td>
<td>rsc3-2</td>
<td>-</td>
<td>YBC1393</td>
</tr>
<tr>
<td>WT</td>
<td>rsc3-3</td>
<td>++++</td>
<td>YBC906</td>
</tr>
<tr>
<td>rsc7Δ</td>
<td>rsc3-3</td>
<td>-</td>
<td>YBC1430</td>
</tr>
<tr>
<td>WT</td>
<td>rsc30Δ</td>
<td>+++</td>
<td>YBC828</td>
</tr>
<tr>
<td>rsc7Δ</td>
<td>rsc30Δ</td>
<td>-</td>
<td>YBC1386</td>
</tr>
<tr>
<td>WT</td>
<td>rsc9-1</td>
<td>+++</td>
<td>YBC1156</td>
</tr>
<tr>
<td>rsc7Δ</td>
<td>rsc9-1</td>
<td>-</td>
<td>YBC1381</td>
</tr>
<tr>
<td>WT</td>
<td>rsc1Δ</td>
<td>+++</td>
<td>YBC849</td>
</tr>
<tr>
<td>rsc7Δ</td>
<td>rsc1Δ</td>
<td>-</td>
<td>YBC1505</td>
</tr>
<tr>
<td>WT</td>
<td>rsc2Δ</td>
<td>+++</td>
<td>YBC79</td>
</tr>
<tr>
<td>-------</td>
<td>-------</td>
<td>-----</td>
<td>-------</td>
</tr>
<tr>
<td>rsc7Δ</td>
<td>rsc2Δ</td>
<td>-</td>
<td>YBC1507</td>
</tr>
</tbody>
</table>

Strains refer to the covered version bearing the \textit{RSC7}^+\textit{URA3}, \textit{RSC14}^+\textit{URA3} or \textit{HTL1}^+\textit{URA3} plasmids. Inviability (-) is determined through a lack of growth ability on media containing 5-FOA.
### TABLE 3

**rsc14Δ rsc mutants are inviable**

<table>
<thead>
<tr>
<th>RSC14 allele</th>
<th>rsc allele</th>
<th>Growth at 28°C</th>
<th>Strain(^1) on 5-FOA</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSC14</td>
<td>WT</td>
<td>++++</td>
<td>YBC62</td>
</tr>
<tr>
<td>rsc14Δ</td>
<td>WT</td>
<td>++++</td>
<td>YBC1927</td>
</tr>
<tr>
<td>WT</td>
<td>htl1Δ</td>
<td>++++</td>
<td>YBC1984</td>
</tr>
<tr>
<td>rsc14Δ</td>
<td>htl1Δ</td>
<td>-</td>
<td>YBC2006</td>
</tr>
<tr>
<td>WT</td>
<td>rsc3-2</td>
<td>++++</td>
<td>YBC842</td>
</tr>
<tr>
<td>rsc14Δ</td>
<td>rsc3-2</td>
<td>-</td>
<td>YBC2028 [p746]</td>
</tr>
<tr>
<td>WT</td>
<td>rsc3-3</td>
<td>++++</td>
<td>YBC906</td>
</tr>
<tr>
<td>rsc14Δ</td>
<td>rsc3-3</td>
<td>-</td>
<td>YBC2028 [p817]</td>
</tr>
</tbody>
</table>

\(^1\) Strains refer to the covered version bearing the RSC7\(^+\)-URA3, RSC14\(^+\)-URA3 or HTL1\(^+\)-URA3 plasmids. Inviability (-) is determined through a lack of growth ability on media containing 5-FOA.
### TABLE 4

Null mutations conferring growth defects in combination with the *rsc7Δ* mutation

<table>
<thead>
<tr>
<th>Broad Category</th>
<th>Processes</th>
<th>Complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transcription/Chromatin</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>ARD1</em></td>
<td>Transcription</td>
<td>ARD1</td>
</tr>
<tr>
<td><em>SDS3</em> <em>DEP1</em></td>
<td>Transcription</td>
<td>ECM16</td>
</tr>
<tr>
<td><em>ELP2</em> <em>ELP3</em> <em>ELP6</em> <em>IKI3</em></td>
<td>Transcription</td>
<td>ELONGATOR</td>
</tr>
<tr>
<td><em>GIM4</em></td>
<td>Transcription</td>
<td>GIM</td>
</tr>
<tr>
<td><em>ARP8</em></td>
<td>Transcription</td>
<td>INO80</td>
</tr>
<tr>
<td><em>MED1</em> <em>SSN8</em> <em>SSN2</em> <em>SRB8</em></td>
<td>Transcription</td>
<td>MEDIATOR</td>
</tr>
<tr>
<td><em>RTF1</em></td>
<td>Transcription</td>
<td>PAF1</td>
</tr>
<tr>
<td><em>BRE1</em></td>
<td>Transcription</td>
<td>RAD6</td>
</tr>
<tr>
<td><em>RSC2</em></td>
<td>Transcription</td>
<td>RSC</td>
</tr>
<tr>
<td><em>SPT3</em> <em>NGG1</em> <em>SGF73</em> <em>GCN5</em></td>
<td>Transcription</td>
<td>SAGA</td>
</tr>
<tr>
<td><em>BRE2</em> <em>SWD1</em></td>
<td>Transcription</td>
<td>SET1</td>
</tr>
<tr>
<td><em>SWR1</em> <em>SWC1</em> <em>ARP6</em></td>
<td>Transcription</td>
<td>SWR1</td>
</tr>
<tr>
<td><em>HTZ1</em> <em>AOR1</em> <em>VPS72</em> <em>VPS71</em></td>
<td>Transcription</td>
<td>SWR1</td>
</tr>
<tr>
<td><em>SET2</em> <em>CTK2</em></td>
<td>Transcription Elongation</td>
<td></td>
</tr>
<tr>
<td><em>LSM6</em> <em>SSF1</em> <em>SNT309</em> <em>SNU66</em></td>
<td>RNA processing</td>
<td></td>
</tr>
<tr>
<td><em>ASF1</em></td>
<td>Chromatin assembly</td>
<td></td>
</tr>
</tbody>
</table>

Chromosome Metabolism
The *rsc7Δ* strain (YBC2039) was mated to the yeast haploid deletion set. Heterozygous double mutants were selected and sporulated on solid media. Haploid double mutant strains (harboring the plasmid-borne *RSC7*+) were then isolated. These strains were scored as sick/lethal by their...
ability to lose the *URA3*-marked *RSC7*<sup>+</sup> plasmid on solid media containing 5-FOA. Boldface genes were confirmed by tetrad dissection and/or random spore analysis, while other genes were identified in our screen but not verified.

* The protein encoded by the *RTT103* gene also has a role in transcription termination
Wilson et al. and Cairns
Figure 2A

A

Wild Type
rsc7Δ
rsc7Δ [RSC7\(^+\)CEN]
YPD 28\(^{\circ}\)  YPD 35\(^{\circ}\)  YPD 35\(^{\circ}\)

Wild Type
rsc7Δ
rsc7Δ [RSC7\(^+\)CEN]
YPD 28\(^{\circ}\)  YPD 28\(^{\circ}\)
+Formamide (2\(\%\))  +NaCl (0.5M)

Wild Type
rsc7Δ
rsc7Δ [RSC7\(^+\)CEN]
YPD 28\(^{\circ}\)  YPD 35\(^{\circ}\)
+UV 8000 uJ/cm\(^2\)  +HU (150mM)

Wild Type
rsc7Δ
rsc7Δ [RSC7\(^+\)CEN]
YPD 28\(^{\circ}\)  YPD 33\(^{\circ}\)

Wild Type
rsc7Δ
rsc7Δ [RSC7\(^+\)CEN]
YPD 28\(^{\circ}\)  YPD 33\(^{\circ}\)
+Benomyl (12.5 ug/ml)  +MMS (0.03\(\%\))

Wild Type
rsc7Δ
rsc7Δ [RSC7\(^+\)CEN]
SC-LEU 28\(^{\circ}\)  SC-LEU 28\(^{\circ}\)
+Congo Red (800μg/ml)
Wilson et al. and Cairns
Figure 2B and 2C

B

Wild Type
rsc7Δ
rsc7Δ [RSC7\(^+\) CEN]
rsc7Δ [RSC3\(^+\) 2\(\mu\)]

SC 28°
SC 35°

C

Wild Type
rsc7Δ [Empty Vector]
rsc7Δ [RSC7\(^+\) CEN]

YPD 35°
YPD 35°
+ Sorbitol (1M)
+ NaCl (1.2 M)
+ Formamide (2%)
Figure 3

Wild Type

\( rsc14\Delta \)

\( rsc14\Delta \ [RSC14^{+}CEN] \)

\( rsc14\Delta \ [RSC14^{+}2\mu] \)

\( rsc14\Delta \ [RSC3^{+}2\mu] \)

YPD 28°

YPD 38°

Wild Type

\( rsc14\Delta \)

\( rsc14\Delta \ [RSC14^{+}CEN] \)

\( rsc14\Delta \ [RSC14^{+}2\mu] \)

\( rsc14\Delta \ [RSC3^{+}2\mu] \)

YPD 28° + NaCl (1.2M)

YPD 38° + NaCl (1.2M)

Wild Type

\( rsc14\Delta \)

\( rsc14\Delta \ [RSC14^{+}CEN] \)

\( rsc14\Delta \ [RSC14^{+}2\mu] \)

\( rsc14\Delta \ [RSC3^{+}2\mu] \)

YPD 28° + Caffeine (15mM)

YPD 38° + Formamide (2%)

Wild Type

\( rsc14\Delta \)

\( rsc14\Delta \ [RSC14^{+}CEN] \)

SC-Leu 38°

SC-Leu 38° + Congo Red (800μg/ml)
Wilson et al. and Cairns
Figure 4A, 4B, and 4C
Figure 5

Wild Type
rsc8 [RSC8^+2μ]
rsc8 [empty vector]
rsc8 [RSC7^+2μ]
rsc8 [RSC3^+2μ]
rsc8 [HTL1^+2μ]

Wild Type
rsc8 [RSC8^+2μ]
rsc8 [empty vector]
rsc8 [RSC7^+2μ]
rsc8 [RSC3^+2μ]
rsc8 [HTL1^+2μ]

Wild Type
rsc8 [RSC8^+2μ]
rsc8 [empty vector]
rsc8 [RSC7^+2μ]
rsc8 [RSC3^+2μ]
rsc8 [HTL1^+2μ]
Wilson et al. and Cairns
Figure 6A and 6B

A

Wild Type
rsc7Δ
mad1Δ
rad9Δ
rsc7Δ mad1Δ
rsc7Δ rad9Δ

B

Wild Type 28°
rsc7Δ 28°