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

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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 *rsc7*Δ and *rsc14*Δ 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 nonessential null mutations (SGA) we identified mutations that are sick/lethal in combination with the *rsc7*Δ 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.

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 (Jenuwein and Allis 2001; Becker and Horz 2002).

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 uses 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 that 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) (Tsukiyama 2002; Martens and Winston 2003; Mohrmann and Verrijzer 2005). 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 with the catalytic subunit enable remodeling within twofold 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

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DNA binding domain and Rsc4 contains bromodomains that 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 (Cao *et al.* 1997; Treich and Carlson 1997; Cairns *et al.* 1998, 1999; Treich *et al.* 1998; Damelin *et al.* 2002; Saha *et al.* 2002; Bungard *et al.* 2004; 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; Romeo *et al.* 2002; Kasten *et al.* 2004). The cell wall integrity pathway is a kinase cascade that 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 *pkc1*Δ *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 nonessential 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 (Romeo *et al.* 2002; Kasten *et al.* 2004). 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 *rsc7*Δ strain to a library of nonessential deletion mutants and identified a broad spectrum of null mutations that conferred growth defects in combination with the *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 *S. cerevisiae* strains are S288C derivatives. Yeast strains are listed in Table 1. Standard procedures were used for mating, sporulation, transformation, and tetrad dissection. All media were prepared as described previously (Rose *et al.* 1990). The null mutations for *rsc7*, *swp82*, and *rsc14* were constructed using standard methods (Baudin *et al.* 1993; Lorenz *et al.* 1995) and verified by PCR analysis. All primer sequences for PCR reactions are available upon request. Alleles of *rsc3*, *htl1*, *rsc9*, *rsc30*, *rsc1*, and *rsc2* have been described previously (Cairns *et al.* 1999; Angus-Hill *et al.* 2001; Damelin *et al.* 2002; Romeo *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 *et al.* 1998). A *rsc7*Δ strain was modified for use in synthetic genetic array analysis. Briefly, a *MFA1pr-HIS3* cassette was integrated into the genomic *CAN1* locus of YBC1968 (using standard procedures, Baudin *et al.* 1993; Lorenz *et al.* 1995) and confirmed by PCR analysis. The mating-type locus of this strain was then converted from *MAT***a** to *MAT*α to generate YBC2039. All other null mutations (including mutations from the nonessential haploid deletion library) were obtained through Research Genetics (Invitrogen) and are isogenic to either BY4741 or BY4742 (Brachmann *et al.* 1998).

Plasmids: Plasmids p137 and p136 (gifts from Pam Silver) harbor a 2.6-kb *Sna*BI fragment of the genomic *RSC7* locus subcloned into YCP50 or YEP352, respectively. Plasmid p1310 was constructed using a *Xho*I–*Spe*I fragment bearing *RSC3* that was isolated from p906 (pRS314.*RSC3*; Angus-Hill *et al.* 2001) and subcloned 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 (*MET25*pr.*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 *Xho*I and *Xba*I or *Spe*I and *Bam*HI, 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 2 min (three pulses) and 5 min 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 μl of a 50% slurry) or Anti-HA beads (50 μl of slurry) were incubated with 200 μg of the indicated protein extract in immunoprecipitation buffer

TABLE 1

Yeast strains

Strain	Genotype	Source
YBC834	*MAT***a** *his3Δ200 leu2Δ1 lys2-128δ trp1Δ63 ura3-52 rsc7Δ::HIS3* [p739b; YCP50.*RSC7.13×MYC.TRP1]*	This study
YBC818	*MATα his3Δ200 leu2Δ0 met15Δ0 ura3Δ0 trp1Δ63 lys2Δ0 SWP82.13×MYC.TRP1*	This study
YBC1946	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 RSC14.13MYC.his3MX6*	This study
YBC62	*MAT***a** *his3Δ200 leu2Δ1 lys2-128δ trp1Δ63 ura3-52*	Cairns lab
YBC63	*MATα his3Δ200 leu2Δ1 lys2-128δ trp1Δ63 ura3-52*	Cairns lab
BY4741	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Research Genetics
BY4742	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	Research Genetics
YBC1332	*MAT***a** *his3Δ200 leu2Δ1 lys2-128δ trp1Δ63 ura3-52 rsc7Δ::HIS3* [p137;YCP50.*RSC7*]	This study
YBC1333	*MATα his3Δ200 leu2Δ1 lys2-128δ trp1Δ63 ura3-52 rsc7Δ::HIS3* [p137;YCP50.*RSC7*]	This study
YBC1333A	*MATα his3Δ200 leu2Δ1 lys2-128δ trp1Δ63 ura3-52 rsc7Δ::HIS3* [p935;*MET25*pr.*rsc7* 248-435.13×*MYC.TRP1*]	This study
YBC76	*MAT***a**/α *his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 lys2-128δ/lys2-128δ trp1Δ63/trp1Δ63 ura3-52/ ura3-52*	Cairns lab
YBC1888	*MAT***a**/α *his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 met15Δ0/MET15 lys2Δ0/LYS2 ura3Δ0/ura3Δ0 rsc14Δ::KanMX/RSC14*	Research Genetics
YBC1926	*MATα his3Δ1 leu2Δ0 ura3Δ0 rsc14Δ::KanMX* [p1402;pRS316.*RSC14*]	This study
YBC1927	*MAT***a** *his3Δ1 leu2Δ0 ura3Δ0 rsc14Δ::KanMX* [p1402;pRS316.*RSC14*]	This study
YBC1887	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 rsc14Δ::KanMX*	Research Genetics
YBC2297	*MAT***a** *his3 leu2Δ ura3 trp1Δ63 lys2 Rsc14.13×MYC.His3MX6*	This study
YBC2281	*MATα his3 leu2Δ ura3 trp1Δ63 lys2 rsc7Δ::LEU2 Rsc14.13×MYC.His3MX6*	This study
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 mad1Δ::KanMX* [p137;YCP50.*RSC7*]	This study
YBC1944	*MATα his3 leu2Δ0 lys2 ura3 rsc7Δ::HIS3 rad9Δ::KanMX*	This study
YBC1918	*MAT***a** *his3Δ1 leu2Δ0 ura3Δ0 rsc14Δ::KanMX rsc7Δ::HIS3* [p137;YCP50.*RSC7*]	This study
YBC1349	*MATα his3Δ200 leu2Δ1 lys2-128δ trp1Δ63 ura3-52 htl1Δ::TRP1*[p1127;pRS316.*HTL1*]	Romeo *et al.* (2002)
YBC1984	*MAT***a** *lys2-128∂ leu2Δ1 ura3-52 trp1Δ63 his3Δ200 htl1Δ::LEU2* [p1127.pRS316.*HTL1*]	This study
YBC1429	*MAT***a** *his3Δ200 leu2Δ1 lys2-128δ trp1Δ63 ura3-52 rsc7Δ::HIS3 htl1Δ::TRP1* [p1127;pRS316.*HTL1*]	This study
YBC842	*MAT***a** *his3Δ200 leu2Δ1 lys2-128δ trp1Δ63 ura3-52 rsc3Δ::HIS3* [p746,pRS315.*rsc3-2*]	Angus-Hill *et al.* (2001)
YBC1393	*MAT***a** *his3Δ200 leu2Δ1 lys2-128δ trp1Δ63 ura3-52 rsc7Δ::HIS3 rsc3Δ::HIS3* [p137;YCP50.*RSC7*] [p746,pRS315.*rsc3-2*]	This study
YBC906	*MAT***a** *his3Δ200 leu2Δ1 lys2-128δ trp1Δ63 ura3-52 rsc3Δ::HIS3* [p817.pRS315.*rsc3-3*]	Angus-Hill *et al.* (2001)
YBC1430	*MAT***a** *his3Δ200 leu2Δ1 lys2-128δ trp1Δ63 ura3-52 rsc7Δ::HIS3 rsc3Δ::HIS3* [p137;YCP50.*RSC7*] [p817;pRS315.*rsc3-3*]	This study
YBC828	*MAT***a** *his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 ade2Δ::hisG rsc30Δ::LEU2* [p731;pRS316.*RSC30*]	Angus-Hill *et al.* (2001)
YBC1386	*MAT***a** *his3Δ200 leu2 lys2 trp1Δ63 ura3 ade2Δ::hisG rsc7Δ::HIS3 rsc30Δ::LEU2* [p137;YCP50.*RSC7*]	This study
YBC1156	*MAT***a** *his3 leu2Δ1 trp1Δ63 ura3-52 rsc9-1*	Damelin *et al.* (2002)
YBC1381	*MAT***a** *his3 leu2Δ1 lys2-128δ trp1Δ63 ura3-52 rsc7Δ::HIS3 rsc9-1*[p137;YCP50.*RSC7*]	This study
YBC849	*MAT***a** *his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 ade2Δ::hisG rsc1Δ::LEU2*	Cairns *et al.* (1999)
YBC1505	*MAT***a** *his3Δ200 leu2Δ0 lys2 trp1Δ63 ura3 rsc7Δ::HIS3 rsc1Δ::LEU2* [p137;YCP50.*RSC7*]	This study
YBC79	*MAT***a** *his3Δ200 leu2Δ1 lys2-128δ trp1Δ63 ura3-52 rsc2Δ::LEU2*	Cairns *et al.* (1999)
YBC1507	*MAT***a** *his3Δ200 leu2Δ1 lys2-128δ trp1Δ63 ura3-52 rsc2Δ::LEU2 rsc7Δ::HIS3* [p137;YCP50.*RSC7*]	This study
YBC2006	*MAT***a** *his3 leu2 trp1Δ63 ura3 htl1Δ::TRP1 rsc14Δ::KanMX* [p1127;pRS316.*HTL1*]	This study
YBC2028 [p746]	*MAT***a** *lys2 leu2 ura3 trp1Δ63 his3 rsc3Δ::HIS3 rsc14Δ::KanMX* [p746;pRS315.*rsc3-2*]	This study
YBC2028 [p817-3A]	*MAT***a** *lys2 leu2 ura3 trp1Δ63 his3 rsc3Δ::HIS3 rsc14Δ::KanMX* [p817.pRS315.*rsc3-3*]	This study

(*continued*)

TABLE 1

(Continued)

Strain	Genotype	Source
YBC1968	*MAT***a** *lys2-128∂ leu2Δ1 ura3-52 trp1Δ63 his3Δ200 rsc7Δ::LEU2* [p137;YCP50.*RSC7*]	This study
YBC2039	*MAT*α *lys2-128∂ leu2Δ1 ura3-52 trp1Δ63 his3Δ200 rsc7Δ::LEU2 can1Δ::MFA1pr-HIS3* [p137;YCP50.*RSC7*]	This study
YBC2014	*MAT***a** *lys2-128∂ leu2Δ1 ura3-52 trp1Δ63 his3Δ200 rsc71-224.13Xmyc.TRP1* [p137;YCP50.*RSC7*]	This study
YBC2048	*MAT***a** *lys2-128∂ leu2Δ1 ura3-52 trp1Δ63 his3Δ200 rsc71-370.13XMYC.TRP1* [p137;YCP50.*RSC7*]	This study
YBC2017	*MAT***a** *lys2-128∂ leu2Δ1 ura3-52 trp1Δ63 his3Δ200 rsc71-414.13×MYC.TRP1* [p137;YCP50.*RSC7*]	This study

[50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 10% glycerol, 100 mM NaCl, 0.05% Tween-20] (total volume was 140 μl) at 4° for 3.5–7.5 hr. When checking RSC stability in *rsc14*Δ extracts, the immunoprecipitation buffer contained 250 mM NaCl (total immunoprecipitation volume was 400 μl). When checking RSC stability in *rsc7*Δ extracts, the total immunoprecipitation volume was 400 μl. 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 polyvinylidine 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, 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 *rsc7*Δ strain (YBC2039) was mated with the yeast haploid deletion set (BY4741) from Research Genetics (Invitrogen) (catalog no. 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 *MAT***a** 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) or 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, 1996). The 50-kDa 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 copurification), we immunoprecipitated an epitope-tagged Rsc7 derivative (Rsc7.13×Myc) and tested for association with known RSC members. Rsc7.13×Myc efficiently coprecipitated 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 (Uetz *et al.* 2000; Gavin *et al.* 2002; Sanders *et al.* 2002; Baetz *et al.* 2004; Graumann *et al.* 2004) 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-kDa subunit of SWI/SNF as *YFL049w*/Swp82 (data not shown). An epitope-tagged Swp82 (Swp82.13×Myc) efficiently coprecipitates with Swi3 and Swp73 and remains associated even under stringent wash conditions (Figure 1D). These data support and extend proteomic approaches by others who observed Swp82 copurifying with SWI/SNF subunits (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 1.36×10^{-4}). In Figure 1D, 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.

On the basis of alignments with orthologs, we have separated Rsc7 into three regions: (1) an acidic amino

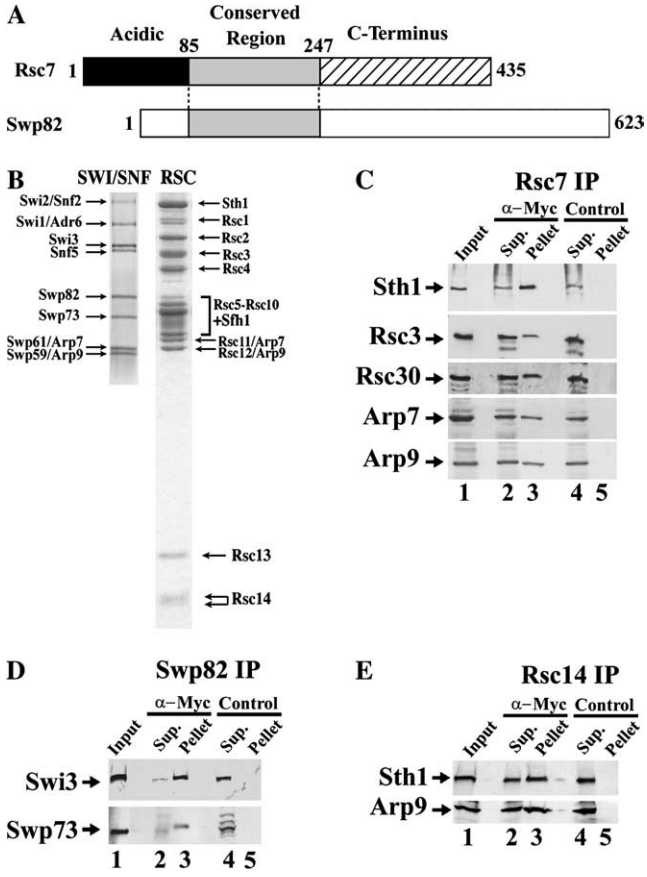

FIGURE 1.—Rsc7 and Swp82 are paralogs found in RSC and SWI/SNF, whereas Rsc14 is unique in RSC. (A) The three regions of Rsc7: (1) a nonconserved acidic amino terminus, (2) a region conserved in Swp82 and other fungi, and (3) a C-terminal region conserved in other fungi with low/no homology to Swp82. (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 250 mM 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 described in 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 described in 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%).

terminus (Figure 1A; black box), (2) a conserved region (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 1.71×10^{-16}), *S. pombe* SPCC1281.05 (BLAST *P*-value 2.02×10^{-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 2.27×10^{-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 2.81×10^{-13}) and CAG60715 (BLAST *P*-value 6.74×10^{-3}) and identified orthologs in other fungal species. Furthermore, searches using the C-terminal nonconserved region of Swp82 helped to identify Swp82 counterparts in closely related hemiascomycete yeast, such as CAG61742 (BLAST *P*-value 1.10×10^{-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 homologs 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 ~15 kDa, 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 13 copies of the Myc epitope (Rsc14.13×Myc). Co-immunoprecipitation

experiments confirmed association between Rsc14 and other members of RSC. Indeed, Rsc14 coprecipitates with Sth1 and Arp9 and remains stably associated under stringent wash conditions (Figure 1E). Our results support recent proteomic studies revealing that Rsc14 copurifies 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 6.07×10^{-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 *rsc7*Δ, *swp82*Δ, and *rsc14*Δ strains: To address the *in vivo* functions of Rsc7, Swp82, and Rsc14 we isolated null alleles and examined their growth characteristics. The *rsc7*Δ haploid spores grew slowly at 28° and were inviable at 37°. Furthermore, the *rsc7*Δ 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 *rsc7*Δ 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 *rsc7*Δ phenotypes, but was produced in the cell (supplemental Table 1 at http://www.genetics.org/supplemental/). To determine the extent to which Rsc7 function depends on 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 *rsc7*Δ phenotypes, showing that the conserved homology region is apparently not required

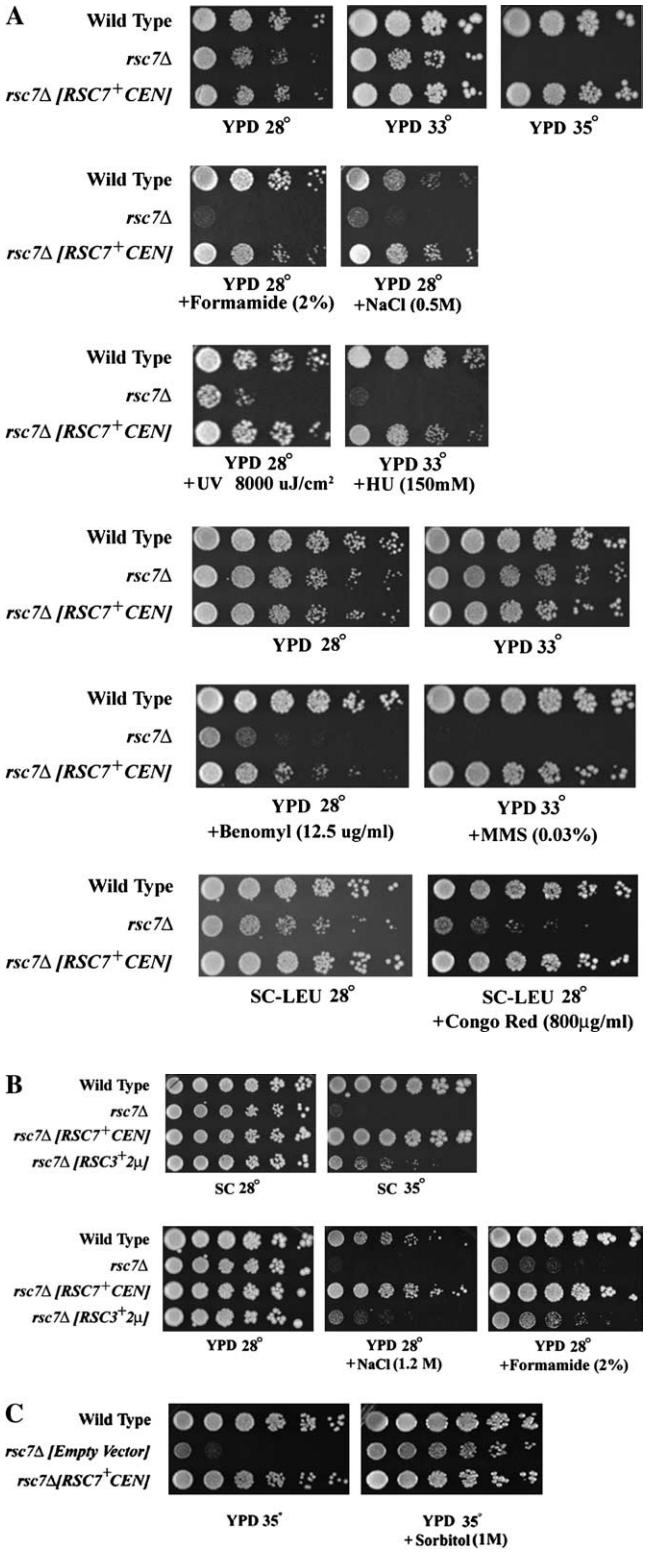

Figure 2.—Growth ability of a *rsc7*Δ strain and dosage suppression by *RSC3* or sorbitol. (A) Growth ability of *rsc7*Δ strains. Wild-type (YBC62) and *rsc7*Δ (YBC1332) strains were grown in liquid medium to log phase, subjected to 10-fold serial dilution (four spots per row) or 5-fold serial dilution (six spots per row), and spotted on solid medium containing the compounds indicated. *rsc7*Δ phenotypes were complemented by a plasmid-borne *RSC7*⁺ (p137). We note that the incomplete complementation of the benomyl phenotype by a low-copy plasmid-borne *RSC7*⁺ is likely due to lower levels of Rsc7 expression as this construct lacks the full promoter/upstream region. (B) An increased dosage of *RSC3*⁺ (p1310) partially suppresses certain *rsc7*Δ phenotypes. (C) Sorbitol suppresses the *rsc7*Δ Ts⁻ phenotype.

for the large spectrum of Rsc7 functions examined (supplemental Table 1 at at http://www.genetics.org/supplemental/).

The *swp82*Δ 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), $ZnSO_4$ (5 mM), NaCl (1.2 M), $MnCl_2$ (6 mM), $CaCl_2$ (200 mM), and $CoCl_2$ (1.5 mM). Furthermore, we tested for sensitivity to ultraviolet radiation (100 μJ/cm^2 × 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), and low pH media (secretory pathway); sensitivity to 6-azauracil (35 μg/ml) (transcription elongation); and sensitivity to cycloheximide (1 μg/ml) (protein synthesis). The *swp82*Δ strain displayed no obvious sporulation or mating defects, and genes displaying altered expression in *swi/snf* mutants (including *SER3*) were not misregulated in a *swp82*Δ strain (data not shown). Finally, the *swp82*Δ *rsc7*Δ double mutant displayed phenotypes indistinguishable from *rsc7*Δ alone (data not shown). Therefore, the role of Swp82 in SWI/SNF remains elusive.

The haploid *rsc14*Δ 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 *rsc14*Δ phenotypes that connect Rsc14 to the cell wall integrity pathway and certain functions shared by Rsc3 and Rsc7.

***rsc7*Δ and *rsc14*Δ mutations are lethal in combination with other *rsc* mutations:** To help identify functional relationships between RSC subunits and Rsc7 we attempted to cross the *rsc7*Δ strain to other *rsc* mutants. However, we found that strains lacking Rsc7 rapidly acquired mutations that conferred spore inviablity (revealed in subsequent mating/sporulation tests, data not shown). To circumvent this problem, *rsc7*Δ*::HIS3/RSC7*$^+$ heterozygous diploids bearing *RSC7*$^+$ on a *URA3*-marked plasmid were sporulated and dissected to generate a haploid *rsc7*Δ 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 *rsc7*Δ double-mutant combinations showed lethality (Table 2).

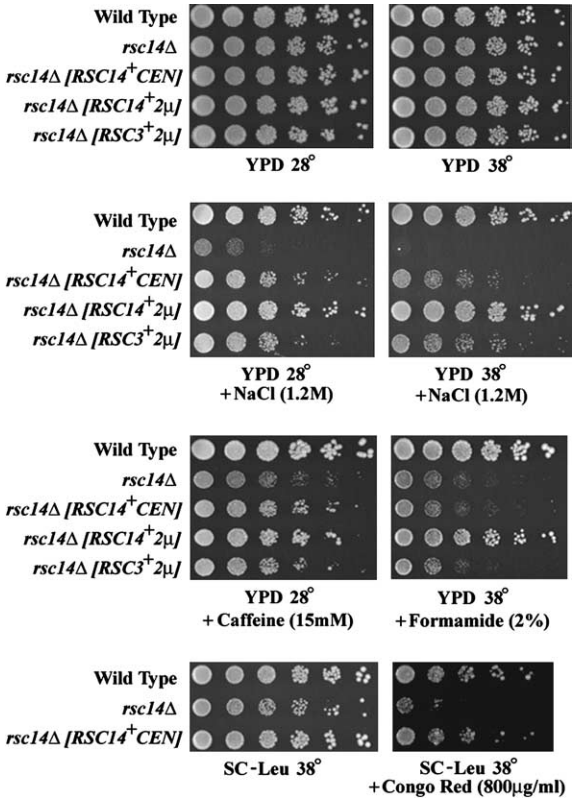

FIGURE 3.—Growth ability of a *rsc14*Δ strain and dosage suppression by *RSC3*. Wild-type (YBC1895) and *rsc14*Δ (YBC1926) strains were grown in liquid medium to log phase, subjected to fivefold serial dilution, and spotted on media containing the compounds indicated. *rsc14*Δ phenotypes were complemented by plasmid-borne *RSC14*$^+$ (p1401, CEN; p1403 2μ), and suppressed by a plasmid-borne *RSC3*$^+$ (p1310). We note that the incomplete complementation by a low-copy plasmid-borne *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 *RSC14*$^+$ fully complements the *rsc14*Δ phenotypes.

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 *rsc14*Δ null with other *rsc* mutants. Similar to the *rsc7*Δ strain, *rsc14*Δ mutants acquired mutations that conferred low spore viability. To circumvent this problem, *rsc14*Δ*::KanMX/RSC14*$^+$ heterozygous diploids bearing *RSC14*$^+$ on a *URA3*-marked plasmid were sporulated and dissected to generate a haploid *rsc14*Δ 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 *rsc7*Δ, *rsc14*Δ 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 *rsc7*Δ raised

TABLE 2

***rsc7*Δ *rsc* double mutants are inviable**

RSC7 allele	*rsc* allele	Growth at 28° on 5-FOA	Strain[a]
RSC7	*WT*	++++	YBC62
*rsc7*Δ	*WT*	+++	YBC1332
WT	*rsc14*Δ	++++	YBC1927
*rsc7*Δ	*rsc14*Δ	−	YBC1918
WT	*htl1*Δ	++++	YBC1349
*rsc7*Δ	*htl1*Δ	−	YBC1429
WT	*rsc3-2*	++++	YBC842
*rsc7*Δ	*rsc3-2*	−	YBC1393
WT	*rsc3-3*	++++	YBC906
*rsc7*Δ	*rsc3-3*	−	YBC1430
WT	*rsc30*Δ	+++	YBC828
*rsc7*Δ	*rsc30*Δ	−	YBC1386
WT	*rsc9-1*	+++	YBC1156
*rsc7*Δ	*rsc9-1*	−	YBC1381
WT	*rsc1*Δ	+++	YBC849
*rsc7*Δ	*rsc1*Δ	−	YBC1505
WT	*rsc2*Δ	+++	YBC79
*rsc7*Δ	*rsc2*Δ	−	YBC1507

[a] 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 3

***rsc14*Δ *rsc* mutants are inviable**

RSC14 allele	*rsc* allele	Growth at 28° on 5-FOA	Strain[a]
RSC14	*WT*	++++	YBC62
*rsc14*Δ	*WT*	++++	YBC1927
WT	*htl1*Δ	++++	YBC1984
*rsc14*Δ	*htl1*Δ	−	YBC2006
WT	*rsc3-2*	++++	YBC842
*rsc14*Δ	*rsc3-2*	−	YBC2028 [p746]
WT	*rsc3-3*	++++	YBC906
*rsc14*Δ	*rsc3-3*	−	YBC2028 [p817]

[a] 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.

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 (Treich and Carlson 1997; Treich *et al.* 1998; Lu *et al.* 2003).We found Rsc8 protein levels greatly reduced in a *rsc7*Δ 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 *rsc7*Δ 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 *rsc7*Δ strain.

The *rsc14*Δ 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; *rsc7*Δ *rsc14*Δ or *rsc3 rsc14*Δ combinations were inviable (Tables 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 *rsc14*Δ strain, Rsc8.3×HA coprecipitates 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) that would predict their co-loss. Together, our data support 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 *rsc7*Δ 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 *rsc7*Δ phenotypes. We found that plasmid-borne *RSC3* in high copy partially suppressed *rsc7*Δ 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 (*rsc1*Δ *rsc2*, *arp7*, *arp9*, *rsc2*Δ, *rsc1*Δ, *rsc8*) could not be suppressed by an increased dosage of Rsc3 (A. Schlichter and B. R. Cairns, unpublished data 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 *pkc1*Δ and display cell wall defects that can be suppressed by an increased dosage of Pkc1 (Angus-Hill *et al.* 2001). These results prompted us to test whether the *rsc7*Δ strain displayed an osmotically remedial temperature sensitive phenotype. Indeed, we found that the temperature sensitivity of the *rsc7*Δ strain can be rescued by the addition of sorbitol (a cell wall

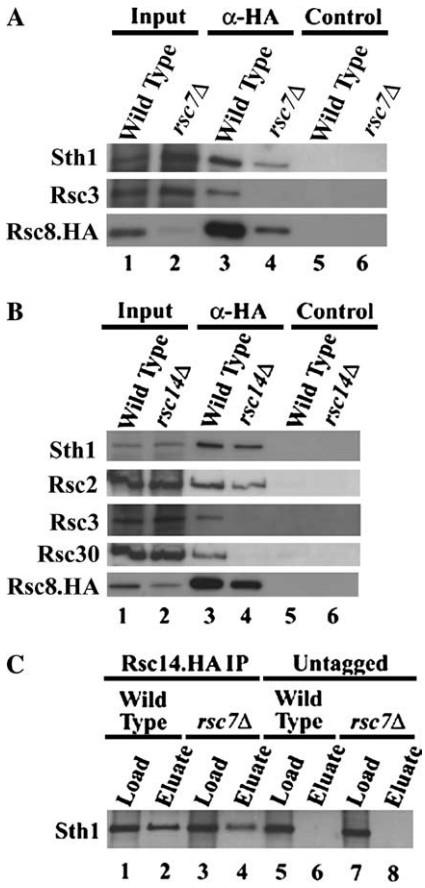

FIGURE 4.—Rsc7 and Rsc14 are structural components of RSC. (A) Loss of Rsc7 leads to Rsc8 instability and failure of Rsc8 to associate with other RSC components. Extracts were derived from wild-type (YBC62) and *rsc7*Δ (YBC1332) strains harboring the plasmid p186 (encoding Rsc8.3×HA). Rsc8.3×HA protein was immunopreciptated using an HA antibody (α-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 *rsc14*Δ (YBC1928) strains harboring the plasmid p186 (encoded Rsc8.3×HA). Rsc8.3×HA protein was immunopreciptated using an HA antibody (α-HA) coupled to magnetic beads, or beads alone (control). Precipitated proteins were analyzed by the methods used in A (input 25%, eluate/pellet 100%). (C) Rsc14 associates with Sth1 in a *rsc7*Δ strain. Extracts were derived from wild-type (YBC2297) and *rsc7*Δ (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 A and B (input 10%, beads 100%).

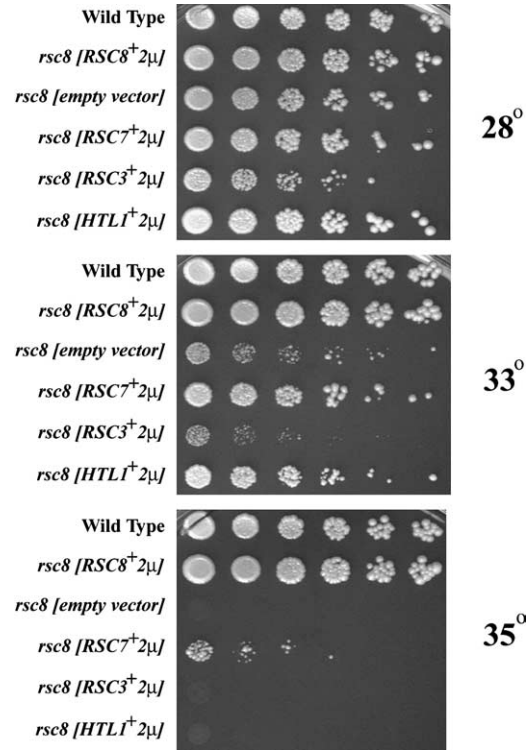

FIGURE 5.—Growth defects conferred by *rsc8* are suppressed through increased dosage of *RSC7* or *HTL1*. A *rsc8* mutant strain (J. LENKART and B. R. CAIRNS, unpublished data) harboring high-copy plasmids, p136 (*RSC7*$^+$), p1310 (*RSC3*$^+$), p1204 (*HTL1*$^+$) p185 (*RSC8*$^+$), or pRS426 (empty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

stabilizer), $CaCl_2$, and $MgCl_2$ to the media (Figure 2C and data not shown), whereas other *rsc* mutants (such as *rsc1*Δ *rsc2* Ts$^-$ mutants) cannot be rescued by the addition of cell wall stabilizers (A. SCHLICHTER and B. R. CAIRNS, unpublished data). 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 *rsc14*Δ phenotypes. Indeed, we found that *rsc14*Δ phenotypes are suppressed by an increased dosage of Rsc3 but not by an increased dosage of Rsc7 (Figure 3 and data not shown). These data are consistent with Rsc14 being physically linked to Rsc3, and *rsc14*Δ 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 data), 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). These data support 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 provide 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 6).

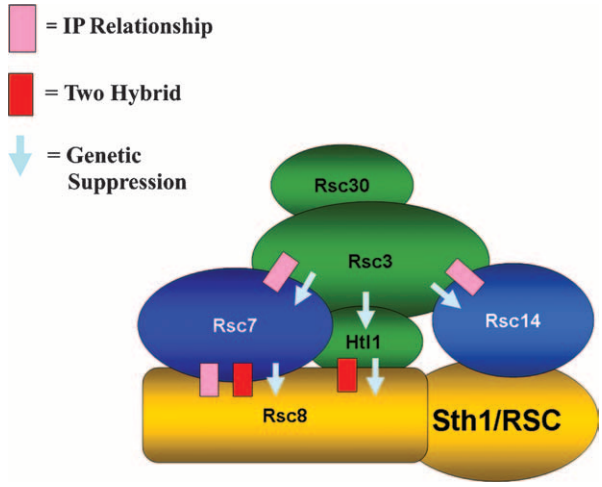

FIGURE 6.—A yeast-specific module containing Rsc7, Rsc14, Htl1, Rsc30, and Rsc3. The physical and functional connections among members are indicated: pink boxes represent IP relationships, red boxes indicate a two-hybrid interaction, and light blue arrows indicate dosage suppression.

Isolation of null mutations conferring growth defects in combination with *rsc7*Δ: Rsc7 is important for general RSC function (via Rsc8 stability) and also for module functions. As *rsc7*Δ strains show moderate phenotypes that encompass all known RSC functions, the *rsc7*Δ allele represented a moderate hypomorph ideal for examining the full scope of RSC function. Therefore, we used the *rsc7*Δ 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 *rsc7*Δ null allele. A *rsc7*Δ strain was modified to enable crossing to each strain of a haploid deletion library, composed of ~4700 strains each bearing a deletion in a nonessential gene and double-mutant phenotypes were determined. Remarkably, the *rsc7*Δ 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, and (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 *asf1*Δ (chromatin assembly); *ctf4*Δ and *ctf8*Δ (cohesion); *bub1*Δ and *mad1*Δ (spindle checkpoint); *cbf1*Δ (kinetochore); *msc1*Δ (recombination); and *rtt103*Δ, *rtt101*Δ, and *rtt109*Δ (Ty transposition). The third class had roles in cell wall organization and biogenesis and includes *bck1*Δ (cell wall integrity pathway), *gas1*Δ (cell wall organization), and *mid1*Δ (calcium transporter). Finally, the fourth class of deletion mutants identified had roles in transport: *nup84*Δ and *sxm1*Δ (nuclear transport); *apg17*Δ (autophagy); and *bre5*Δ, *ric1*Δ, and *rgp1*Δ (intracellular transport) (Table 4). Taken together, these data reveal the broad scope of RSC function by identifying nonessential deletion mutants that are sick/lethal in combination with the *rsc7*Δ 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 suggest that RSC has roles in chromosome metabolism and the spindle checkpoint pathway (TSUCHIYA *et al.* 1998; HSU *et al.* 2003; BAETZ *et al.* 2004; HUANG *et al.* 2004). 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 (MUSACCHIO and HARDWICK 2002; LEW and BURKE 2003). Mutations in several RSC components, *sth1*, *rsc3*, *rsc9*, and *htl1*, all confer an arrest or delay in the G_2/M phase of the cell cycle (DAMELIN *et al.* 2002; ROMEO *et al.* 2002; HSU *et al.* 2003). The G_2/M arrest observed with certain *rsc* mutants (*rsc3* and *sth1*) can be relieved by the removal of spindle checkpoint components (Mad proteins) (TSUCHIYA *et al.* 1998; ANGUS-HILL

TABLE 4

Null mutations conferring growth defects in combination with the *rsc7*Δ mutation

Broad category	Processes	Complex
Transcription/chromatin		
ARD1	Transcription	ARD1
SDS3, DEP1	Transcription	ECM16
ELP2, *ELP3*, *ELP6, IKI3*	Transcription	ELONGATOR
GIM4	Transcription	GIM
ARP8	Transcription	INO80
MED1, *SSN8, SSN2, SRB8*	Transcription	MEDIATOR
RTF1	Transcription	PAF1
BRE1	Transcription	RAD6
RSC2	Transcription	RSC
SPT3, *NGG1*, *SGF73, GCN5*	Transcription	SAGA
BRE2, *SWD1*	Transcription	SET1
SWR1, *SWC1, ARP6*	Transcription	SWR1
HTZ1, *AOR1, VPS72, VPS71*	Transcription	SWR1
SET2, *CTK2*	Transcription elongation	
LSM6, *SSF1, SNT309, SNU66*	RNA processing	
ASF1	Chromatin assembly	
Chromosome metabolism		
RTT103[a], *RTT101*, *RTT109*	Ty transposition	
BUB1, *MAD1*	Spindle checkpoint	
MSC1	Recombination	
CBF1	Kinetochore	
CTF4, *CTF8*	Cohesion	
EAP1	Genetic stability	
Cell wall integrity		
BCK1, *GAS1*, *MID1*		
FYV5, *FYV1*		
Transport		
APG17, *BRE5*		
RIC1, *RGP1*		
VPS1, *VPS61*, *VPS51, GOS1*		
NUP84, *SXM1*		
Other		
IFM1, *MDM10*, *RSM7*	Mitochondria	
IMG2, MRPS5, CEM1, ALD5	Mitochondria	
AAT2, *HOM2, ILV1*	Amino acid metabolism	
SIC1, *MEC3*	Cell cycle	

The *rsc7*Δ 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*$^+$ plasmid on solid media containing 5-FOA. Underlined genes were confirmed by tetrad dissection and/or random spore analysis, while other genes were identified in our screen but not verified.

[a] The protein encoded by the *RTT103* gene also has a role in transcription termination.

et al. 2001; Hsu *et al.* 2003). To test for Rsc7 involvement in the spindle checkpoint pathway we performed FACS analysis on the *rsc7*Δ, which revealed a slight G_2/M bias (Figure 7B). These data suggest that the spindle checkpoint pathway has become partially activated in the *rsc7*Δ strain. Furthermore, although the removal of key checkpoint components can relieve the G_2/M growth arrest, these double mutants often display synergistic growth defects due to loss of viability (Tsuchiya *et al.* 1998; Hsu *et al.* 2003). Consistent with this notion, we find that combining spindle checkpoint pathway mutations (*bub1*Δ and *mad1*Δ) with *rsc7*Δ confers very slow growth (Figure 7A and Table 4). These results link Rsc7 to the spindle checkpoint pathway and strengthen the genetic connections between RSC and this pathway.

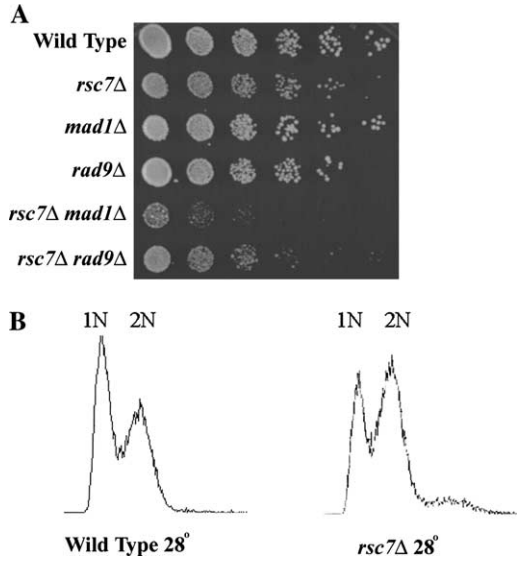

FIGURE 7.—Links between Rsc7 and the spindle checkpoint pathway. (A) The *rsc7*Δ *mad1*Δ double mutation confers severe growth defects. Wild-type (YBC62), *rsc7*Δ (YBC1332), *mad1*Δ (YBC936), *rad9*Δ (YBC939), *rsc7*Δ *mad1*Δ (YBC1470), and *rsc7*Δ *rad9*Δ (YBC1944) strains were grown to log phase, subjected to a fivefold serial dilution, and spotted onto rich media. (B) The *rsc7*Δ mutation confers a G_2/M bias. Wild-type (YBC62) and *rsc7*Δ (YBC1332) strains were grown to log phase in rich medium and analyzed by FACS.

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 noncatalytic 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 *swp82*Δ 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 4, A and B). 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*Δ, *rsc14*Δ, and *htl1*Δ mutations (Figures 2B and 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*Δ with either *rsc14*Δ or *htl1*Δ 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*Δ 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 nonessential null mutations that conferred increased sickness when combined with the *rsc7*Δ 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.

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.

Chromosome metabolism and the spindle checkpoint pathway: The *rsc7*Δ mutation confers a G_2/M bias (Figure 7), as do mutations in other RSC members such as Rsc3, Htl1, Rsc9, Sth1, and Sfh1 (Cao *et al.* 1997; Angus-Hill *et al.* 2001; Chai *et al.* 2002; Damelin *et al.* 2002; Romeo *et al.* 2002; Hsu *et al.* 2003; Baetz *et al.* 2004), 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 *rsc7*Δ mutant does not display significant chromosome missegregation defects, in contrast to many other *rsc* mutations (Tsuchiya *et al.* 1998; Lanzuolo *et al.* 2001; Hsu *et al.* 2003; Baetz *et al.* 2004; Huang *et al.* 2004). Therefore, we propose that the low level of RSC assembly in *rsc7*Δ mutants confers 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 (Tsuchiya *et al.* 1998; Hsu *et al.* 2003). 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; Hosotani *et al.* 2001; Chai *et al.* 2002; Romeo *et al.* 2002; Jones *et al.* 2003; Corbacho *et al.* 2004; Kasten *et al.* 2004). Here, we show that *rsc7*Δ and *rsc14*Δ 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 (B. Wilson and B. Cairns, 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 nonhomologous 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 *rsc7*Δ mutation strengthens this link as the *rsc7*Δ 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 7).

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 *rsc7*Δ and *rsc14*Δ 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 that cooperate physically/functionally in RSC, and strengthened links between RSC and many important cellular processes.

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LITERATURE CITED

Altschul, S. F., W. Gish, W. Miller, E. W. Myers and D. J. Lipman, 1990 Basic local alignment search tool. J. Mol. Biol. **215:** 403–410.

Angus-Hill, M. L., A. Schlichter, D. Roberts, H. Erdjument-Bromage, P. Tempst *et al.*, 2001 A Rsc3/Rsc30 zinc cluster dimer reveals novel roles for the chromatin remodeler RSC in gene expression and cell cycle control. Mol. Cell **7:** 741–751.

Baetz, K. K., N. J. Krogan, A. Emili, J. Greenblatt and P. Hieter, 2004 The ctf13–30/CTF13 genomic haploinsufficiency modifier screen identifies the yeast chromatin remodeling complex RSC, which is required for the establishment of sister chromatid cohesion. Mol. Cell. Biol. **24:** 1232–1244.

Baudin, A., O. Ozier-Kalogeropoulos, A. Denouel, F. Lacroute and C. Cullin, 1993 A simple and efficient method for direct gene deletion in Saccharomyces cerevisiae. Nucleic Acids Res. **21:** 3329–3330.

Becker, P. B., and W. Horz, 2002 ATP-dependent nucleosome remodeling. Annu. Rev. Biochem. **71:** 247–273.

Bossie, M. A., C. DeHoratius, G. Barcelo and P. Silver, 1992 A mutant nuclear protein with similarity to RNA binding proteins interferes with nuclear import in yeast. Mol. Biol. Cell **3:** 875–893.

Brachmann, C. B., A. Davies, G. J. Cost, E. Caputo, J. Li *et al.*, 1998 Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast **14:** 115–132.

Bungard, D., M. Reed and E. Winter, 2004 RSC1 and RSC2 are required for expression of mid-late sporulation-specific genes in *Saccharomyces cerevisiae*. Eukaryotic Cell **3:** 910–918.

Cairns, B. R., Y. J. Kim, M. H. Sayre, B. C. Laurent and R. D. Kornberg, 1994 A multisubunit complex containing the SWI1/ADR6, SWI2/SNF2, SWI3, SNF5, and SNF6 gene products isolated from yeast. Proc. Natl. Acad. Sci. USA **91:** 1950–1954.

Cairns, B. R., Y. Lorch, Y. Li, M. Zhang, L. Lacomis *et al.*, 1996 RSC, an essential, abundant chromatin-remodeling complex. Cell **87:** 1249–1260.

Cairns, B. R., H. Erdjument-Bromage, P. Tempst, F. Winston and R. D. Kornberg, 1998 Two actin-related proteins are shared functional components of the chromatin-remodeling complexes RSC and SWI/SNF. Mol. Cell **2:** 639–651.

Cairns, B. R., A. Schlichter, H. Erdjument-Bromage, P. Tempst, R. D. Kornberg *et al.*, 1999 Two functionally distinct forms of the RSC nucleosome-remodeling complex, containing essential AT hook, BAH, and bromodomains. Mol. Cell **4:** 715–723.

Cao, Y., B. R. Cairns, R. D. Kornberg and B. C. Laurent, 1997 Sfh1p, a component of a novel chromatin-remodeling complex, is required for cell cycle progression. Mol. Cell. Biol. **17:** 3323–3334.

Chai, B., J. M. Hsu, J. Du and B. C. Laurent, 2002 Yeast RSC function is required for organization of the cellular cytoskeleton via an alternative PKC1 pathway. Genetics **161:** 575–584.

Chai, B., J. Huang, B. R. Cairns and B. C. Laurent, 2005 Distinct roles for the RSC and Swi/Snf ATP-dependent chromatin remodelers in DNA double-strand break repair. Genes Dev. **19:** 1656–1661.

Corbacho, I., I. Olivero and L. M. Hernandez, 2004 Identification of low-dye-binding (ldb) mutants of *Saccharomyces cerevisiae*. FEMS Yeast Res. **4:** 437–444.

Damelin, M., I. Simon, T. I. Moy, B. Wilson, S. Komili *et al.*, 2002 The genome-wide localization of Rsc9, a component of the RSC chromatin-remodeling complex, changes in response to stress. Mol. Cell **9:** 563–573.

Du, J., I. Nasir, B. K. Benton, M. P. Kladde and B. C. Laurent, 1998 Sth1p, a *Saccharomyces cerevisiae* Snf2p/Swi2p homolog, is an essential ATPase in RSC and differs from Snf/Swi in its interactions with histones and chromatin-associated proteins. Genetics **150:** 987–1005.

Funk, M., R. Niedenthal, D. Mumberg, K. Brinkmann, V. Ronicke *et al.*, 2002 Vector systems for heterologous expression of proteins in *Saccharomyces cerevisiae*. Methods Enzymol. **350:** 248–257.

Gavin, A. C., M. Bosche, R. Krause, P. Grandi, M. Marzioch *et al.*, 2002 Functional organization of the yeast proteome by systematic analysis of protein complexes. Nature **415:** 141–147.

Graumann, J., L. A. Dunipace, J. H. Seol, W. H. McDonald, J. R. Yates III *et al.*, 2004 Applicability of tandem affinity purification MudPIT to pathway proteomics in yeast. Mol. Cell Proteomics **3:** 226–237.

Heinisch, J. J., A. Lorberg, H. Schmitz and J. J. Jacoby, 1999 The protein kinase C-mediated MAP kinase pathway involved in the maintenance of cellular integrity in *Saccharomyces cerevisiae*. Mol. Microbiol. **32:** 671–680.

Hosotani, T., H. Koyama, M. Uchino, T. Miyakawa and E. Tsuchiya, 2001 PKC1, a protein kinase C homologue of *Saccharomyces cerevisiae*, participates in microtubule function through the yeast EB1 homologue, BIM1. Genes Cells **6:** 775–788.

Hsu, J. M., J. Huang, P. B. Meluh and B. C. Laurent, 2003 The yeast RSC chromatin-remodeling complex is required for kinetochore function in chromosome segregation. Mol. Cell. Biol. **23:** 3202–3215.

Huang, J., J. M. Hsu and B. C. Laurent, 2004 The RSC nucleosome-remodeling complex is required for Cohesin's association with chromosome arms. Mol. Cell **13:** 739–750.

Jenuwein, T., and C. D. Allis, 2001 Translating the histone code. Science **293:** 1074–1080.

Jones, D. L., J. Petty, D. C. Hoyle, A. Hayes, E. Ragni *et al.*, 2003 Transcriptome profiling of a *Saccharomyces cerevisiae* mutant with a constitutively activated Ras/cAMP pathway. Physiol. Genomics **16:** 107–118.

Kasten, M., H. Szerlong, H. Erdjument-Bromage, P. Tempst, M. Werner *et al.*, 2004 Tandem bromodomains in the chromatin remodeler RSC recognize acetylated histone H3 Lys14. EMBO J. **23:** 1348–1359.

Koyama, H., M. Itoh, K. Miyahara and E. Tsuchiya, 2002 Abundance of the RSC nucleosome-remodeling complex is important for the cells to tolerate DNA damage in *Saccharomyces cerevisiae*. FEBS Lett. **531:** 215–221.

Lanzuolo, C., S. Ederle, A. Pollice, F. Russo, A. Storlazzi *et al.*, 2001 The HTL1 gene (YCR020W-b) of *Saccharomyces cerevisiae* is necessary for growth at 37°C, and for the conservation of chromosome stability and fertility. Yeast **18:** 1317–1330.

Lee, K. K., P. Prochasson, L. Florens, S. K. Swanson, M. P. Washburn *et al.*, 2004 Proteomic analysis of chromatin-modifying complexes in *Saccharomyces cerevisiae* identifies novel subunits. Biochem. Soc. Trans. **32:** 899–903.

Levin, D. E., 2005 Cell wall integrity signaling in *Saccharomyces cerevisiae*. Microbiol. Mol. Biol. Rev. **69:** 262–291.

Lew, D. J., and D. J. Burke, 2003 The spindle assembly and spindle position checkpoints. Annu. Rev. Genet. **37:** 251–282.

Longtine, M. S., A. McKenzie, III, D. J. Demarini, N. G. Shah, A. Wach *et al.*, 1998 Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. Yeast **14:** 953–961.

Lorenz, M. C., R. S. Muir, E. Lim, J. McElver, S. C. Weber *et al.*, 1995 Gene disruption with PCR products in *Saccharomyces cerevisiae*. Gene **158:** 113–117.

Lu, Y. M., Y. R. Lin, A. Tsai, Y. S. Hsao, C. C. Li *et al.*, 2003 Dissecting the pet18 mutation in *Saccharomyces cerevisiae*: HTL1 encodes a 7-kDa polypeptide that interacts with components of the RSC complex. Mol. Genet. Genomics **269:** 321–330.

Martens, J. A., and F. Winston, 2003 Recent advances in understanding chromatin remodeling by Swi/Snf complexes. Curr. Opin. Genet. Dev. **13:** 136–142.

Mohrmann, L., and C. P. Verrijzer, 2005 Composition and functional specificity of SWI2/SNF2 class chromatin remodeling complexes. Biochim. Biophys. Acta **1681:** 59–73.

Musacchio, A., and K. G. Hardwick, 2002 The spindle checkpoint: structural insights into dynamic signalling. Nat. Rev. Mol. Cell Biol. **3:** 731–741.

Narlikar, G. J., H. Y. Fan and R. E. Kingston, 2002 Cooperation between complexes that regulate chromatin structure and transcription. Cell **108:** 475–487.

Peterson, C. L., A. Dingwall and M. P. Scott, 1994 Five SWI/SNF gene products are components of a large multisubunit complex required for transcriptional enhancement. Proc. Natl. Acad. Sci. USA **91:** 2905–2908.

Phelan, M. L., S. Sif, G. J. Narlikar and R. E. Kingston, 1999 Reconstitution of a core chromatin remodeling complex from SWI/SNF subunits. Mol. Cell **3:** 247–253.

Romeo, M. J., M. L. Angus-Hill, A. K. Sobering, Y. Kamada, B. R. Cairns *et al.*, 2002 HTL1 encodes a novel factor that interacts with the RSC chromatin remodeling complex in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **22:** 8165–8174.

Rose, M. D., F. Winston and P. Hieter, 1990 *Methods in Yeast Genetics: A Laboratory Course Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Saha, A., J. Wittmeyer and B. R. Cairns, 2002 Chromatin remodeling by RSC involves ATP-dependent DNA translocation. Genes Dev. **16:** 2120–2134.

Sanders, S. L., J. Jennings, A. Canutescu, A. J. Link and P. A. Weil, 2002 Proteomics of the eukaryotic transcription machinery: identification of proteins associated with components of yeast TFIID by multidimensional mass spectrometry. Mol. Cell. Biol. **22:** 4723–4738.

Shim, E. Y., J. L. Ma, J. H. Oum, Y. Yanez and S. E. Lee, 2005 The yeast chromatin remodeler RSC complex facilitates end joining repair of DNA double-strand breaks. Mol. Cell. Biol. **25:** 3934–3944.

Swanson, M. J., H. Qiu, L. Sumibcay, A. Krueger, S. J. Kim *et al.*, 2003 A multiplicity of coactivators is required by Gcn4p at individual promoters in vivo. Mol. Cell. Biol. **23:** 2800–2820.

Taneda, T., and A. Kikuchi, 2004 Genetic analysis of RSC58, which encodes a component of a yeast chromatin remodeling complex, and interacts with the transcription factor Swi6. Mol. Genet. Genomics **271:** 479–489.

Tong, A. H., M. Evangelista, A. B. Parsons, H. Xu, G. D. Bader *et al.*, 2001 Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science **294:** 2364–2368.

Treich, I., and M. Carlson, 1997 Interaction of a Swi3 homolog with Sth1 provides evidence for a Swi/Snf- related complex with an essential function in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **17:** 1768–1775.

Treich, I., L. Ho and M. Carlson, 1998 Direct interaction between Rsc6 and Rsc8/Swh3, two proteins that are conserved in SWI/SNF-related complexes. Nucleic Acids Res. **26:** 3739–3745.

Tsuchiya, E., T. Hosotani and T. Miyakawa, 1998 A mutation in NPS1/STH1, an essential gene encoding a component of a novel chromatin-remodeling complex RSC, alters the chromatin structure of *Saccharomyces cerevisiae* centromeres. Nucleic Acids Res. **26:** 3286–3292.

Tsukiyama, T., 2002 The in vivo functions of ATP-dependent chromatin remodelling factors. Nat. Rev. Mol. Cell Biol. **3:** 422–429.

Uetz, P., L. Giot, G. Cagney, T. A. Mansfield, R. S. Judson *et al.*, 2000 A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae*. Nature **403:** 623–627.

Vignali, M., A. H. Hassan, K. E. Neely and J. L. Workman, 2000 ATP-dependent chromatin-remodeling complexes. Mol. Cell. Biol. **20:** 1899–1910.

Whitehouse, I., C. Stockdale, A. Flaus, M. D. Szczelkun and T. Owen-Hughes, 2003 Evidence for DNA translocation by the ISWI chromatin-remodeling enzyme. Mol. Cell. Biol. **23:** 1935–1945.

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