QSR1, an Essential Yeast Gene with a Genetic Relationship to a Subunit of the Mitochondrial Cytochrome bc1 Complex, Codes for a 60 S Ribosomal Subunit Protein*

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QSR1 (quinol-cytochrome c reductase subunit-requiring) is a highly conserved, essential gene in Saccharomyces cerevisiae that was identified through a synthetic lethal screen by its genetic relationship to QCR6, the gene for subunit 6 (Qcr6p) of the mitochondrial cytochrome bc1 complex. The function of the QSR1-encoded protein (Qsr1p) and its relationship to the QCR6-encoded protein are unknown.

When yeast cell lysates are fractionated by density gradient centrifugation, Qsr1p separates from organelles and sediments with a uniformly sized population of particles that are similar to eukaryotic ribosomes upon velocity gradient centrifugation. When 40 S and 60 S ribosomal subunits are separated on velocity gradients, Qsr1p is found exclusively with the 60 S subunits, where it is a stoichiometric component. Extracts prepared from qsr1-1 cells are defective in in vitro translation assays relative to the wild type.

In yeast cell lysates in which QCR6 rescues an otherwise lethal qsr1-1 mutation, Qcr6p is found only in mitochondria, both in respiratory-competent cells and in rho0 cells in which the bc1 complex is no longer present. These results suggest that suppression of the qsr1-1 mutation by QCR6 occurs by a trans-relationship across the outer mitochondrial membrane.

All cytochrome bc1 complexes contain three redox proteins, cytochrome b, cytochrome c1, and an iron-sulfur protein, which are essential for the electron transfer and energy transduction functions of this enzyme in respiration and photosynthesis (1). The cytochrome bc1 complexes of mitochondria also contain seven or eight additional subunits that lack prosthetic groups and that are not present in the bc1 complexes of prokaryotes (2). The functions of these supernumerary subunits in the mitochondrial enzymes are largely unknown.

QCR6 is the nuclear encoded gene for subunit 6 (Qcr6p) of the mitochondrial cytochrome bc1 complex. Deletion of QCR6 does not impair growth of yeast on respiratory substrates at temperatures up to 35 °C, indicating that the supernumerary subunit 6 is not essential for respiration, although the deletion does result in a temperature-sensitive petite phenotype at 37 °C (3). To test whether the deletion of QCR6 might be covered by another, functionally redundant gene, we screened for mutants that require the nonessential subunit 6 and identified quinol-cytochrome c reductase subunit-requiring mutants in two complementation groups, which we named qsr1 and qsr2 (4). Surprisingly, the qsr mutants require QCR6 to grow on either fermentable or nonfermentable carbon sources. This suggests that subunit 6 of the cytochrome bc1 complex has a role outside of respiration.

QCR6 suppresses an otherwise lethal missense mutation in a qsr1-1 mutant. We cloned QSR1 by complementing the qsr1-1 mutant for growth in the absence of QCR6 and showed that QSR1 is an essential gene in yeast (4). The protein encoded by QSR1 is highly conserved and has been identified in at least 10 different eukaryotic organisms (4–10). Comparisons of the amino acid sequences have shown the QSR1-encoded proteins to be at least 60% identical between the most unrelated homologues (10). Despite being identified in numerous organisms, little is known about the function of the QSR1-encoded protein. For the most part, the only information available has been the details of the molecular cloning of these genes, and the deduced amino acid sequences give few clues as to Qsr1p function.

In addition to showing that QSR1 is essential, we showed that Qsr1p is localized throughout the cytoplasm in a punctate staining pattern (4). In an effort to better understand the relationship between Qcr6p and Qsr1p, we have investigated the localization of these two proteins. Here we report that Qsr1p is a 60 S ribosomal subunit protein and that Qcr6p is found only inside mitochondria in yeast cells in which QCR6 rescues the qsr1-1 mutation.

EXPERIMENTAL PROCEDURES

Materials—Yeast extract, peptone, Tryptone, and yeast nitrogen base without amino acids were purchased from Difco. Dextrose was obtained from Fisher. Yeast lytic enzyme was from ICN Biochemicals. Sucrose was from Life Technologies, Inc. Cycloheximide, heparin (sodium salt, M, 3000), protease inhibitors, and Nycodenz were purchased from Sigma. DNA-modifying enzymes were purchased from New England Biolabs Inc., Life Technologies, Inc., and Boehringer Mannheim. Oligonucleotides were purchased from DNA Express (Fort Collins, CO) and the Molecular Biology Core Facility at Dartmouth College.

Yeast and Bacterial Strains—Wild-type cell lysates were prepared from Saccharomyces cerevisiae strains W303-1A (MATa) and W303-1B (MATa, ade2-1, his3-11,15, ura3-1, leu2-3,112, trpl-1, can1-100), which were obtained from Dr. R. Rothstein (Columbia University). The qsr1-1 mutant strain used in this study was LR10(qsr1-1) with pLK57 instead of pMES32 (4), except in Figs. 6 and 7, where MMY3-3B was used (4). The qcr6 deletion strain used was MESS (4). rho0 strains were created from the relevant rho+ parents by treatment with ethidium bromide (11) and genotyped on selective media. S. cerevisiae strains were grown in media prepared as described (12), except that amino acid dropout mixtures were purchased from BIO 101, Inc. Plasmids were amplified in Escherichia coli strain DH5α (pBR322) and selected in E. coli, and the appropriate plasmid was purified using a QiaGen kit.

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Fractionation of Yeast Lysates—An 800-ml culture of W303-1B cells was grown in YPD medium (1% yeast extract, 2% peptone, and 2% dextrose) to a density of 0.6 absorbance units at 600 nm. Cells were harvested by centrifugation at 1500 g for 15 min, resuspended in 1 ml of H$_2$O containing 0.5% $\beta$-mercaptoethanol, and centrifuged as before. Pellets were resuspended in 20 ml of 1.2 M sorbitol and 50 mM KH$_2$PO$_4$, pH 7.5, containing 0.1% $\beta$-mercaptoethanol and transferred to a 250-ml flat-bottomed flask, and yeast lytic enzyme was added to a final concentration of 0.25 mg/ml. The cell suspension was placed on a shaker at 30 °C for 20–30 min. Spheroplast formation was monitored by checking for osmotic lysis of 1 ml of cells diluted in 10 ml of H$_2$O (14). Spheroplasts were harvested by centrifugation at 2500 x g for 1 min at 4 °C; resuspended in 15 ml of 25 mM HEPES, 5 mM magnesium acetate, and 10% Nycodenz, pH 7.6, supplemented with 1 mg/ml pepstatin A, 10 $\mu$g leupeptin, 0.5 mM 1,10-phenanthroline, and 1 mM disopropyl fluorophosphate and transferred to a cold Dounce homogenizer.

Spheroplasts were lysed on ice with 15 strokes of the loose plunger and 10 of the tight plunger. This resulted in ~90% lysis as judged by viewing the lysate under a light microscope. The crude lysate was centrifuged at 2500 x g for 5 min to clear debris and unlysed cells. The supernatant was removed, and samples were saved for SDS-PAGE, protein determinations, and enzyme assays.

Nycodenz density gradients were prepared by adding a 6-ml aliquot of HEND15 buffer (HEND15, HEND20, HEND25, HEND30, and HEND35 buffers contain 25 mM HEPES; 1 mM EDTA; 15, 20, 25, 30, and 35% Nycodenz, respectively; and 1 mM diithothreitol, pH 7.6) to the bottom of a centrifuge tube. This buffer was then underlaid with HEND20 buffer, which was in turn underlaid with HEND25, HEND30, and HEND35 buffers. A 6-ml sample of the medium-speed supernatant was layered on top of this gradient and centrifuged at 120,000 x g in an SW 28 rotor for 10 h. The bottom of the centrifuge tube was punctured; 25 x 1.4 ml fractions were collected; and aliquots were taken for SDS-PAGE, protein determinations, refractometry, and enzyme assays (15, 16).

Fractions containing Qsr1p from the equilibrium density gradient were combined, and the Nycodenz concentration was adjusted to 35% by adding a 10% Nycodenz. Two ml of this sample was used to lay a gradient composed of 2 ml each of HEND30, HEND25, HEND20, and HEND15. This gradient was centrifuged at 180,000 x g in an SW 40 rotor for 16 h, after which 10 x 1-ml samples were collected, and aliquots were withdrawn for SDS-PAGE, protein determinations, refractometry, and enzyme assays (16).

Ribosomes were isolated as described by Hartl et al. (20). 

Ribosome Velocity Gradients—Velocity gradient centrifugations of ribosomes were performed by the Bio-Rad method according to the manufacturer’s instructions.

The GDPase marker for the Golgi apparatus was assayed enzymatically (19), and the maximum activity was scaled to 100%. Marker recoveries were calculated as activity per $\mu$l volume divided by activity per $\mu$l of lysate x volume of lysate for each marker. Nycodenz concentrations were determined on an Abbrefractometer, and protein determinations were performed by the Bio-Rad method according to the manufacturer’s instructions.

Ribosome Velocity Gradients—Velocity gradient centrifugations of lysates were done essentially as described by Ramirez et al. (20). Lysates of W303-1B cells were prepared in 20 ml Tris, pH 7.5, 50 mM NaCl, 1 mM diithothreitol, 0.2 mg/ml hemin, 1 mg/ml peptatin A, 10 $\mu$g leupeptin, and 1 mM disopropyl fluorophosphate. The lysate was then centrifuged at 15,000 x g for 30 min to remove debris and most organelles. Supernatant equivalent to 35 absorbance units at 600 nm was loaded onto 5–47% sucrose gradients prepared in 20 ml Tris, pH 7.5, 50 mM NaCl, 1 mM dithothreitol, 1 mg/ml peptatin A, and 10 $\mu$g leupeptin. The gradients were then centrifuged at 220,000 x g for 3 h and fractions were collected in an SW 28 rotor and were combined into 1.5-ml fractions. Absorbance was monitored at 254 nm. Yeast lysates and gradient buffer were supplemented with 50 mg/ml cycloheximide, 10 mg MgCl$_2$, or 0.5 M KCl where indicated. Samples were analyzed by SDS-PAGE and Western blotting as described above. Blots were probed with rabbit polyclonal antibodies to Qsr1p or mouse monoclonal antibodies to the ribosomal L3 protein (diluted 1:2500).

The relative amounts of Qsr1p and L3 associated with 60 S subunits, 80 S ribosomes, and polysomes were determined by scanning Western blots of fractions from a gradient of polysomes from yeast cells whose protein synthesis was arrested by addition of cycloheximide. Band intensities of the two proteins were quantified with the NIH Image program as described above.

Limited Trypsinolysis—Ribosomes were isolated as described by Rave et al. (21). 60 S subunits equivalent to 2 absorbance units at 260 nm were treated with 20 mg/ml trypsin for 0, 5, 15, and 30 min, either in the absence or presence of equimolar amounts of 40 S subunits as controls. Reactions were stopped by adding SDS-PAGE sample buffer preheated to 95 °C.

In Vitro Translation Assays—S100 extracts were prepared from wild-type or qsr1-1 mutant cells (23) and stored at a concentration of 10 mg/ml. Thawed extracts were treated with nucleases, and reactions were carried out at 20 °C at a final protein concentration of 6 mg/ml in the reaction buffer described by Leibowitz et al. (24). Total yeast RNA was prepared from spheroplasts using the RNeasy midikit from QIAGEN Inc., and poly(A)+ RNA was purified on oligo(dT)-cellulose (12). Reactions were stopped by spotting on trichloroacetic acid-soaked filter paper. Filters were washed extensively with 10% trichloroacetic acid and ethanol and dried, and incorporation of [35S]methionine into tri-chloroacetic acid-insoluble material was quantitated by liquid scintillation.

Construction of Plasmids—DNA manipulations were carried out according to the manufacturers’ directions and standard procedures (11). DNA was sequenced with the Prism dye terminator cycle sequencing kit from Applied Biosystems Inc. (ABI) or the modified FS kit. Reactions were performed on an ABI 373 automated sequencer at the Dartmouth Molecular Biology Core Facility, and the data were interpreted using Sequence Editor™ (ABI).

The plasmid pLK57 was obtained from the original library screening to complement the qsr1-1 mutant (4). It was identified as a QCR6-containing suppressor because it contains the QCR6 genomic 1.9-kilo-base SapI fragment. pLK13, which expresses the glutathione S-transf erase-QCR6p fusion, was constructed by amplifying the QCR6 open reading frame by polymerase chain reaction. The polymerase chain reaction mixtures were initially denatured at 95 °C for 3 min and then cycled 25 times at 95 °C for 30 s, 55 °C for 1 min, and 72 °C for 2 min, and the resulting product was subcloned into the EcoRI sites of pGEX-1xT. The primers used were 1QCR6 (5'-GAA TTC ATG TTG GAA CTA-3') and 2QCR6 (5'-GAA TTC CTA CTT AAT TTT GTC AAA TAA TCT AGG-3').

Preparation of Qsrp Antiserum—Antibodies to a glutathione S-transferase-Qsrp fusion protein were raised in rabbits (4), affinity-purified (22), and used at a dilution of 1:500.

Isolation of Mitochondria—Lysates of wild-type and qsr1-1 mutant strains were prepared by resuspending cells from a 5-ml culture in 50 ml Tris-Ci, pH 7.4, 400 mM mannitol, and 2 mM EDTA and vortexing with glass beads. Mitochondria were then pelleted by centrifugation at 16,000 x g for 15 min in a microcentrifuge. Samples for SDS-PAGE were prepared from the supernatant (cytosol) and the pellet (mitochondria).

Mitochondria with bound polysomes were purified with the buffers adjusted to pH 6.0 to minimize contamination by microsomes (25, 26). The pellet fraction containing mitochondria with bound polysomes was loaded onto a 30–80% sucrose step gradient in buffer containing 200 mg/ml cycloheximide and centrifuged at 120,000 x g for 16 h, and gradients were fractionated as described above. Mitochondria were treated with proteinase K as described by Hartl et al. (27).
RESULTS

Subcellular Fractionation of Qsr1p and Organelles—The punctate cytoplasmic localization of Qsr1p (4) implies that it is concentrated in some subcellular compartment or is oligomerized to create regions of high density when viewed by immunofluorescence microscopy. When lysates from exponentially grown yeast cells were centrifuged to remove nuclei, plasma membranes, and larger organelles, 95% of the Qsr1p was recovered in the supernatant (Table I). When this medium-speed supernatant was fractionated on a Nycodenz gradient, Qsr1p sedimented as a uniform population as shown in Fig. 1. The distribution of Qsr1p overlaps with, but does not exactly match that of Sec61p, an integral membrane protein that is part of the protein translocation complex of the endoplasmic reticulum.

To investigate the possibility that Qsr1p is associated with a subpopulation of endoplasmic reticulum, the fractions containing Qsr1p from the first Nycodenz gradient were combined and applied to the bottom of a flotation gradient. In this procedure, membranes floated up through the gradient until they came to equilibrium, while denser proteins and protein complexes remained on the bottom. Under these conditions, Qsr1p remained at the bottom of the gradient while Sec61p floated up (data not shown). Qsr1p was thus separated completely from all markers, except for 2% of the Atp2p mitochondrial marker, which was recovered at the bottom of the second gradient (Table I). That Qsr1p did not float in the second gradient indicates that it is not associated with any membranes. During the 10-h centrifugation of the first density gradient, membranes will reach their equilibrium density, but proteins and protein complexes will still be sedimenting toward their equilibrium position at the bottom of the tube and will be recovered according to their sedimentation rate rather than density. Since Qsr1p is predicted from the deduced amino acid sequence to be ~25 kDa and the cytosolic markers Hxk1p and Hxk2p are 100-kDa dimers in solution (28), Qsr1p appears to be part of a large complex that sediments significantly faster than Hxk1p/Hxk2p in the first density gradient (Fig. 1). Quantitation of the amount of Qsr1p relative to that in the initial lysate (Table I) indicates that ~80% of the Qsr1p was recovered after the second density gradient centrifugation as a large membrane-free complex under the standard conditions used to lyse and fractionate yeast cells.

Qsr1p Is a 60 S Ribosomal Subunit Protein—The size of the putative particle identified by the density gradient centrifugations was investigated by velocity gradient centrifugation and determined to be between 80 and 100 S under conditions that maintain association of ribosomal subunits (data not shown).

TABLE I

Recovery of Qsr1p and organelle markers after fractionation of yeast cell lysates

Recoveries were calculated by dividing the activity per μl × volume for each marker by the activity per μl × volume of the lysate. The percent recovery of each marker in the lysate is scaled to 100 for comparison. Pool 1 is fractions 11–15 from the first Nycodenz gradient (Fig. 1), and Pool 2 is the bottom two fractions from the flotation gradient described under “Experimental Procedures.”

| Marker | Organelle/compartment | Marker recovery |
|--------|------------------------|-----------------|
| Qsr1p  | 100                    | 95 85 83        |
| Sec61p | Endoplasmic reticulum  | 100 26 6 0      |
| Atp2p  | Mitochondria           | 100 34 5 2      |
| Hxk1p  | Cytosol                | 100 95 0 0      |
| GDPase | Golgi                  | 100 95 0 0      |
| Pho8p  | Vacuole                | 100 55 4 0      |

* MSS, medium-speed supernatant (described under “Experimental Procedures”).

FIG. 1. Qsr1p separates from organelle markers during density gradient centrifugation. Fractions from density gradient centrifugation were assayed enzymatically for GDPase and immunologically for Pho8p, Atp2p, Sec61p, Hxk1p/Hxk2p, and Qsr1p. Fractions were also assayed for protein content and Nycodenz concentration. Relative activities are shown to allow comparison between markers.

This size estimate led us to investigate whether Qsr1p is associated with ribosomes.

Rapidly sedimenting organelles and membranes were removed from yeast cell lysates by centrifugation for 30 min at 15,000 × g (20), after which polysomes were separated by gradient centrifugation. The distribution of 40 and 60 S subunits, 80 S ribosomes, and polysomes was monitored by absorbance at 254 nm, and the distribution of Qsr1p and L3, a large ribosomal subunit protein (29), was monitored by Western analysis.

The association of large and small ribosomal subunits is dependent upon the presence of Mg2+, and cycloheximide arrests ribosomes during translation on an mRNA template (20). We thus varied these parameters to test the association of Qsr1p with ribosomes. As shown in Fig. 2a, when protein
synthesis was inhibited with cycloheximide and Mg\(^{2+}\) was included, there were small amounts of 40 S and 60 S subunits followed by larger amounts of 80 S ribosomes and polysomes. L3 was localized to the 60 S and 80 S peaks and with the polysomes, as expected. A small amount of Qsr1p was with the 60 S subunit, and its distribution in 80 S and polysome fractions matched that of L3.

When Mg\(^{2+}\) was omitted from the buffers and cycloheximide was included, the ribosomes were able to finish translating in lysates, and translation initiation factors like Gcn2p are associated with the 40 S subunit (20). Under these conditions, Qsr1p was found with the 60 S subunit, 80 S ribosomes, and polysomes with the same distribution as the large ribosomal subunit marker L3.

Quantitative Western blots indicate that Qsr1p constitutes between 0.1 and 0.5% of total cell protein (data not shown), which is in agreement with the abundance of other ribosomal proteins (29). Although Qsr1p was consistently found in 60 S subunits, visual comparison of the relative amounts of L3 and Qsr1p in the 60 S subunits and 80 S ribosomes in Fig. 2 (a and c) indicated that proportionately less Qsr1p than L3 was present in free 60 S subunits. To investigate the stoichiometry of Qsr1p on 60 S subunits, the amounts of Qsr1p and L3 were quantitated by Western analysis of fractions from a gradient.

**Fig. 2.** Qsr1p is associated with ribosomes during velocity gradient sedimentation. Supernatants from 15,000 × g centrifugations of yeast cell lysates were fractionated on 5–47% sucrose gradients. The lysate and gradient buffers were prepared with 50 μg/ml cycloheximide (Cyc.) and 10 mM MgCl\(_2\) (a), with 50 μg/ml cycloheximide alone (b), or with 10 mM MgCl\(_2\) alone (c). During fractionation, absorbance at 254 nm was recorded to locate ribosomal subunits, 80 S couples, and polysomes. Western blots of L3 and Qsr1p are aligned below the tracings to show their distribution in the corresponding fractions. The relative amounts of Qsr1p and L3 in 60 S subunits, 80 S couples, and polysomes were determined by scanning a Western blot from a gradient fractionation identical to that in a and are shown in d.
identical to that in Fig. 2a. As shown in Fig. 2d, the ratio of Qsr1p to L3 was relatively constant and was close to 1 in fractions containing 80 S ribosomes and polysomes. In fractions 10 and 11, which included the 60 S subunits, Qsr1p was diminished 5-fold relative to L3. This suggests that although Qsr1p is quantitatively recovered with the ribosomal fraction (Fig. 1 and Table I), a portion of free 60 S subunits lack Qsr1p.

To further characterize the association of Qsr1p with the large ribosomal subunit, lysates and gradient buffers were prepared that contained 0.5M KCl and Mg$^{2+}$, but lacked cycloheximide. These conditions distinguish core ribosomal proteins from contaminants (21, 30). Under these conditions, Qsr1p remained with the 60 S subunit, 80 S ribosomes, and polysomes as shown in Fig. 3a. When 40 S and 60 S subunits were purified by standard procedures (21) and blotted for Qsr1p and L3, Qsr1p was with the 60 S subunits (Fig. 3b).

The profile in Fig. 4a shows a variation of the previous gradients in which Mg$^{2+}$ was omitted from the lysates and buffers and 0.5 M KCl was included. The absorbance profile indicates that the 40 S and 60 S subunits were intact, although somewhat smaller, and the L3 marker was still associated with the large subunit. Qsr1p, however, was now at the top of the gradient, indicating that it is extracted off of the 60 S subunit by 0.5M KCl if Mg$^{2+}$ is omitted.

If purified 60 S subunits were subjected to limited trypsinolysis, Qsr1p was more sensitive to degradation than L3 (Fig. 4b). However, when 60 S subunits were incubated with 40 S subunits to form 80 S couples, Qsr1p became significantly more resistant to trypsin, suggesting that it may be located between the large and small subunits in 80 S dimers. This agrees with findings that subunit joining is impaired in qsr1 mutants. Interestingly, under these same conditions, L3 became slightly more accessible to the protease. By these operational criteria, we conclude that Qsr1p is a peripherally located 60 S ribosomal subunit protein, and its association with the subunit is dependent on Mg$^{2+}$ in high ionic strength buffer.

**Colocalization of Qsr1p and L3 by Indirect Immunofluorescence Microscopy**—Double label indirect immunofluorescence microscopy was performed to investigate whether Qsr1p also colocalizes with the L3 ribosomal protein in vivo. The left panel in Fig. 5 shows Qsr1p detected by fluorescein staining, and the center panel shows the L3 protein detected by Texas Red staining. The two proteins clearly colocalize, as indicated by spots that have been labeled with arrows to point out double labeling by both antibodies, and their staining patterns agree with previously published staining patterns for yeast ribosomal proteins (31, 32).

**In Vitro Protein Synthesis Is Diminished in qsr1-1 Mutants**—Since the previous results indicate that Qsr1p is a 60 S ribosomal subunit protein, we tested whether Qsr1p is involved in protein synthesis or whether it serves some other purpose.

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while located on the 60 S subunit. As shown in Fig. 6, mRNA-dependent translation was greatly diminished in cell-free extracts prepared from the qsr1-1 mutant, suggesting that Qsr1p plays a role in protein synthesis. Although it is difficult to correlate rates of protein synthesis in vitro with growth rates, it is surprising that the in vitro protein synthesis activity of the mutant is approximately 5% of that of wild-type, while the doubling time of the mutant cells in YPD medium is only increased to 3.6 h compared with 1.6 h for the wild-type cells (data not shown). This may indicate that the extracts are no longer suppressed by Qcr6p since the mitochondria were removed during preparation or that in vitro extracts do not accurately reflect the activity of the translational machinery in vivo.

Localization of Qcr6p—Since Qcr6p is a subunit of the mitochondrial bc1 complex, located in the inner mitochondrial membrane, and it suppresses the qsr1-1 mutation in a cytoplasmic ribosomal protein, we investigated whether Qcr6p might also exist in a soluble cytosolic form, where it could carry out its suppressor role. Affinity-purified antibodies, raised against a glutathione S-transferase-Qcr6p fusion protein, were used to probe a blot of cell lysates from wild-type and qcr6 deletion strains. As shown in Fig. 7a, the antibodies detected a single band in the lysate from the wild-type strain and detected nothing in the qcr6 deletion strain, indicating that they are specific for Qcr6p. The Qcr6p in the lysate was compared with Qcr6p from the purified bc1 complex and was found to be of mature size, indicating that it had been proteolytically processed (data not shown).

When lysates from wild-type and qsr1-1 cells were centrifuged to obtain a supernatant containing cytosol and a pellet containing mitochondria, Qcr6p was found only in the pellet and not in the cytosol (Fig. 7b). In mitochondria from wild-type and qsr1-1 strains, Qcr6p was protected from degradation by proteinase K (Fig. 7c), a criterion that indicates that it is not on the cytoplasmic surface of the outer mitochondrial membrane. Solubilization of the membranes with detergent followed by treatment with proteinase K resulted in degradation of Qcr6p, indicating that it was protected from digestion by a membrane and not inherently protease-resistant (data not shown). These results show that Qcr6p, although synthesized on cytoplasmic polysomes, is undetectable in the cytoplasm under steady-state conditions and is found in the mitochondria.

Qcr6p Is Localized in rho0 Mitochondria—Since Qcr6p is a subunit of the cytochrome bc1 complex, its obligate presence in this membranous enzyme might obscure its detection elsewhere. We thus investigated the location of Qcr6p in wild-type, qcr6 deletion, and qsr1-1 yeast strains that were converted to rho0 by growth on media containing ethidium bromide (11). All of these strains lack the cytochrome bc1 complex due to the absence of the mitochondrial genome. As shown in Fig. 8a, when lysates from these rho0 strains were fractionated to separate a cytosolic supernatant from the rho0 mitochondria, Qcr6p was found exclusively in the pellet fraction, and treatment of the rho0 mitochondria with proteinase K showed that Qcr6p is protected (Fig. 8b). Western blots of severalfold greater amounts of supernatant also failed to reveal any Qcr6p (data not shown). These results indicate that, in cells in which it suppresses the mutation in a cytosolic ribosomal protein, Qcr6p is only detectable in mitochondria; it is sequestered inside the organelle and its location in mitochondria is independent of the cytochrome bc1 complex.

Qsr1p Remains Associated with Ribosomes Bound to Mitochondria—Treatment of cells with cycloheximide before harvesting traps ribosomes that are synthesizing mitochondrial proteins on the outer mitochondrial membrane (26). These ribosomes are docked at sites on the outer mitochondrial membrane that are in proximity to the inner membrane (33). Under these conditions, Qcr6p was again found only in the pellet mitochondria; however, Qsr1p was now also found extensively in the pellet (Fig. 9). The extent of this redistribution of Qsr1p can be appreciated by comparing the contents of the cytosol and pellet fractions in Fig. 9 with the Qsr1p content of the lysate and medium-speed supernatant shown in Table I. When these mitochondria were purified on a sucrose gradient, Qsr1p and Qcr6p comigrated (Fig. 9), further indicating that Qsr1p remains associated with ribosomes that are bound to mitochondria.

DISCUSSION

The subcellular localization of Qsr1p and its possible association with any organelles are of special interest because of the novel genetic relationship between QSR1 and QCR6, which encodes a subunit of the mitochondrial cytochrome bc1 complex (34). QSR1 is an essential gene, and a G194D missense mutation in QSR1 is lethal unless QCR6 is present. A single chromosomal copy of QCR6 will rescue the otherwise lethal qsr1-1 mutation. However, QCR6 cannot substitute for QSR1, either as a chromosomal or high copy plasmid gene, and QCR6 will rescue the qsr1-1 mutation in a rho0 cell (4).

These previous results indicate a functional interaction between Qsr1p and Qcr6p in which the presence of QCR6 somehow allows the qsr1-1 mutant to survive, and this rescue is independent of the role of the QCR6-encoded protein in mitochondrial respiration. The only known location of Qcr6p is in the cytochrome bc1 complex, a respiratory enzyme located in the inner mitochondrial membrane. Qsr1p was previously shown to be localized in punctate arrays throughout the cyto-
plasm, and it did not colocalize with mitochondria, as detected by staining of mitochondrial DNA (4).

In this report, we have shown that Qsr1p is a 60 S ribosomal subunit protein. Ribosomal proteins are typically small (10–30 kDa), basic (pI > 8.6), and moderately abundant (~0.2% of total cell protein). Qsr1p is a 25.4-kDa protein with a calculated isoelectric point of 10.1 (4). The codon bias of QSR1 indicates that it is highly translated (4), and we estimated that Qsr1p constitutes 0.1–0.5% of total cell protein. Furthermore, the yeast QSR1 gene contains a ribosomal protein gene upstream activating site. This site, followed by a thymidine-rich sequence 200–450 base pairs 5' of the transcription start site (29), has been found to be essential for complementing a qsr1 deletion strain (4).

Qsr1p remains associated with 60 S subunits in 0.5 M KCl, an operational criterion for distinguishing core ribosomal proteins from contaminants (21, 30). Since Qsr1p is washed off the 60 S subunit with 0.5 M KCl in the absence of Mg$^{2+}$, while 60 S subunits remain relatively intact, we suggest that Qsr1p is a peripheral protein on the 60 S subunit, and the magnesium dependence may reflect an interaction with rRNA. Limited trypsinoysis further supports the peripheral location of Qsr1p. The fact that Qsr1p is protected by the 40 S subunit from digestion argues that it is located between the large and small subunits in 80 S couples.

The abundance of Qsr1p, coupled with the fact that it is found exclusively with ribosomes, argues that Qsr1p is a stoichiometric component of ribosomes, although a portion of free 60 S subunits lack Qsr1p. This suggests the possibility that Qsr1p may be one of the last proteins assembled onto the 60 S subunit, which would also be consistent with a peripheral location of Qsr1p on the 60 S subunit.

In light of evidence that Qsr1p is a ribosomal protein, it is useful to re-evaluate what is known about QSR1 homologues in other species. The findings that QSR1 homologues appear to be more highly expressed in undifferentiated mouse (6) and plant (8) cells are consistent with the fact that ribosomal genes are highly expressed in growing cells (29). In the cloning of jif-1, the chicken homologue of QSR1, it was found that Jif-1 interacts with c-Jun and that Jif-1 could disrupt Jun-Jun dimers (7). While it is conceivable that a cytoplasmic protein could translocate to the nucleus under some conditions and activate or repress transcription, it is also possible that the Jif-1/c-Jun interaction in vitro is a nonspecific interaction of the very basic Jif-1 with an acidic domain on c-Jun.

QSR1 was recently again identified in yeast by its ability to complement a temperature-sensitive mutation. This “growth control” gene was named GRC5 (35). The grc5-1 temperature-sensitive yeast mutant (35), which encodes a ribosomal protein, is rescued by QSR1, accumulates cell wall material at the septum, exhibits an aberrant actin cytoskeleton, and is not viable on rich media. Given that these defects seem distantly related and were observed 24 h after a shift to restrictive temperature, they could be pleiotropic effects secondary to a lesion in this ribosomal protein, affecting protein synthesis.

The relationship between QSR1 and QCR6, which encodes subunit 6 of the mitochondrial cytochrome bc1 complex, remains unexplained. The data presented on the localization of Qcr6p indicate that it is found only in mitochondria in cells in which it rescues the otherwise lethal qsr1-I mutation, even in qsr1-I mutant cells that lack the cytochrome bc1 complex.

FIG. 6. Rates of protein synthesis are diminished in cell-free extracts from qsr1-1 mutant cells. Extracts from yeast strains MMY3-3A (QSR1) and MMY3-3B (qsr1-1) were treated with nuclease and assayed for $^{35}$S incorporation into trichloroacetic acid-insoluble material with or without addition of poly(A)$^+$ RNA.

FIG. 7. Qcr6p is localized exclusively within mitochondria. Affinity-purified antibodies were used to probe a Western blot of whole cell lysates prepared from wild-type (WT) and qcr6 deletion strains (a). Lysates from wild-type and qcr6-I mutant strains were centrifuged at 16,000 × g to obtain a cytosolic supernatant (S) and a pellet containing mitochondria (P), which were then analyzed by Western blotting (b). Mitochondria from wild-type, qcr6 deletion, and qsr1-I strains were treated with proteinase K before Western analysis (c).
which implies that unidentified factors play a role in this process. Ribosomes dock with the mitochondrial outer membrane in a protein synthesis-dependent manner (36, 37), and we have shown that Qsr1p remains associated with mitochondrial bound polysomes. It is thus possible that Qcr6p, which is located on the outer surface of the inner mitochondrial membrane (34), interacts indirectly across the outer membrane with polysomes bound to the surface of the outer membrane. Further experiments to test the possible involvement of Qcr6p in cotranslational import and the relationship between Qcr6p and Qsr1p are in progress.

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