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SQT1, Which Encodes an Essential WD Domain Protein of *Saccharomyces cerevisiae*, Suppresses Dominant-Negative Mutations of the Ribosomal Protein Gene *QSR1*

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***QSR1* is an essential *Saccharomyces cerevisiae* gene, which encodes a 60S ribosomal subunit protein required for joining of 40S and 60S subunits. Truncations of *QSR1* predicted to encode C-terminally truncated forms of Qsr1p do not substitute for *QSR1* but do act as dominant negative mutations, inhibiting the growth of yeast when expressed from an inducible promoter. The dominant negative mutants exhibit a polysome profile characterized by 'half-mer' polysomes, indicative of a subunit joining defect like that seen in other *qsr1* mutants (D. P. Eisinger, F. A. Dick, and B. L. Trumpower, Mol. Cell. Biol. 17:5136–5145, 1997.) By screening a high-copy yeast genomic library, we isolated several clones containing overlapping inserts of a novel gene that rescues the slow-growth phenotype of the dominant negative *qsr1* truncations. The suppressor of *qsr1* truncation mutants, *SQT1*, is an essential gene, which encodes a 47.1-kDa protein containing multiple WD repeats and which interacts strongly with Qsr1p in a yeast two-hybrid system. *SQT1* restores growth and the "half-mer" polysome profile of the dominant negative *qsr1* mutants to normal, but it does not rescue temperature-sensitive *qsr1* mutants or the original *qsr1-1* missense allele. In yeast cell lysates, Sqt1p fractionates as part of an oligomeric protein complex that is loosely associated with ribosomes but is distinct from known eukaryotic initiation factor complexes. Loss of *SQT1* function by down regulation from an inducible promoter results in formation of half-mer polyribosomes and decreased Qsr1p levels on free 60S subunits. Sqt1p thus appears to be involved in a late step of 60S subunit assembly or modification in the cytoplasm.**

The functions of individual eukaryotic ribosomal proteins are not well understood. We have undertaken a biochemical and genetic approach to elucidate the function of the recently discovered ribosomal protein, Qsr1p, in *Saccharomyces cerevisiae* (13). Qsr1p is a eukaryotic 60S ribosomal protein encoded by the essential single-copy *S. cerevisiae* gene *QSR1* (13, 54) and is the mammalian homolog of rat ribosomal protein L10 (9).

Characterization of Qsr1p and of temperature-sensitive *qsr1* mutants has revealed that Qsr1p is required for joining of 40S and 60S subunits into either active 80S ribosomes or inactive 80S couples (14). Mutant alleles that are either temperature sensitive or conditional for the synthesis of Qsr1p have 60S subunits that are devoid of Qsr1p and are unable to join with 40S subunits. Whereas most conditional mutants of 60S ribosomal protein genes are defective in the biogenesis of 60S subunits (11, 12, 35, 38, 39, 45, 47, 48), which precludes insights into any postassembly functions, the biogenesis of 60S subunits is only slightly impaired in *qsr1* mutants (14). Further investigation of Qsr1p is thus likely to lead to a better understanding of the late stages of translation initiation or ribosomal subunit joining, of which little is currently known.

To learn more about the function of Qsr1p, we constructed dominant-negative *qsr1* mutants and isolated a novel essential gene (*SQT1*) as a high-copy suppressor of the dominant-negative slow-growth phenotype. The dominant-negative mutants have a defect in ribosomal subunit joining that is rescued by *SQT1*. By directed two-hybrid analysis, we show that Sqt1p can physically interact with Qsr1p. *SQT1* encodes a cytosolic protein that appears to be loosely associated with ribosomes and involved in a late stage of large ribosomal subunit assembly or maturation.

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MATERIALS AND METHODS

Construction of plasmids. Plasmid pDEGQ2 (*CEN URA3 GAL*$_{UAS}$::*QSR1*), which has the *GAL1-10* upstream activating sequence (UAS) modulating the expression of *QSR1* from a minimal promoter (14), was used to construct three C-terminal truncations of the *QSR1*-encoded protein as follows. pDEGQ2 was digested with *Hin*dIII to remove an internal 474-bp fragment of the *QSR1* open reading frame encoding amino acids 64 to 221, immediately preceding the stop codon. Self-ligation of the remaining plasmid backbone generated pDEGQ64, in which the N-terminal 64 amino acids of the *QSR1*-encoded protein are retained. This truncation is referred to as *qsr1-64*Δ. Constructs pDEGQ133 and pDEGQ187 were made by ligating *Hin*dIII-digested *QSR1* PCR fragments into the *Hin*dIII pDEGQ2 backbone. The fragment for making pDEGQ133 was synthesized by PCR with primers DEQ133HIII (5′-GCGTAAGCTTGACCAA TGTCGACA [coding strand]) and FDYGST5′ (5′-AACGGATCCAAGATGG CTAGAAGACC [noncoding strand]). The resulting construct has the N-terminal 133 amino acids encoded by the open reading frame and is referred to as *qsr1-133*Δ. The PCR fragment for making pDEGQ187 was synthesized with primers DEQ187HIII (5′-CCTCACAAGCTTCTCTTC [coding strand]) and FDYGST5′ (5′-AACGGATCCAAGATGGCTAGAAGACC [noncoding strand]). The resulting construct has the N-terminal 187 amino acids encoded by the *QSR1* open reading frame and is referred to as *qsr1-187*Δ.

A 1.8-kb PCR fragment encompassing the *SQT1* open reading frame was made with High Fidelity *Taq* polymerase (Boehringer Mannheim) with the primers DESQTP3 (5′-CAAGGATCCCCATTCAAACCCCATC [coding strand]) and DESQTP1 (5′-AGAGAAGGATCCCCAAAG [noncoding strand]) which contain *Bam*HI sites for cloning. The PCR fragment has 277 bp of 3′-flanking sequence and 258 bp of 5′-flanking sequence and was cut with *Bam*HI and cloned into the *Bam*HI sites of pFL36 and pFL46S (5) to generate pDESQT2 (*SQT1 CEN LEU2*) and pDESQT1 (*SQT1* 2μm *LEU2*).

To make a myc-tagged version of the *SQT1*-encoded protein, two primers were synthesized for long-inverse PCR (15) such that the myc epitope recognized by the monoclonal antibody 9E10 (31) was added to the C terminus of Sqt1p. Primer DSQTM1 (5′-AAGCTTTTGTTCACCACCGTTTGGTACCTCGAAC AC [noncoding strand]) and primer DSQTM2 (5′-ATTTCTGAAGAAGACT TGTGATAGATAGTCGCGTATACATA [coding strand]) add the myc epitope (EQKLISEEDL) preceded by two glycines between the C-terminal asparagine

TABLE 1. Yeast strains used in this study

Strain	Genotype	Source
W303-1A	**a** *ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 can1-100*	54
W303-1B	α *ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 can1-100*	54
HFF10	**a**/α W303 markers, *QSR1*/*qsr1*Δ*1::HIS3*	54
DEH64	**a** W303 markers, *QSR1*, + pDEGQ64 (*GAL1-10*$_{UAS}$-*qsr1-64*Δ *CEN URA3*)	This study
DEH133	α W303 markers, *QSR1*, + pDEGQ133 (*GAL1-10*$_{UAS}$-*qsr1-133*Δ *CEN URA3*)	This study
DEH187	α W303 markers, *QSR1*, + pDEGQ187 (*GAL1-10*$_{UAS}$-*qsr1-187*Δ *CEN URA3*)	This study
DEH221$^-$	**a** W303 markers, *QSR1*, + pDEGQ2 (*GAL1-10*$_{UAS}$-*QSR1 CEN URA3*)	This study
DESQT1B	**a** DEH64, + pSQT1B (*SQT1* 2μm *LEU2*)	This study
DEQSR4	**a** DEH64, + pQSR-4 (*QSR1* 2μm *LEU2*)	This study
YPH500 X 499	**a**/α *ura3-52 lys2-801 ade2-101 trp1-*Δ*63 his3-*Δ*200 leu2-*Δ*1*	49
DPE1	**a**/α YPH markers, *SQT1*/*sqt1*Δ*1::HIS3*	This study
DPESM1	**a**/α DPE1 + pSQTMYC1 (*SQT1-myc* 2μm *LEU2*)	This study
DPESM2-1A	α YPH markers, *sqt1*Δ*1::HIS3* + pSQTMYC2 (*SQT1-myc CEN LEU2*)	This study
FAD13-2B	**a** YPH markers, *sqt1*Δ*1::HIS3* + pFAD30 (*GAL-SQT1-myc CEN URA3*)	This study
EGY48	**a***trp1 ura3-52 his3 leu2 LEU2::pLexAop6-Leu2*	20
Y190	**a***leu2-3,112 ura3-52 trp1-901 his3-*Δ*200 ade2-101 gal4*Δ*gal80*Δ (*URA3GAL-lacZ LYSGAL-HIS3* Cyh^r)	22

and the stop codon of *SQT1*. pDESQT1 and pDESQT2 were used as templates for addition of the myc tag, creating 2μm (pSQTMYC1) and *CEN* (pSQTMYC2) versions, respectively, of myc-tagged *SQT1*. Primers FDSQTP1 (5′-GGATCCCCAAAGAACTTACC [coding strand]) and FDSQTP2 (5′-GGATCCGCGTAATGGAACCTCAAG [noncoding strand]), which both contain *Bam*HI sites, were used to amplify SQT1-myc from plasmid pSQTMYC2, using the High Fidelity *Taq* polymerase (Boehringer Mannheim). The resulting 1.6-kb fragment was ligated into pCR2.1 with the TA cloning kit (Invitrogen). The 1.6-kb insert was excised with *Bam*HI and ligated into the unique *Bam*HI site in pBM258. The resulting plasmid, which contains *SQT1-MYC* under the control of the *GAL1-10* promoter, was named pFAD30. All of the plasmids were amplified in *Escherichia coli* DH5α (21), and the DNA constructs were verified by sequencing with an Applied Biosystems no. 373 DNA sequencer.

***S. cerevisiae* strains and growth conditions.** Yeast cells were grown in yeast extract-peptone-dextrose (YPD), synthetic complete (SC), or synthetic dropout (SD) medium in which the relevant nutritional supplements were omitted. Where indicated, galactose was substituted for dextrose as a carbon source. A lithium acetate procedure was used to transform the yeast cells (1). The yeast strains used are listed in Table 1 and constructed as described below. Haploid strains wild type for chromosomal *QSR1* and harboring galactose-inducible C-terminal *qsr1* truncation mutants were made by separately transforming the centromeric plasmid pDEGQ64, pDEGQ133, or pDEGQ187 into HFF10, a hemizygous *QSR1* diploid, and isolating Ura$^+$ His$^-$ spores to generate strains DEH64, DEH133, and DEH187, respectively. DEH221$^-$ was isolated in the same manner but has a full-length galactose-inducible wild-type *QSR1* gene. DESQT1B is derived from DEH64 and has an additional plasmid (pSQT1B) which contains the *SQT1* locus and was isolated from the high-copy genomic library. DESQR4 is also derived from DEH64 and has a plasmid (pDEQSR4) which contains the *QSR1* locus and was isolated from the genomic library.

A null allele of *SQT1* was made in the isogenic diploid YPH499 × YPH500 by targeted deletion of the *SQT1* open reading frame (3). Two primers, each with 36 bp (34) of *SQT1* sequence flanking *HIS3* gene sequence, were used to amplify the *HIS3* gene from pRS303 (49). Primer SQT1HIS1 (5′-CAAGACGTTCCC GTTGATATTGAAGGAGAGAATGAC*GGCCTCCTCTAGTACACTC* [coding strand]) contains nucleotides 73 to 108 of *SQT1* fused to the 5′ *HIS3* sequence (shown in italics). Primer SQT1HIS2 (5′-TCAGTTTGGTACCTCGAA CACCAGAGATACACCTTC*GCGCGCCTCGTTCAGAATG* [noncoding strand]) contains nucleotides 1296 to 1261 of *SQT1* fused to the 3′ *HIS3* sequence (shown in italics). The amplified fragment was transformed directly into strain YPH499 × YPH500 (49), and His$^+$ transformants were selected and screened by colony PCR (3) to generate strain DPE1. Proper integration of the *HIS3* gene in the *SQT1* locus was confirmed by PCR genotyping such that predicted unique fragments were generated by internal *HIS3* primers and external *SQT1* primers.

Strain DPESM2-1A, in which plasmid pSQTMYC2 (*SQT1-MYC CEN LEU2*) contains the *SQT1-MYC* tag allele covering the chromosomal *SQT1* deletion (*sqt1*Δ*1::HIS3*), was made by isolating a Leu$^+$ His$^+$ spore derived from diploid strain DPE1 transformed with pSQTMYC2. Strain DPESM1 is diploid strain DPE1 with plasmid pSQTMYC1 (*SQT1-MYC* 2μm *LEU2*). DPE1 was transformed with pFAD30 and sporulated. The spores were dissected on YPGal plates, and a spore carrying the *sqt1*Δ*1::HIS3* allele complemented by the *GAL1-10*-controlled *SQT1-MYC* was identified and named FAD13-2B.

Isolation of a high-copy suppressor of a dominant-negative *qsr1* truncation. Strain DEH64 was grown in SD medium lacking uracil, transformed with a *LEU2*-marked YEp13 yeast genomic library (43), and allowed to recover in YPD medium for 1 h at 30°C before plating on SD galactose plates lacking uracil and leucine. The transformation efficiency was 1.2×10^5 per μg of library plasmid, and the plating density was estimated to be 15,000 transformants per 10-cm petri dish. After 4 days at 30°C, 15 large colonies were restreaked to fresh plates. Total yeast DNA was purified and transformed into *E. coli*, and purified plasmids were retransformed into DEH64 to link rescue of the growth inhibition caused by the galactose-induced *qsr1-64*Δ to the library-derived plasmid. Nine clones rescued the growth defect and were analyzed by restriction mapping and/or partial sequencing. Deletions in chromosome 9 genomic clone 12 were made by long-inverse PCR (15) as follows. The 12.6-kb pSQT12A was made by blunt-end self-ligation of a PCR product made with vector primers YEPB3 (5′-GGAGGA TCCACAGGACGGGTGTGGTC) and DESQTP1 (see above), while the 11-kb pSQT12B was made with primers YEPB3 and DESQTP2 (5′-CTAGGATCCT GCTCAACTTCTTCGG [noncoding strand]).

Polysome and ribosome subunit analysis. Ribosomal subunits, 80S ribosomes, and polysomes were fractionated on sucrose velocity gradients (14). The cells were treated with cycloheximide to arrest translating ribosomes, and the gradients were run with Mg and cycloheximide. To dissociate ribosomal subunits, the cells were collected without cycloheximide treatment, and Mg and cycloheximide were omitted from all solutions.

Directed two-hybrid interaction analysis of *SQT1* and *QSR1*. The yeast strain EGY48, the *LexA* fusion plasmid pEG202, the positive control *LexA-GAL4* plasmid, and the *LexAop-lacZ* reporter plasmid pSH18-34 were provided by Roger Brent (1, 20). Strain Y190 (22) was a gift from Steve Elledge. The *GAL4* DNA binding domain vector pAS2-1 and the *GAL4* activation domain vector pACT2-1 were from Clontech Laboratories, Inc. pEG202-*QSR1*, pAS2-1-*QSR1*, and pACT2-*QSR1* contain a *Bam*HI-*Bgl*II PCR fragment encoding the full-length QSR1 protein. The fragment was generated by PCR amplification of pMY7 (54) with primers HFF1 (5′-CTGGATCCAGATGGCTCAAGACC) and HFF2 (5′-GAAAAGATCTTAAGCTTGAGCAGC [noncoding strand]) to create *Bam*HI and *Bgl*II sites, respectively, which are underlined in the primer sequences. The *Bam*HI-*Bgl*II fragment was ligated into the *Bam*HI sites of pEG202, pAS2-1, and pACT2. To construct pEG202-*SQT1*, pAS2-1-*SQT1*, and pACT2-*SQT1*, a PCR fragment was generated with plasmid pSQT1B and primers DESQTP5 (5′-CAAGGATCCCTCAAGAAGAGTTTATAAC) and DESQ TP6 (5′-CTTGGATCCTTGGTACCTCGA.ACACCA [noncoding strand]) to create *Bam*HI sites, after which the fragment was cloned into pEG202, pAS2-1, and pACT2. The open reading frames for both proteins are in translational phase with the DNA binding and activation domains of the vectors. The accuracy of the reading frames was verified by sequencing the junctions.

Cells were prepared from exponentially growing cultures. To monitor interaction of the fusion proteins in Y190, 50 mM 3-amino-1,2,4-triazole was added to the His dropout plates to inhibit low levels of leaky *HIS3* expression in this reporter strain. Colony color β-galactosidase assays were performed either directly on plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) (1) or by a colony lift filter assay (Clontech). β-Galactosidase activity in solution was assayed by the *o*-nitrophenyl-β-D-galactopyranoside method (Clontech) in cells that were permeabilized by freezing in liquid nitrogen and thawing at 37°C. β-Galactosidase activity is expressed in units as described by Miller (37).

Cell fractionation and immunoblot analysis of proteins. Yeast cells were recovered from 25-ml cultures by centrifugation when the absorbance at 600 nm was 1.0. The cells were washed once in 1.5 ml of 20 mM Tris (pH 7.5)–50 mM NaCl–0.4 M sorbitol (lysis buffer); resuspended in 250 μl of lysis buffer plus 10 μM leupeptin, 1 μg of pepstatin A per ml, and 1 mM diisopropyl fluorophosphate; and glass beads were added to 50% of the total volume. The cells were broken by vortexing for 5 min at 4°C, and the lysates were cleared by centrifugation at 500 × *g* for 5 min at 4°C. A 30-μl volume of this supernatant was kept as whole cell lysate. The remainder was centrifuged at 14,000 rpm for 20 min, and the supernatant was retained as a crude cytosolic fraction. The pellet was washed

by being resuspended in 200 μl of lysis buffer and recentrifuged, and the supernatant was discarded. The crude membrane pellet was resuspended in 30 μl of lysis buffer. Protein concentrations were determined by a dye-binding assay (7). Protein fractions were denatured for electrophoresis by incubation with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (32) at 55°C for 15 min.

Fractionation of yeast cells to isolate nuclei was carried out essentially as described by Wise (57). The proteins were resolved on 10% SDS-PAGE gels (1), unless otherwise stated, and analyzed by Western blotting (13). After being electroblotted to nitrocellulose, the membrane was blocked with 5% nonfat dry milk in 20 mM Tris (pH 7.5)–150 mM NaCl–0.3% Tween 20 (TTBS) for at least 1 h and incubated for 1 h with the primary antibody diluted 1:2,000 in 1% milk in TTBS. The primary antibodies used were rabbit polyclonal anti-Qsr1p (13), rabbit polyclonal anti-Prt1p (10), rabbit polyclonal anti-Nop1p (52), and mouse monoclonal anti-Myc (31). After the blots were washed with TTBS, they were incubated with horseradish peroxidase-conjugated goat anti-rabbit antibodies at a 1:2,500 dilution or with horse anti-mouse antibodies at a 1:10,000 dilution in 1% milk in TTBS for 1 h, washed again, and then visualized with the Lumiglo chemiluminescent system (Kirkegaard & Perry, Gaithersburg, Md.) by exposure to Hyperfilm-ECL (Amersham, Arlington Heights, Ill.).

Gel filtration of Sqt1p from salt-washed ribosomes. Ribosomes were purified by a modification of a procedure used to isolate translation initiation factors (10). Strain DPESM2-1A (16 liters) was grown in YPD to an absorbance at 600 nm of 1.2, and the cells were collected by centrifugation. The cells were washed once in water and once in 20 mM Tris (pH 7.5)–10 mM $MgSO_4$–100 mM KCl–1 mM dithiothreitol (ribosome lysis buffer), resuspended in 20 ml of ribosome lysis buffer, and frozen and lysed with liquid N_2 in a Waring blender (1).

The broken cells were thawed on ice, and protease inhibitors were added as described above. The lysate was cleared by sequential centrifugation at 5,000 × *g* for 15 min and 22,000 × *g* for 15 min in a Sorvall SS34 rotor, after which the ribosomes were pelleted by centrifugation at 200,000 × *g* for 2 h in a Beckman Ti60 rotor. The postribosomal supernatant was collected and stored at −70°C. The ribosomal pellet was resuspended in 15 ml of 20 mM Tris (pH 7.5)–10 mM $MgSO_4$–500 mM KCl–1 mM dithiothreitol (ribosome wash buffer) plus protease inhibitors and again centrifuged for 2 h at 200,000 × *g*. The supernatant (ribosomal salt wash) was dialyzed overnight against 2 liters of ribosome lysis buffer and frozen at −70°C until further use.

A 2-mg sample of the ribosomal salt wash or the post ribosomal supernatant was loaded onto a Superose 6 gel filtration column on a Pharmacia fast protein liquid chromatography system equilibrated with ribosome lysis buffer. Forty 0.5-ml fractions were collected and analyzed by Western blotting. The column was calibrated with thyroglobulin (670 kDa), β-galactosidase (540 kDa), gamma globulin (158 kDa), ovalbumin (44 kDa), myoglobin (17 kDa), and vitamin B_{12} (1.3 kDa).

Nucleotide sequence accession number. The *SQT1* nucleic acid sequence has been assigned GenBank accession no. U75717.

RESULTS

Isolation of dominant-negative *QSR1* mutants. To investigate how much of the *QSR1*-encoded protein is essential, three C-terminal truncations of Qsr1p were constructed by deleting portions of *QSR1* (Fig. 1) and placing the resulting constructs under the control of the *GAL1-10* upstream activating sequence (UAS). The truncated peptides are 64 (encoded by GAL_{UAS}-*qsr1-64*Δ), 133 (encoded by GAL_{UAS}-*qsr1-133*Δ), and 187 (encoded by GAL_{UAS}-*qsr1-187*Δ) residues in length compared to 221 residues (encoded by GAL_{UAS}-*QSR1*) in the full-length Qsr1p. These four plasmids were transformed into HFF10, a strain hemizygous for *QSR1* (54), and tetrads were dissected from the sporulated diploid. None of the deletion alleles could cover the *QSR1* chromosomal deletion, while the full length GAL_{UAS}-*QSR1* allele covered the deletion strain when grown on galactose but not on dextrose (data not shown). Examination of spores that failed to grow showed that they did not divide even once, indicating that the nonconserved C-terminal 34 residues (16) are essential for *QSR1* function.

We noticed that diploids or haploids harboring the longest deletion (GAL_{UAS}-*qsr1-64*Δ) and the shortest deletion (GAL_{UAS}-*qsr1-187*Δ) grew much slower than those carrying full-length *QSR1* when spread on galactose plates. The dominant-negative slow growth phenotype was stable throughout multiple switches between galactose and dextrose media without the occurrence of spontaneous revertants, and the growth defect was evident at temperatures from 16 to 37°C. In liquid culture, the doubling times increased from 1.5 to 3.5 h, and the cellular morphology appeared normal (data not shown).

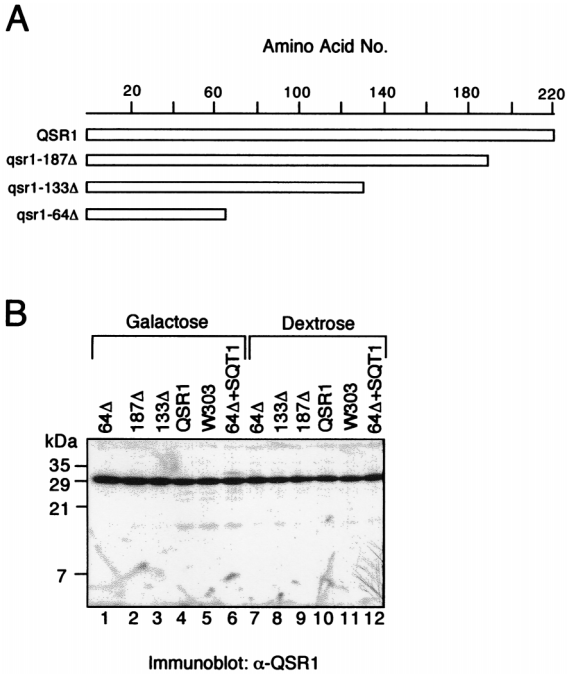

FIG. 1. (A) C-terminal truncation mutations of the 221-amino-acid Qsr1p were constructed by making three in-frame deletions fused to the last residue before the stop codon of *QSR1*. (B) Immunoblot analysis of dominant-negative *qsr1* truncation strains with anti-Qsr1p antibodies. The strains are DEH64 (GAL_{UAS}-*qsr1-64*Δ), DEH133 (GAL_{UAS}-*qsr1-133*Δ), DEH187 (GAL_{UAS}-*qsr1-187*Δ), DEH221-(GAL_{UAS}-QSR1), W303, and DESQT1B (DEH64 rescued with *SQT1* expressed from a 2μm plasmid). Western blots of lysates from cells grown on galactose and on dextrose are shown in lanes 1 to 6 and lanes 7 to 12, respectively.

In an effort to quantitate the relative amounts of Qsr1p and truncated Qsr1 proteins, haploid *QSR1* strains carrying the various dominant-negative alleles on plasmids were grown on galactose or dextrose and analyzed by Western blotting. The blot in Fig. 1B is overexposed to show that none of the truncated proteins, predicted to be 21.6, 15.3, and 7.5 kDa, can be detected when induced by growth on galactose. Furthermore, the endogenous Qsr1p levels remain unchanged even when an extra wild-type copy of galactose-inducible *QSR1* is present. The apparently invariant amount of Qsr1p is consistent with previous findings that unassembled ribosomal proteins have short half-lives and are degraded rapidly (56). Although the antibodies do not detect any of the truncated forms of Qsr1p, the shortest (qsr1-64Δp) and longest (qsr1-187Δp) truncated proteins apparently are present in sufficient quantities to exert a dominant-negative effect. The failure of the antibodies to detect the truncated proteins may be because the antibodies recognize only the C-terminal portion of Qsr1p that is deleted in all three constructs. This is also the least conserved region of the protein (15).

Isolation and characterization of *SQT1*. We exploited the dominant-negative effect of the Qsr1p truncations on growth to search for high-copy suppressors in a genetic screen. Strain DEH64 (*QSR1* pDEGQ64[GAL_{UAS}-*qsr1-64*Δ *CEN URA3*]) was transformed with a *LEU2*-marked high-copy genomic library, and fast-growing colonies were picked. Screening of 10^5 transformants yielded 16 large colonies. After passage through *E. coli*, nine of the library plasmids recovered from the fast-growing colonies suppressed the slow-growth phenotype when

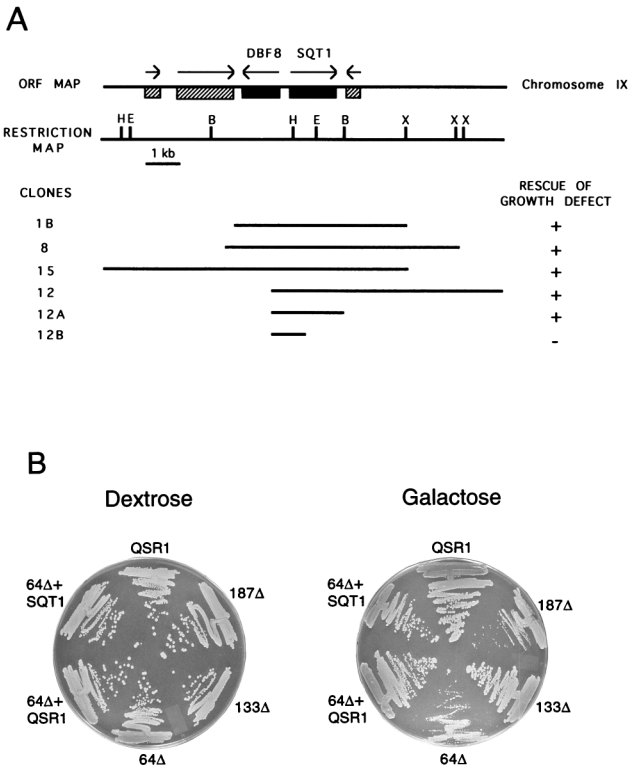

FIG. 2. Rescue of the dominant-negative growth defect by *SQT1*. (A) Map of the *SQT1* locus and genomic clones carrying *SQT1*. The top of the figure shows the open reading frames and restriction map in the vicinity of *SQT1* on the long arm of chromosome IX. The *SQT1* open reading frame is separated from the divergently transcribed gene *DBF8* (27) by 242 bp. Clones 1B, 8, 15, and 12 were isolated by complementing the slow-growth phenotype of the *qsr1-64*Δ mutant strain DEH64. Clones 12A and 12B are subclones of clone 12. The ability of the clones to rescue the slow-growth phenotype of strain DEH64 is indicated on the right. A restriction map is given for *Hin*dIII (H), *Eco*RI (E), *Bam*HI (B), and *Xho*I (X). (B) Effect of *qsr1* truncations on yeast growth. The strains are DEH64 (GAL_{UAS}-*qsr1-64*Δ), DEH133 (GAL_{UAS}-*qsr1-133*Δ), DEH187 (GAL_{UAS}-*qsr1-187*Δ), DEH221$^-$ (GAL_{UAS}-*QSR1*), DESQT1B (DEH64 rescued with *SQT1* expressed from a 2μm plasmid), and DESQT4 (DEH64 rescued with *QSR1* expressed from a 2μm plasmid).

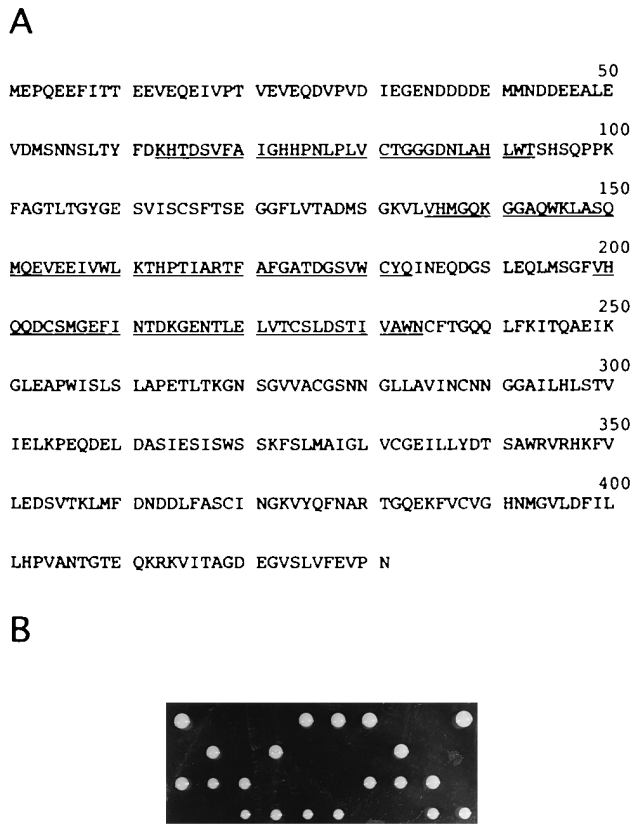

FIG. 3. (A) The deduced amino acid sequence of Sqt1p with the sequences of three WD repeats underlined. The middle repeat (residues 135 to 183) conforms to the consensus sequence of Neer et al. (44), except for an insertion of 10 residues instead of 3 after the initial VH. (B) Tetrad analysis of the hemizygous *sqt1*Δ*1* strain DPE1 showing that the *SQT1* null allele (*sqt1*Δ*1*) segregates 2:2 with lethality.

retransformed into strain DEH64. Restriction digestion analysis and partial sequencing of the genomic inserts revealed that five of the plasmids carried three identical and two overlapping clones of *QSR1* and the remaining four carried overlapping clones of a novel DNA fragment that mapped to a region on the long arm of chromosome IX (54a). The four genomic chromosome IX clones and two subclones are depicted in Fig. 2A and show that a single open reading frame that we have named *SQT1* (suppressor of *QSR1* truncations) suppresses the slow-growth phenotype caused by the dominant-negative *QSR1* truncations in the presence of a chromosomal copy of *QSR1*. Examination of the DNA sequence surrounding the open reading frame reveals that there are several TATA-like elements (consensus TATAA) in the region 50 to 120 bp upstream of the open reading frame (51) and a putative polyadenylation signal (55) centered at position 1349.

The dominant-negative effect on growth of the GAL_{UAS}-*qsr1-64*Δ and GAL_{UAS}-*qsr1-187*Δ alleles and rescue of the GAL_{UAS}-*qsr1-64*Δ allele by high-copy *QSR1* and *SQT1* are shown in Fig. 2B. *SQT1* is an allele-specific suppressor since it does not rescue the lethality of the *QSR1* chromosomal deletion, nor does it suppress temperature-sensitive *qsr1* mutants or the original *qsr1-1* missense mutation (data not shown). When a minimal 1.8-kb fragment encompassing the *SQT1* open reading frame was cloned into a centromeric vector, it also suppressed the growth defect on galactose (data not shown), suggesting that the relationship of *SQT1* and *QSR1* is specific, since a weaker nonspecific interaction would most probably require overexpression typical of a high-copy plasmid.

The *SQT1* open reading frame encodes a predicted protein of 431 amino acids and a molecular mass of 47.1 kDa with a calculated pI of 4.16 (Fig. 3A). Inspection of the deduced amino acid sequence and a search of the databases reveals that *SQT1* encodes a novel protein with sequence similarity to the β-transducin WD repeats (44). The deduced Sqt1p sequence includes at least two, and possibly three, consensus WD repeats (Fig. 3A).

Originally identified in the β-subunit of heterotrimeric GTP-binding proteins, WD-repeat motifs are present in a family of conserved eukaryotic proteins that seem to have a common function in that all known members are regulatory proteins and none are enzymes (44). Searches of the nucleic acid and protein data banks revealed significant homologies of Sqt1p limited to other proteins through the two major WD repeats. The homologous proteins include known WD proteins such as Tup1p, which is involved in gene regulation (30); Ret1p, a coatamer protein involved in vesicular traffic (44); and AAMP, a mammalian mediator of heparin-sensitive cell adhesion (4).

***SQT1* is an essential gene.** A hemizygous *SQT1/sqt1Δ1::HIS3* diploid strain (DPE1) was created by replacing one chromosomal copy of *SQT1* with the *HIS3* gene. Sporulation of the diploid and dissection of tetrads yielded two viable spores from each diploid, as shown in Fig. 3B, and all of the viable spores were His^- (data not shown), indicating that *SQT1* is an essential gene. Microscopic examination of the spores that failed to grow revealed that they had germinated but failed to progress beyond a two- to six-cell stage. The 1.8-kb *SQT1* complementing fragment on a centromeric plasmid (pDESQT2) transformed into strain DPE1 rescued the lethality with a frequency compatible with segregation of a centromeric plasmid when the hemizygous diploid was sporulated (data not shown).

***SQT1* rescues a defect in 40S to 60S ribosomal-subunit joining caused by the dominant-negative *qsr1-64Δ* mutant.** Since previous studies have shown that *QSR1* is essential for the ability of 60S subunits to dock with 40S subunits to form 80S ribosomes (14), we investigated the effect of the dominant-negative *qsr1-64*Δ allele on the distribution of ribosomal subunits and polysomes. Figure 4 shows polysome profiles of the *qsr1-64*Δ strain repressed on dextrose, induced on galactose, and rescued by *SQT1*. The *qsr1-64*Δ strain grown on dextrose has a polysome profile typical of wild-type cells in that there is a large 80S peak and polysome peaks towards the bottom of the gradient. When the dominant-negative qsr1-64Δ protein is induced by galactose, the 80S and polysome peak sizes are dramatically reduced, and half-mer polysomes are seen on the heavier side of the 80S peak and smaller polysomes (Fig. 4B). The polysome profile is restored to that of the dextrose condition when *SQT1* is present on a centromeric plasmid (Fig. 4C).

Half-mer polysomes are a result of a 60S-40S subunit-joining defect such that stalled 43S preinitiation complexes on an mRNA template containing at least one translating 80S ribosome have not joined with a 60S subunit (23, 47). A defect in the assembly of 60S subunits (stoichiometric imbalance) or a subunit joining defect leads to half-mers. Most reported half-mers occur in mutants that display depleted or mutated 60S ribosomal proteins and are thus defective in assembling 60S subunits (11, 12, 35, 38, 39, 45, 47, 48). We have previously shown that temperature-sensitive mutations in *QSR1* or depletion of Qsr1p by down regulation of an inducible promoter results in a population of 60S subunits lacking Qsr1p that are defective in subunit joining as opposed to a deficiency of 60S subunits (14). The degree of half-mer formation is greater in the temperature-sensitive strains than in the strain expressing the induced dominant-negative *qsr1-64*Δ allele.

To investigate whether the dominant-negative *qsr1-64*Δ mutation causes a subunit imbalance, we examined the ratio of 40S to 60S subunits by running polysome gradients that lack Mg, which causes 80S ribosomes to dissociate (14, 18, 46). As shown in Table 2, there is a small but significant increase in the ratio of 40S to 60S subunits when the *qsr1-64*Δ allele is induced on galactose, and the ratio is restored to essentially that of the dextrose condition when high-copy *SQT1* is present. Although the subunit imbalance is slight, it is difficult to say whether the half-mers result from a subunit imbalance caused by the truncated Qsr1p or whether the truncated Qsr1p inhibits subunit joining by one of the mechanisms discussed below.

***SQT1* and *QSR1* interact in a two-hybrid assay.** To test for interactions between Qsr1p and Sqt1p, we used two different bait vectors expressing fusion proteins with either the *LexA* DNA-binding protein or the *GAL4* DNA binding domain. To express the second hybrid, we fused the open reading frames encoding both proteins to the *GAL4* activation domain. The full-length Qsr1p and Sqt1p were fused to the *LexA* DNA binding protein in pEG202 (20) and to the *GAL4* activation domain in pACT2 (17). Combinations of different bait and prey plasmids were cotransformed together with the *LexA-lacZ* reporter plasmid pSH18-34 into the EGY48 reporter strain, and transformants were identified by growth on selective medium.

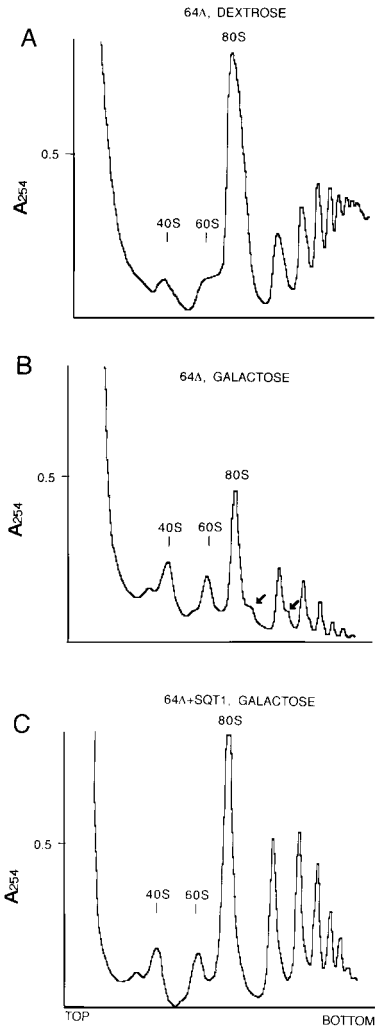

FIG. 4. Changes in the polysome profiles resulting from expression of the dominant-negative *qsr1-64*Δ allele and rescue by *SQT1*. Cells were pretreated with cycloheximide, and lysates were fractionated on 5 to 47% sucrose gradients containing Mg and cycloheximide. The 40S, 60S, and 80S subunit peaks are marked in the absorbance profile. The arrows in panel B mark the location of half-mer polysomes. (A) DEH64, GAL_{UAS}-*qsr1-64*Δ grown on dextrose; (B) DEH64, GAL_{UAS}-*qsr1-64*Δ grown on galactose; (C) DESQT1B, DEH64, GAL_{UAS}-*qsr1-64*Δ grown on galactose and rescued with *SQT1* expressed from a 2μm plasmid.

Interaction of the fusion proteins was monitored first by streaking colonies on X-Gal medium and then by performing β-galactosidase assays. As shown in Fig. 5, when the *LexA-QSR1* fusion or the *LexA-SQT1* fusion was coexpressed with the *GAL4* activation domain alone, the cells showed only basal-level β-galactosidase activity, which was also observed when the LexA protein was expressed together with the *GAL4* activation domain. This basal-level activity corresponded to white colonies on the X-Gal plates and demonstrates that the LexA hybrids of Qsr1p and Sqt1p do not interact with the *GAL4* portion of the prey hybrid or induce β-galactosidase activity

TABLE 2. Ratio of 40S to 60S subunits in wild-type cells, *qsr1-64*Δ mutants, and *qsr1-64*Δ mutants rescued by *SQT1*

Strain	Medium supplement	Mean 40S-to-60S ratio[a] ± SD
W303-1B	Dextrose	0.470 ± 0.04
DEH64 (GAL_{UAS}-*qsr1-64*Δ)	Dextrose	0.419 ± 0.016
DEH64 (GAL_{UAS}-*qsr1-64*Δ)	Galactose	0.534 ± 0.037
DESQT1B (GAL_{UAS}-*qsr1-64*Δ, +2μm *SQT1*)	Galactose	0.464 ± 0.008

[a] Ratios of 40S to 60S subunits were calculated by determining the corresponding areas under the 254-nm absorbance profiles.

themselves. The same basal level of reporter gene activity was observed when the *GAL4-QSR1* and *GAL4-SQT1* prey plasmids were coexpressed with the LexA protein. When the *GAL4-SQT1* prey plasmid was introduced into cells bearing the *LexA-QSR1* fusion construct, it resulted in β-galactosidase activity approximately 100-fold higher than basal levels (Fig. 5). Coexpression of *LexA-SQT1* and *GAL4-QSR1* prey plasmids resulted in a 25-fold increase in β-galactosidase activity. In both cases, the colonies were blue on X-Gal medium.

Similar results were obtained when *QSR1* and *SQT1* were fused to the *GAL4* DNA binding domain in the bait vector pAS2-1 (data not shown). Coexpression in strain Y190 of either the *QSR1* or the *SQT1* bait fusion together with the *GAL4* activation domain alone did not cause expression of the *HIS3* or *lacZ* reporter genes. When the *QSR1* bait fusion was transformed into yeast cells bearing the *SQT1* prey fusion, the cells grew on minimal medium without histidine and formed blue colonies in the β-galactosidase filter assay. Expression of the reporter genes was also observed when *SQT1* was fused to the DNA binding domain and *QSR1* was coexpressed as the prey fusion.

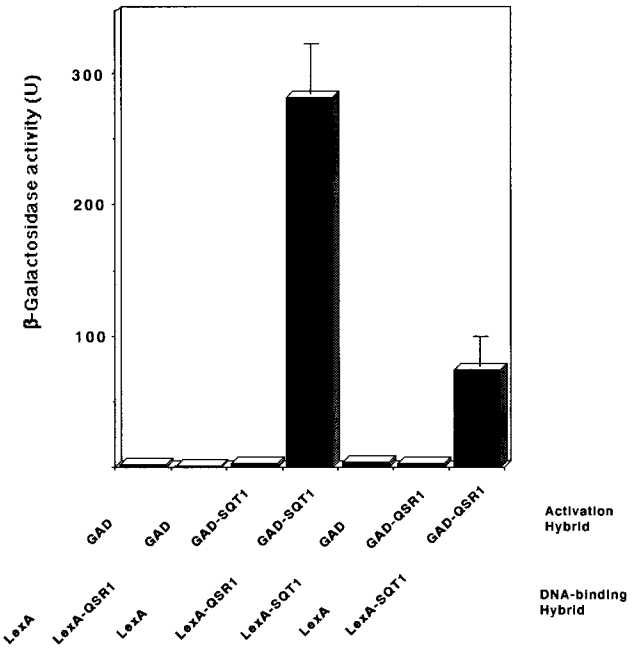

FIG. 5. Directed two-hybrid analysis of *QSR1* and *SQT1*. Proteins were expressed from the LexA fusion vector pEG202 and its derivatives pEG202-QSR1 and pEG202-SQT1 and from the *GAL4* activation domain fusion vector pACT2 and its derivatives pACT2-QSR1 and pACT2-SQT1. LexA, full-length LexA protein; GAD, *GAL4* activation domain. Transformants were assayed for β-galactosidase activity, and the activities are means of 10 to 15 independent transformants, each assayed in triplicate. Standard errors of the basal activities were below 30% and are not depicted. The *GAL4* activation domain LexA plasmid pSH17-4 was used as a positive control and showed an activity of 590 U.

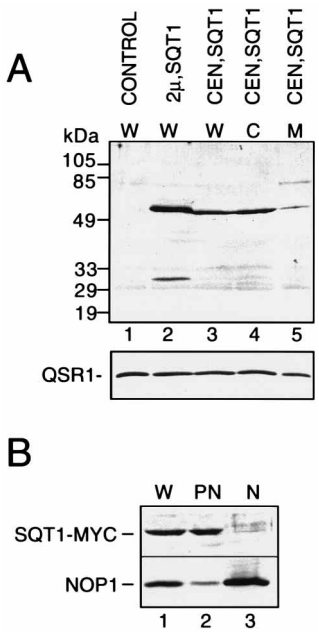

FIG. 6. Sqt1p is a cytosolic protein. Protein fractions from strains expressing a myc-tagged Sqt1p were visualized by immunoblotting and detection with the 9E10 mouse monoclonal anti-myc antibody. (A) Whole-cell lysates (W), cytosol (C), and membrane (M) fractions from the indicated yeast strains. The Western blot was first probed with anti-myc antibodies and then stripped and reprobed with anti-Qsr1p antibodies. Lanes: 1, the parent strain DPE1, in which Sqt1p is not myc tagged; 2, strain DPESM1 (*SQT1*/*sqt1*Δ*1*) expressing Sqt1-myc protein from a 2μm plasmid; 3, strain DPESM2-1A (*sqt1*Δ*1*) expressing Sqt1-myc protein from a CEN plasmid; 4, cytosolic fraction of DPESM2-1A; 5, membrane fraction of DPESM2-1A. (B) Strain DPESM2-1A was fractionated to obtain nuclei, and blots were probed with anti-myc and anti-Nop1 antibodies. Lanes: 1, whole-cell lysate; 2, postnuclear supernatant; 3, nuclear fraction.

Sqt1p is a cytosolic protein with an affinity for ribosomes. Sqt1p was myc tagged at the C terminus by fusion of the myc epitope (31) to the last residue of the *SQT1* open reading frame, and a CEN plasmid carrying the myc-tagged *SQT1* was transformed into the hemizygous *SQT1* strain DPE1. When this diploid was sporulated and tetrads were dissected, the *SQT1-myc* allele covered the *SQT1* chromosomal deletion with a frequency compatible with segregation of a centromeric plasmid, and the deletion strain covered by the CEN plasmid carrying *SQT1-myc* grew with a normal doubling time in liquid culture at 30°C.

Western blot analysis of the *SQT1-myc* strains with a mouse monoclonal anti-myc antibody reveals a specific protein which is not present in the control strain lacking the myc-tagged protein (Fig. 6A) and which is more abundant in the strain expressing the myc-tagged protein from a 2μm plasmid. The myc-tagged protein migrates at approximately 52 kDa, compared to its predicted molecular mass of 48.5 kDa. Subcellular fractionation of cytosolic and membrane fractions from the *SQT1-myc* strain DPESM2-1A [*sqt1*Δ*1* pSQTMYC2(*SQT1-MYC CEN LEU2*)] shows Sqt1p to be predominantly in the cytosolic fraction (Fig. 6A). The levels of Qsr1p are not affected by the gene dosage of *SQT1* in the different strains, and the relative amount of Qsr1p in the membrane fraction is greater than the amount of Sqt1p in that fraction (Fig. 6A). The association of Qsr1p with the membrane fraction is con-

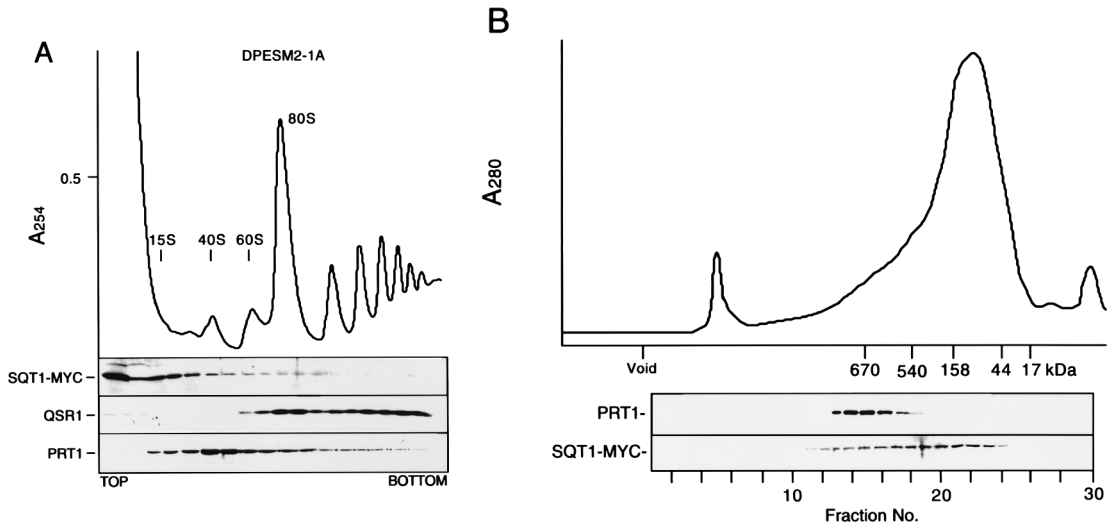

FIG. 7. Characterization of Sqt1p with the translational machinery. (A) Analysis of polysome profiles and distribution of Sqt1p, Qsr1p, and Prt1p in strain DPESM2-1A. Cell lysates were fractionated by centrifugation on 5 to 47% sucrose gradients. Immunoblots of Sqt1p and Prt1p are aligned below the tracings. (B) Fractionation of a 0.5 M KCl ribosomal salt wash fraction by gel filtration chromatography on a Superose 6 column. The 280-nm absorbance (A_{280}) profile is shown with the location of known protein standards. Immunoblots of Sqt1p and Prt1p are aligned below the corresponding column fractions.

sistent with the fact that 60S subunits bind to the endoplasmic reticulum with relatively high affinity (6, 29).

One possible explanation for the rescue of the subunit-joining defect in the mutants is that 60S subunit assembly is impaired by the truncated Qsr1p and rescued by *SQT1*. Since 40S and 60S subunits are assembled in the nucleolus (36, 56, 58), we investigated whether Sqt1p is a nuclear protein. When cell lysates were fractionated to recover nuclei and postnuclear supernatants, Sqt1p was recovered in the supernatant fraction, while Nop1p, a nucleolar marker (52), was enriched in the nuclear fraction, as shown in Fig. 6B.

Since Sqt1p is a cytosolic protein that physically interacts with Qsr1p, a 60S ribosomal subunit protein, we analyzed the distribution of Sqt1p in polysome gradients. As shown in Fig. 7A, the majority of Sqt1p is recovered in the top 0.5 ml of the polysome gradient, which contains most of the free cytosolic proteins. However, Sqt1p does migrate significantly into the gradient and is present even in the 80S ribosome and small polysome fractions. This is unlikely to be nonspecific tailing, since cytosolic markers such as hexokinase are recovered solely in the top of these gradients (13). Furthermore, when a strain (DPESM1) expressing *SQT1* from a high-copy plasmid was similarly analyzed, all of the excess Sqt1p accumulated at the top of the gradient, while the lower fractions contained the same relative amounts of Sqt1p as observed with the low-copy strain (data not shown). These properties of Sqt1p are consistent with a loose association with ribosomes.

The apparently loose association of Sqt1p with ribosomes resembles the behavior of some translational initiation factors (24). Translational initiation factor eIF-3 is a stable complex of at least eight polypeptides with an aggregate mass of approximately 600 kDa and is one of the least understood translational initiation factors (for a review, see reference 24). eIF-3 is involved in several steps of the initiation pathway; it is one of the first factors to bind to the 40S subunit, shifting the equilibrium of 40S subunits away from association with 60S subunits, and is thought to be the last factor to leave upon subunit joining. A recent description of the mammalian eIF-3 subunits lists a 47-kDa protein (24), the molecular mass of Sqt1p, and although there has not been any report of a 47-kDa protein in yeast eIF-3, SDS-PAGE of yeast eIF-3 does show a protein in this molecular mass range (41). We thus monitored the distribution of Prt1p, a subunit of the eIF-3 complex (19, 41, 42), in these gradient fractions to explore the possible colocalization of Sqt1p with the eIF-3 complex. The distribution of Sqt1p does not match that of Prt1p, which is concentrated around the 40S subunit (Fig. 7A). The smallest form of Prt1p is in fraction 3, in agreement with where the 600-kDa eIF-3 not bound to 40S subunits would run and where the 540-kDa β-galactosidase marker was also recovered.

To further test the possible association of Sqt1p with the eIF-3 complex, we washed ribosomes with 0.5 M KCl to obtain an extract enriched for translational initiation factors (10, 41). By quantitative Western blot analysis, we estimated that 5% of the Sqt1p from the cell lysate was recovered with this fraction, with the remaining 95% in the postribosomal supernatant (data not shown). When the salt-washed ribosomal extract was fractionated by gel filtration on a Superose 6 column, Sqt1p was concentrated in fraction 21, corresponding to a molecular mass of less than 158 kDa but significantly larger than the 47 kDa predicted for Sqt1p (Fig. 7B). The elution profile of Sqt1p does not match that of Prt1, which eluted at the same position as the 670-kDa marker, in agreement with the size of the eIF-3 complex (10). We also fractionated the postribosomal supernatant on a Superose 6 column and found that both Sqt1p and Prt1p localized in the same fractions as from the ribosomal salt wash (data not shown). These results show that Sqt1p is distinct from the eIF-3 complex. Sqt1p behaves as if it is either oligomeric or associated with a novel complex of 90 to 150 kDa.

Depletion of Sqt1p results in a subunit-joining defect. We analyzed the phenotype of cells depleted of Sqt1p by constructing a yeast strain (FAD13-2B) in which *SQT1* is controlled by the *GAL1-10* promoter on a plasmid. The *GAL-SQT1* plasmid covers the *SQT1* chromosomal deletion when grown on galactose, and the cells covered by myc-tagged Sqt1p grow at the same rate as those covered by Sqt1p (data not shown). By Western analysis, it was determined that Sqt1p was undetectable 8 h after a shift to dextrose medium (data not shown). The *GAL-SQT1* strain was pregrown in dextrose for 8 h to deplete

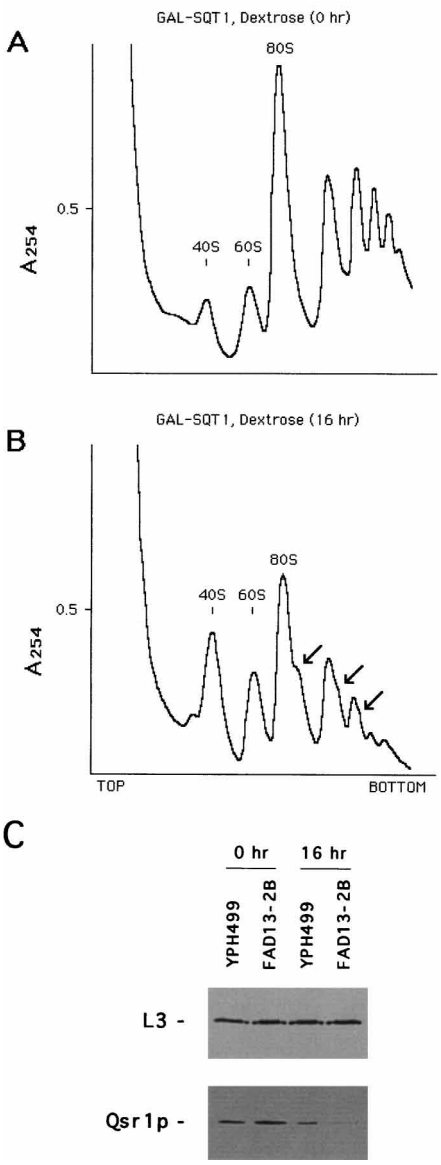

FIG. 8. Loss of *SQT1* function results in half-mer polysomes. FAD13-2B cells were grown on galactose medium followed by 8 h of preculture on dextrose medium, before being reinoculated into fresh dextrose medium. Extracts were prepared from cycloheximide-arrested cells at 0 h (A) or 16 h (B) after reinoculation into dextrose medium and analyzed on 5 to 47% sucrose velocity gradients. Fractions containing 60S subunits were pooled from gradients of FAD13-2B prepared as above (A and B) and a wild-type control (YPH499) for the 0- and 16-h time points and were used to prepare SDS-PAGE samples, which were probed for Qsr1p and L3 (C).

Sqt1p and then reinoculated into fresh dextrose medium. A growth defect became evident 12 h after reinoculation into the dextrose medium, and eventually the cells ceased to grow (data not shown). Polysome profiles of cells immediately after reinoculation were indistinguishable from those of wild-type cells (Fig. 8A). However, whereas the polysome profiles of the wild-type cells remained unchanged after 16 h on dextrose (data not shown), a half-mer phenotype is clearly present in polysomes from the *GAL-SQT1* cells and there is a decrease in the 80S and polysome peaks (Fig. 8B). The altered polysome profile mimics that of the dominant-negative *qsr1* truncation mutants, including the slight decrease in 60S abundance (compare Fig. 8B with Fig. 4B). Western blots of 60S subunit fractions from wild-type cells and the *GAL-SQT1* strain indicate that Qsr1p is significantly depleted in the 60S fraction 16 h after depletion of Sqt1p (Fig. 8C).

DISCUSSION

Truncations of *QSR1* which are unable to substitute for the full-length gene inhibit growth when expressed in yeast which contain a wild-type chromosomal copy of *QSR1*. Of the three truncations tested, only those encoding the longest (qsr1-187Δp) and shortest (qsr1-64Δp) forms of the protein acted as dominant-negative mutations, while a truncation predicted to encode a protein of intermediate length (qsr1-133Δp) had no detectable effect on growth, possibly because this form of the protein is rapidly degraded and thus is not present in sufficient amounts to exert a negative effect. It was not possible to determine the relative amounts of the three truncated proteins, since antibodies raised against Qsr1p did not detect any of the truncated proteins. We suspect that this is because the antibodies recognize epitopes only in the nonconserved C terminus (16), which is absent in all three truncated proteins.

The *QSR1* truncations were placed under the control of a galactose-inducible promoter, thus permitting their controlled expression. When the dominant-negative *qsr1-64*Δ allele was induced by growth on galactose, examination of the polysome profile revealed a ribosomal subunit-joining defect, as evidenced by the presence of half-mer polysomes. When the *qsr1-64*Δ allele was repressed on dextrose, a wild-type polysome profile was restored. The half-mer phenotype of the *qsr1-64*Δ allele is consistent with disruption of wild-type Qsr1p function, since temperature-sensitive *qsr1* mutants and the original *qsr1-1* mutant exhibit similar half-mer polysome profiles (14).

By screening a yeast genomic library, we isolated a novel suppressor of *qsr1* truncations, *SQT1*, which could rescue the growth inhibition and the half-mer polysome profile caused by the *qsr1-64*Δ allele. Although *SQT1* was initially isolated as a high-copy suppressor, subsequent tests established that expression from a CEN plasmid was equally effective at rescuing the dominant-negative mutants, indicating that a slight excess of Sqt1p in addition to that derived from the chromosomal gene is sufficient to reverse the toxic effects of the truncated proteins.

The screen for high-copy genomic suppressors also revealed that *QSR1* can reverse the dominant-negative effect of the *qsr1-64*Δ allele. This suggests that an excess of the wild-type protein can outcompete the dominant-negative truncated protein. In general, a dominant-negative polypeptide needs to be in excess for its inhibitory effects to be substantial (25).

Although *SQT1* rescued both of the dominant-negative *qsr1* truncations, it could not rescue temperature-sensitive *qsr1* mutants. This indicates that *SQT1* cannot function as a bypass suppressor of the temperature-sensitive alleles and cannot substitute for *QSR1*. *SQT1* also could not rescue the original *qsr1-1* mutation, a G194D missense mutation in the C terminus of the protein which is also semidominant against *QSR1* (54). *SQT1* thus appears to be an allele-specific suppressor of *qsr1* dominant-negative truncation mutations. The *qsr1-1* allele is rescued by a single chromosomal copy of *QCR6*, the gene which encodes subunit 6 of the mitochondrial cytochrome bc_1 complex (54). It is not known how a gene for a protein whose only known location is in the inner mitochondrial membrane can rescue an otherwise lethal missense mutation in a cytoplasmic ribosomal protein. The failure of *SQT1* to substitute for *QCR6* indicates different roles for *SQT1* and *QCR6* in their relationship to *QSR1*.

There are several mechanisms by which truncations of *QSR1* could exert a dominant-negative effect which is suppressed by *QSR1* or *SQT1*. One possibility is that the truncated Qsr1p causes reduced assembly of 60S subunits, resulting in a stoichiometric imbalance of ribosomal subunits and formation of half-mer polysomes. This seems unlikely, since there is only a slight change in the ratio of 40S to 60S subunits when the *qsr1-64*Δ allele is induced and the decrease in the proportion of 60S subunits does not seem sufficient to account for the appearance of half-mers, especially in comparison to previously reported mutants that are grossly defective in the assembly of 60S subunits (11, 12, 35, 38, 39, 45, 47, 48). It also seems unlikely that *SQT1* rescues a defect in assembly of 60S subunits. Whereas ribosomal subunits are assembled in the nucleolus and factors affecting the assembly of subunits have been found to be localized to the nucleolus (36, 56, 58), Sqt1p is excluded from the nucleus. Additionally, the loss of *SQT1* function in an otherwise normal genetic background does not appear to impair the assembly of 60S subunits to the degree expected from such a prolonged depletion of Sqt1p.

A second explanation for the dominant-negative effect is that the truncated Qsr1p is assembled onto the 60S subunit, where it inactivates or sequesters one or more closely associated ribosomal proteins and then falls off, leaving a 60S subunit incapable of subunit joining. Since Sqt1p seems to be loosely associated with ribosomes, it is conceivable that it stabilizes Qsr1p on the 60S subunit. This hypothesis is consistent with the observation that loss of *SQT1* function results in smaller amounts of Qsr1p on free 60S subunits. However, it is not obvious how *SQT1* could rescue the dominant-negative effect of the *qsr1-64*Δ and *qsr1-187*Δ mutations, since the corresponding truncated proteins could not substitute for Qsr1p.

A third possibility is that Sqt1p is involved in subunit joining and interacts with Qsr1p on the 60S subunit. In this case, truncated forms of Qsr1p might not be assembled onto the 60S subunit but could sequester Sqt1p and prevent its interaction with Qsr1p on the 60S subunit. The distribution of Sqt1p in polysome gradients and its interaction with Qsr1p in the two-hybrid system indicate that it interacts with ribosomes. We explored the possibility that Sqt1p is a subunit of a known translation initiation factor. Eukaryotic initiation factors are multiprotein complexes that have been defined biochemically by reconstituting in vitro translation. For reviews, see reference 24 and 53. Five initiation factors associate with the 40S subunit in a specific temporal order and, with the possible exception of eIF-2, dissociate before joining of the 60S subunit. The peptide subunits of eIF-1, eIF-1A, eIF-2, eIF-4, and eIF-5 are known, and the factors function in the initiation cycle between dissociation of 40S and 60S subunits and final 60S joining (8, 26, 33, 50). It thus seemed possible that Sqt1p is a subunit of eIF-3 (24), an initiation factor whose peptide composition is not well characterized (19, 40–42). However, Sqt1p does not colocalize with Prt1p, a known subunit of eIF-3, in polysome gradients or during size exclusion chromatography but behaves as if it were oligomeric or part of a previously unreported protein complex. Furthermore, inactivation of eIF-3 through transcriptional repression of a known subunit results in a large inactive 80S peak (40), not half-mer polysomes as described here.

The most plausible explanation is that Sqt1p is involved in assembling Qsr1p onto the 60S subunit late in the assembly pathway, and the truncated Qsr1p competes with full-length Qsr1p for assembly onto the 60S subunit. The lack of functional Qsr1p would result in a half-mer phenotype, as is the case for temperature-sensitive *qsr1* mutants (14). If truncated forms of Qsr1p sequester the essential Sqt1p, such a defect could be suppressed by extra expression of either *QSR1* or *SQT1*. Several of the known WD-repeat proteins participate in the assembly of multiprotein complexes, and it has been proposed that this is a common function for all WD proteins (44). Consistent with this interpretation is the fact that a defect in translational initiation would be expected to be manifested much sooner than 12 h after the disappearance of Sqt1p (40). Interestingly, pronounced growth defects in the FAD13-2B strain were found only when Sqt1p was depleted with an 8-h preculture before starting the growth curves. This indicates that Sqt1p may function early in log-phase growth, when most ribosomal biogenesis occurs (28). However, the cytosolic location of Sqt1p argues against a role in ribosome assembly in the nucleolus, suggesting that it may be important in a maturation or modification of 60S subunits that occurs in the cytoplasm. A delay in assembly of Qsr1p onto immature 60S subunits could result in their degradation and account for the slight 40S-60S subunit imbalance observed. If this explanation applies, the cytosolic location of Sqt1p would imply that Qsr1p is one of the small group of proteins which exchanges on and off of ribosomes in the cytoplasm (12, 59).

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