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Heterologous complementation reveals that mutant alleles of *QSR1* render 60S ribosomal subunits unstable and translationally inactive

Frederick A. Dick and Bernard L. Trumpower*

Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03755, USA

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ABSTRACT

***QSR1* is a highly conserved gene which encodes a 60S ribosomal subunit protein that is required for joining of large and small ribosomal subunits. In this report we demonstrate heterologous complementation of a yeast *QSR1* deletion strain with both the human and corn homologs and show that the human and corn proteins are assembled into hybrid yeast/human and yeast/corn ribosomes. While the homologous genes complement lethality of the *QSR1* deletion, they also result in a diminished growth rate. Analyses of the translation rates of ribosomes containing the human and corn proteins reveal a partial loss of function. Velocity gradient analyses of the hybrid ribosomes after exposure to high concentrations of salt indicate that the decreased activity is due to lability of the hybrid 60S subunits.**

INTRODUCTION

QSR1 is an essential *Saccharomyces cerevisiae* gene that encodes a 60S ribosomal subunit protein (1). This gene has been identified in numerous other eukaryotes (2) and the high degree of sequence homology among the *QSR1*-encoded proteins (2) is similar to that observed with other eukaryotic ribosomal proteins (3,4), suggesting that the function of Qsr1p will undoubtedly be the same in all organisms in which it is found.

At present there is very little information about the functions of individual ribosomal proteins in translation. Numerous studies have identified proteins necessary for the assembly of ribosomes (5–7) and genes for ribosomal proteins have also been identified as suppressors of translational initiation defects (8,9). However, there are only a few examples of functional biochemical studies of ribosomal proteins (7,10–12). Perhaps the best understood ribosomal proteins are the P proteins, which compose the stalk region of the large ribosomal subunit (13). These proteins have been shown to be important in translational elongation (10) and are believed to be sites of interaction between elongation factors and large ribosomal subunits (13). The P proteins also appear to regulate the ability of ribosomes to translate certain messages, suggesting that they may play a role in translational control (10).

We are attempting to elucidate the function of Qsr1p, a large ribosomal subunit protein (1). Previously we demonstrated that Qsr1p is necessary for joining of large and small ribosomal subunits, both at translational initiation and to form inactive 80S couples (14). Qsr1p also appears to be one of the last ribosomal proteins assembled onto the 60S subunit and we recently identified a cytosolic protein, Sqt1p, that is involved in this assembly (15).

Despite the high degree of homology and the one to one existence of homologs between yeast and mammalian ribosomal proteins, there has been only one report of heterologous complementation of a yeast ribosomal protein gene with the corresponding gene from a mammal (4). In the present study we demonstrate heterologous complementation of a *QSR1* null allele with corn and human homologs of this gene and show that the heterologous proteins are assembled into hybrid ribosomes. These strains grow slowly and display a half-mer polyribosome phenotype reminiscent of *qsr1* mutants. We also examine translation elongation rates of ribosomes from the strains complemented with the heterologous genes and compare these results with those of the constitutive mutant *qsr1-1*. From this analysis we conclude that the heterologous proteins are able to function at nearly wild-type levels if the rates of translation are corrected for 60S subunit lability that results from assembly of the corn and human proteins into the hybrid ribosomes.

MATERIALS AND METHODS

Construction of plasmids and yeast strains

All DNA manipulations were carried out according to standard procedures (16). A plasmid containing the *QSR1* locus was initially constructed to complement the *QSR1* deletion. This plasmid was created by inserting a 3.4 kb *Kpn*I–*Cla*I fragment containing *QSR1* into pRS316Δ*Spe*I. This pRS316 derivative had its single *Spe*I site deleted by digestion with *Spe*I, filling in with Klenow fragment and re-ligating with T4 DNA ligase. This vector was then digested with *Kpn*I and *Cla*I. A 3.4 kb *Kpn*I–*Cla*I fragment containing *QSR1* was ligated into pRS316Δ*Spe*I to create pFAD12. This vector contains the entire *QSR1* locus, which has been shown to complement the *qsr1*-Δ*1*::*HIS3* deletion (17). This construction strategy allows the 1.2 kb *Spe*I–*Cla*I fragment containing the entire *QSR1* open reading frame and part of the promoter to be removed and replaced as a cassette.

The 1.2 kb *Spe*I–*Cla*I fragment of pFAD12 was silently altered at codons 4 and 5 of the *QSR1* open reading frame to contain a *Stu*I site by two-stage PCR-directed mutagenesis (18). Introduction of

*To whom correspondence should be addressed. Tel: +1 603 650 1621; Fax: +1 603 650 1389; Email: trumpower@dartmouth.edu

a unique *Stu*I site allows the *QSR1* open reading frame to be removed from a plasmid containing the 1.2 kb *Spe*I–*Cla*I fragment of *QSR1* by digestion with *Stu*I and *Hin*dIII. The primers used were MQM1 (5′-GTA GCG GTT ATT TCC GTG GGG TGC-3′), FDSTU1 (5′-CAT CTA GCA GGC CTT CTA GCC ATC TTG-3′) and FDCLA1 (5′-TAT CAA GCT TAT CGA TTG CTC CC-3′). For the first stage PCR primers MQM1 and FDSTU1 were used with Vent DNA polymerase to amplify a 321 bp fragment of pFAD12. The product from stage one was purified and used as a primer with FDCLA1 for the stage two reaction, again with pFAD12 as template, but amplified by Taq DNA polymerase. The final 1.2 kb product was gel purified, digested with *Spe*I and *Cla*I and ligated into the same sites of pBluescript SK. This plasmid was named pFAD15 and was sequenced to confirm that no unwanted mutations were introduced by PCR.

Plasmid pCB41 was digested with *Stu*I and *Not*I to release a 0.65 kb fragment containing the corn *QM* open reading frame. The *Not*I 5′-overhang was filled in with Klenow fragment. This corn *QM* fragment was ligated into pFAD15 at the *Stu*I and blunted *Hin*dIII sites, creating pFAD16. The 1.15 kb *Spe*I–*Cla*I fragment of pFAD16 was cut out and ligated into the *Spe*I–*Cla*I sites of pFAD12. This plasmid was named pFAD18.

To construct the human *QM* plasmid for exchange with the yeast *QSR1* gene *Stu*I and *Hin*dIII sites were introduced into the human *QM* sequence by PCR. The primers used were FDQMSTU1 (5′-AGG CCT GCC CGT TGT TAC CGG-3′) and FDQMHIND3 (5′-AAG CTT TCA TGA GTG CAG GGC C-3′). FDQMSTU1 anneals to the antisense strand at the 5′-end of the human *QM* open reading frame and silently changes codons 4 and 5 to code for a *Stu*I site. FDQMHIND3 anneals to the sense strand at the 3′-end of the human *QM* open reading frame and introduces a *Hin*dIII site immediately after the stop codon.

PCR was performed with these primers and Taq DNA polymerase with p201.3 as the template. The 0.65 kb product was ligated with the TA clone kit into the pCRII vector. This new construct was named pFAD22 and was sequenced to ensure that PCR did not introduce any mutations. The 0.65 kb *Stu*I–*Hin*dIII human *QM* open reading frame was cut out of pFAD22 and ligated into the *Stu*I and *Hin*dIII sites of pFAD15. The resulting plasmid, pFAD23, was then digested with *Spe*I and *Cla*I to obtain a 1.15 kb fragment containing the human *QM* open reading frame. This fragment of DNA was ligated into the *Spe*I and *Cla*I sites of pFAD12, creating a new plasmid called pFAD24.

The diploid yeast strain HFF10 containing the *qsr1*-Δ*1*::*HIS3* deletion at one of its *QSR1* loci was constructed from a W303 diploid (17). This strain was transformed with plasmids pFAD12, pFAD18 and pFAD24 and sporulated. Tetrads were dissected (19) and spores were allowed to germinate at room temperature. Spores were genotyped on standard selective medium (20) to isolate haploid strains containing the chromosomal deletion of *QSR1* and a plasmid-borne copy of *QSR1* or *QM*. All liquid cultures were grown in YPD medium at 30°C (20).

Velocity gradient analysis of yeast polysomes

Yeast cell lysates were analyzed by velocity gradient centrifugation essentially as described by Ramirez *et al*. (21). Cells were arrested in mid log growth with cycloheximide by adding it to cultures 5 min before harvesting at a concentration of 50 µg/ml. Lysates of yeast cells were prepared in 20 mM Tris, pH 7.5, 50 mM NaCl, 1 mM DTT, 0.2 mg/ml heparin, 50 µg/ml cycloheximide and 1 mM diisopropylfluorophosphate by glass bead lysis. The lysate was then centrifuged at 16 000 *g* for 30 min to remove debris and most organelles. Lysate equivalent to 15 absorbance units at 260 nm was loaded onto 5–47% sucrose gradients prepared in 20 mM Tris, pH 7.5, 50 mM NaCl, 1 mM DTT, 50 µg/ml cycloheximide. The gradients were then centrifuged at 220 000 *g* for 3 h 45 min in an SW40 rotor and were fractionated on an ISCO model UA6 gradient fractionator while monitoring absorbance at 254 nm.

80S couples were dissociated by incubation in buffer E (10 mM Tris, pH 7.5, 8 mM $MgCl_2$, 0.5 M KCl, 6 mM 2-mercaptoethanol and 0.2 mM EDTA) for 2 h. Subunits were resolved on 10–40% sucrose gradients in buffer E in an SW60 rotor at 280 000 *g* for 2.5 h and gradients were fractionated as above. Ratios of 40S to 60S subunits were calculated by determining peak areas in pixels from the 254 nm absorbance profile with NIH Image v.1.52.

Poly(U) translation

Cytosol was prepared for translation assays essentially as described by Deshaies *et al*. (22). Mid log phase cells were pelleted, resuspended in a half volume of lysis buffer (20 mM Tris, pH 7.5, 100 mM KCl, 10 mM $MgCl_2$ and 5 mM 2-mercaptoethanol) and lysed with seven rounds of glass bead disruption. Debris was removed by centrifuging for 10 min at 3000 *g*. The supernatant was removed and centrifuged at 85 000 *g* for 1.5 h to pellet ribosomes. Between 1 and 1.5 ml supernatant was then loaded onto a Sephadex G-25 column (1.2 × 15 cm) which had been equilibrated with lysis buffer containing 14% glycerol. The protein concentrations of fractions were determined and peak fractions were pooled and diluted to a final concentration of 5 mg/ml. Aliquots were snap frozen in liquid nitrogen and stored at –75°C.

80S ribosomes were purified from yeast by the method of Raue *et al*. (23). Mid log phase cells were lysed with glass beads in buffer A (10 mM Tris, pH 7.5, 8 mM $MgCl_2$, 50 mM KCl, 6 mM 2-mercaptoethanol and 0.2 mM EDTA). Lysates were cleared with successive centrifugations of 10 000 *g* for 15 min and 41 000 *g* for 30 min. Ribosomes were purified on 10–40% sucrose gradients in buffer A and centrifuged for 17 h at 73 000 *g* in an SW28 rotor. The gradients were fractionated into 24 × 1.5 ml fractions while monitoring the absorbance at 254 nm. The fractions containing 80S couples were pooled and diluted at least 2-fold with buffer C (10 mM Tris, pH 7.5, 30 mM $MgCl_2$ and 20 mM 2-mercaptoethanol) and pelleted at 170 000 *g* for 10 h. Ribosomes were stored in buffer D (10 mM Tris, pH 7.5, 8 mM $MgCl_2$, 50 mM KCl, 6 mM 2-mercaptoethanol, 0.1 mM EDTA and 50% glycerol) at –20°C (24).

The translation of poly(U) message was carried out essentially as described (10). The reaction mixture consisted of 50 mM Tris, pH 7.6, 15 mM $MgCl_2$, 90 mM KCl and 0.02 mM EDTA containing 0.2 mg/ml yeast phenylalanine-specific tRNA (Sigma R4018), 0.5 mM GTP, 1 mM ATP, 2 mM phosphocreatine, 5.6 mM 2-mercaptoethanol, 0.3 mg/ml poly(U), 20 µM [^{3}H]phenylalanine (100 nCi/µl), 0.8 U/µl placental ribonuclease inhibitor, 0.2 mg/ml creatine phosphokinase, 1 mg/ml ribosome-free cytosol and either 40 or 20 nmol/µl 80S ribosomes. The reactions were started by addition of 80S ribosomes and incubated at 30°C. Aliquots were withdrawn at various times and spotted on 10% TCA-soaked filter paper to end the reaction. Filters were washed twice with 10% TCA at room temperature,

once with 10% TCA at 95°C and once with ethanol. The filters were dried and then immersed in liquid scintillation fluid and counted. Translation was quantitated as c.p.m. TCA-insoluble radioactivity/min pmol 80S ribosomes and as c.p.m./min pmol 0.5 M KCl-resistant 60S subunits.

RESULTS

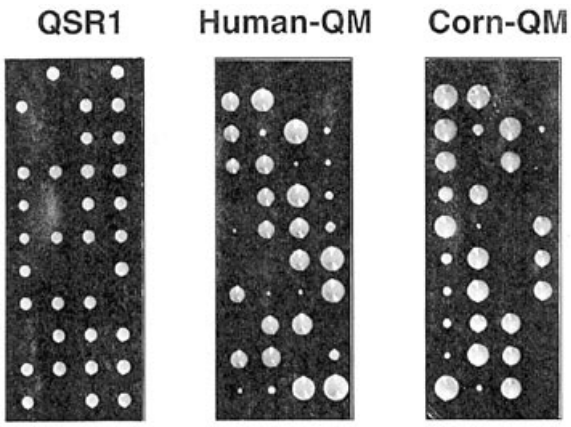
The lethal *qsr1-Δ1::HIS3* allele can be rescued by either human or corn *QM*

The ribosomal protein gene *QSR1* is a highly conserved gene that has been found in all kingdoms except eubacteria. In an effort to investigate the conservation of function between these homologous genes yeast *QSR1* was replaced with the human and corn *QM* genes. Despite the conservation of most ribosomal protein genes there is only one other example of heterologous complementation of a yeast ribosomal protein gene (4). There are, however, a number of examples of hybrid ribosomes assembled from different mammalian species (25,26).

The diploid yeast strain HFF10, containing the *qsr1-Δ1::HIS3* deletion at one of its *QSR1* loci, was transformed with plasmids pFAD12, pFAD18 (corn *QM*) and pFAD24 (human *QM*) and sporulated. Tetrads were dissected and spores were allowed to germinate at room temperature. Photographs of these spores are shown in Figure 1. Inheritance of centromeric plasmids during meiosis has been shown to be less faithful than true chromosomal segregation (27). As a result, complementation depends foremost on inheritance of the plasmid as seen in the left panel of Figure 1. *QSR1* complementation of the *qsr1-Δ1::HIS3* null allele occurs randomly and results in two, three or four viable spores per tetrad. Plasmids containing human and corn *QM* complement the deletion strain with the same frequency as yeast *QSR1*, as judged by the frequency of tetrads with two, three or four viable spores. Genotyping of the viable spores indicated that in all cases the small colonies carried the chromosomal deletion of *QSR1* complemented by *QM*. Interestingly, the colony size indicates a difference between the efficacy of complementation of *qsr1-Δ1::HIS3* by human and corn *QM* versus complementation by *QSR1*. From this we conclude that while the human and corn *QM* genes function sufficiently to rescue the yeast from lethality, these genes are effectively mutant alleles of *QSR1*, as they cause a slow growth phenotype.

Yeast strains rescued by heterologous complementation display the same phenotype as *qsr1-1* mutants

In order to better understand the function of *QSR1*, lysates from strains heterologously complemented with *QM* were analyzed by velocity gradient centrifugation to determine if there were any abnormalities in their cycloheximide-arrested polysome profiles and compared with lysates from a yeast *qsr1-1* mutant and the wild-type parent strain W303-1B. As shown in Figure 2, the profiles for the wild-type (Fig. 2A) and *qsr1-1* (Fig. 2B) strains match the previously reported profiles for these cells (14). The strains complemented with the corn and human genes (Fig. 2C and D) both exhibit the same half-mer phenotype as the *qsr1-1* mutant. In previous work, investigation of the apparently inactive free 60S subunits in *qsr1-1* revealed that they lack qsr1-1p, while translating ribosomes contain stoichiometric amounts of the protein (14). The data presented here is consistent with the previously reported defect, which appears to be at the level of assembly of Qsr1p onto the 60S subunit (14).

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Human   MGRRPARCYR YCKNKPYPKS RFCRGVPDAK IRIFDLGRKK AKVDEFPLCG
Yeast   MARRPARCYR YQKNKPYPKS RYNRAVPDSK IRIYDLGKKK ATVDEFPLCV
Corn    MGRRPARCYR QIKNKPCPKS RYCRGVPDPK IRIYDVGMKR KGVDEFPYCV

        HMVSDEYEQL SSEALEAARI CANKYMVKSC GKDGFHIRVR LHPFHVIRIN
        HLVSFELEQL SSEALEAARI CANKYMTTVS GRDAFHLRVR VHPFHVLRIN
        HLVSWERENV SSEALEAARI ACNKYMTKSA GKDAFHLRVR VHPFHVLRIN

        KMLSCAGADR LQTGMRGAFG KPQGTVARVH IGQVIMSIRT KLQNKEHVIE
        KMLSCAGADR LQQGMRGAWG KPHGLAARVD IGQIIFSVRT KDSNKDVVVE
        KMLSCAGADR LQTGMRGAFG KPQGTCARVD IGQVLLSVRC KDNNAAHASE

        ALRRAKFKFP GRQKIHISKK WGFTKFNADE FEDMVAEKRL IP----DGCG
        GLRRARYKFP GQQKIILSKK WGFTNLDRPE YLK-KREAGE VKDDGAFVKF
        ALRRAKFKFP ARQKIIESRK WGFTKFSRAD YLKYKSEGRI VPD-GVNAKL

        VKYIPSRGPL DKW-R---A- LHS
        LSKKGSLENN IREFPEYFA- AQA
        LGNHGRLEK- -RAPGKAFLD AVA
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Figure 1. Complementation of the yeast *qsr1-Δ1::HIS3* allele by human and corn *QM* genes. Haploid spores were derived from the diploid parent HFF10, carrying plasmids with the yeast *QSR1* (left), human *QM* (center) or corn *QM* (right) open reading frames flanked by *QSR1* 5′- and 3′-untranslated regions. Tetrads show 2, 3 or 4 viable spores, indicating that the centromeric plasmid carried by the diploid parent is inherited at the expected frequency. The sequences of the proteins encoded by the human and corn *QM* genes are compared with that encoded by the yeast *QSR1* gene at the bottom of the figure. Amino acids in the human protein and likewise amino acids in the corn protein which are identical to those in the yeast protein are underlined in the human protein or corn protein respectively. Amino acids that are identical in all three proteins are underlined in the yeast protein.

Heterologously complemented strains have the same defect as the *qsr1-1* strain and other previously investigated *qsr1* mutants (14). Since the defect causing the half-mer is at the level of assembly of Qsr1p onto the 60S subunit, 60S subunits would need to contain the *QM*-encoded protein in order for these subunits to participate in subunit joining and translation. To confirm this 80S couples were purified and analyzed by Western blotting to determine whether the heterologously expressed proteins are incorporated into ribosomes. Antiserum against yeast Qsr1p is weakly cross-reactive with the human and corn proteins. For this reason large amounts of 80S ribosomes, equivalent to ~0.3 µg Qsr1p or its human or corn homolog, were loaded per gel lane. As shown in Figure 3, the *QM*-encoded proteins migrate with apparent molecular weights slightly greater than Qsr1p and all of them migrate with apparent molecular weights that are larger than predicted by their amino acid composition. The Western blots

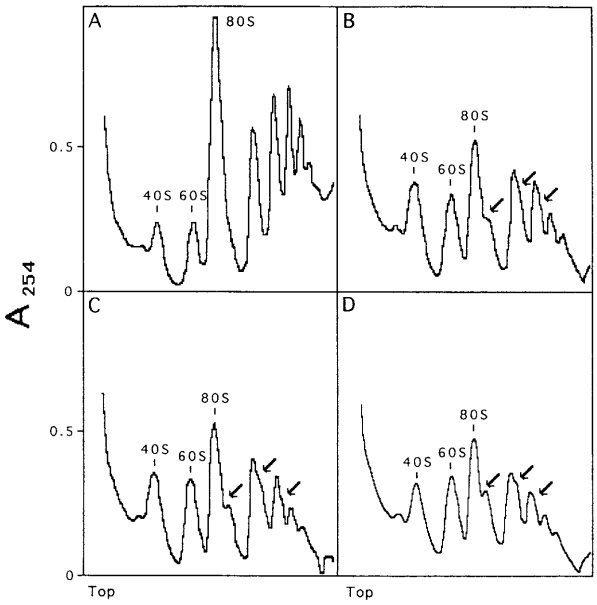

Figure 2. Yeast *qsr1-Δ1*::*HIS3* deletion strains complemented with human and corn *QM* genes have a half-mer phenotype similar to *qsr1-1* mutants. Cells were arrested with cycloheximide and lysates were prepared from the wild-type yeast strain W303-1B (**A**), the *qsr1-1* mutant strain (**B**), the yeast *qsr1-Δ1*::*HIS3* strain complemented with corn *QM* (**C**) and the yeast *qsr1-Δ1*::*HIS3* strain complemented with human *QM* (**D**). Polysomes were resolved on 5–47% sucrose gradients. Half-mers are indicated by arrows.

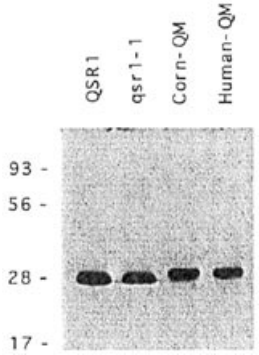

Figure 3. Western blots of purified 80S couples from the yeast strains shown in Figure 2 were probed with antibodies to Qsr1p.

confirm that the heterologously expressed proteins are incorporated into large ribosomal subunits and that complementation of the *qsr1-Δ1* strain occurs by participation of the heterologous proteins in an essential function on the 60S subunit.

Mutant forms of Qsr1p render some 60S subunits salt-labile and inactive

Although the *QM*-encoded proteins complement the lethality of the *qsr1-Δ1* deletion, these strains also display mutant phenotypes, as shown above. Since these heterologously complemented strains and *qsr1-1* are constitutive mutants we decided to test whether mutations in *QSR1* affect the function of ribosomes in translation. Previous defects in Qsr1p have been characterized in relation to its function before the catalysis of peptide bonds.

Poly(U) translation assays were designed to test the elongation rates of ribosomes carrying defective forms of Qsr1p. The source of ribosomes used for these reactions were purified 80S couples isolated from the relevant strains. Using 80S couples ensures that the analysis was carried out on subunits that contain Qsr1p or a mutant form of it, since only these subunits are capable of joining (14) and large ribosomal subunits that do not contain Qsr1p will be removed during this isolation. Purification of 80S couples also ensures that large and small subunits are present in stoichiometric quantities. Elongation factors were provided in a cytosolic protein preparation depleted of ribosomes that was prepared from wild-type yeast. Control reactions in which the cytosol or the 80S couples were omitted did not show activity above background levels (data not shown). The amounts of ribosomes in these assays were titrated until they limited the translation rate.

The results of the poly(U) translation assays in Figure 4 indicate that ribosomes from all strains tested show a defect in their elongation rates compared with the wild-type W303-1B control. The amounts of radioactivity incorported into TCA-insoluble material with either 7.5 or 3.75 pmol 80S ribosomes show that the reactions are limited by ribosome concentration.

These results would seem to indicate that mutant forms of Qsr1p slow translation. This can be interpreted as Qsr1p being important in mediating the functions of elongation factors or being involved in catalysis of peptide bonds. Alternatively, a population of 60S subunits in these purified couples may not be fully active, which would obscure the actual translation rate of the active population.

In order to determine whether ribosomes carrying a mutant form of Qsr1p have decreased elongation rates, two experiments were undertaken. In the first experiment 80S couples were analyzed on 10–40% sucrose velocity gradients under conditions that promote subunit joining, in order to evaluate whether there had been any denaturation of subunits during storage. The profiles of these gradients indicated that all of the 60S subunits from the mutant and heterologous strains remained intact, as judged by their ability to join with 40S subunits. Any imbalance in subunit stoichiometry resulting from denaturation of 60S subunits would be manifest in the presence of a free 40S subunit peak and this was not observed (data not shown).

In the second experiment 80S couples were dissociated in 0.5 M KCl and analyzed on sucrose gradients containing the same salt concentration. Absorbance profiles of these gradients, shown in Figure 5, indicate a diminished abundance of 60S subunits relative to 40S subunits in the mutant and heterologous strains compared with the wild-type control (compare 60S subunits in Fig. 5B–D with A). In this experiment it appears that a proportion of 60S subunits from the mutant and heterologous strains are denatured by 0.5 M KCl. Surprisingly, the decreased absorbance of the 60S peaks is not readily accounted for by an increase elsewhere in the gradient. Denaturation into smaller components, like proteins and nucleotides, prior to gradient centrifugation would result in an increase in absorbance at 254 nm at the top of the gradients. This seems unlikely to be the explanation, since there is only a marginal increase in absorbance in this region of the gradient profiles of the mutant and heterologous strains (compare Fig. 5B–D with A). It seems most likely that these 60S subunits are denatured during centrifugation, which would

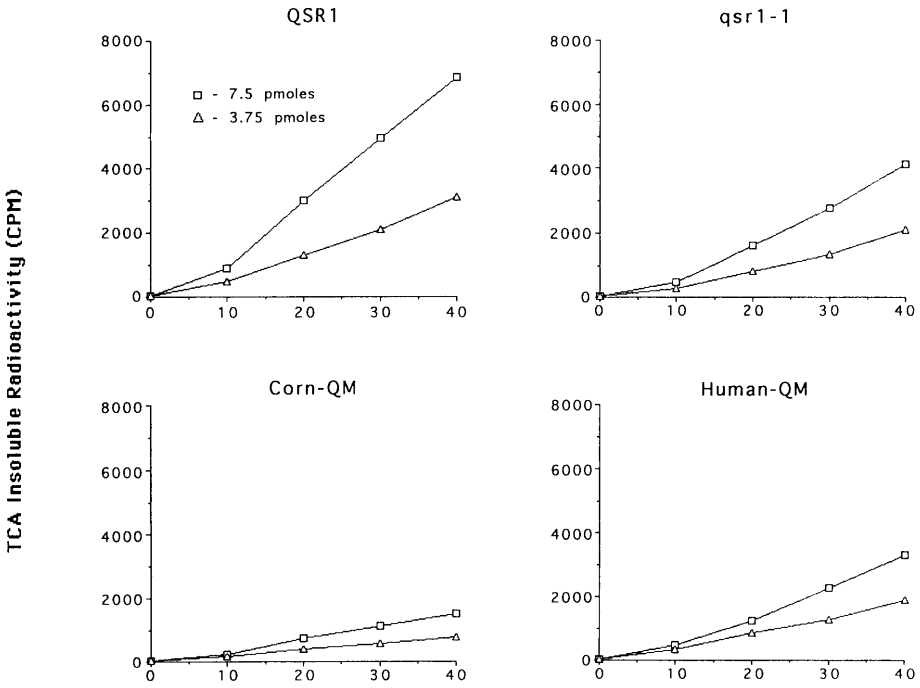

Figure 4. Mutations in *QSR1* impair poly(U) translation rates. Purified 80S couples from the yeast strains described in Figure 2 were analyzed in poly(U) translation assays. Reactions were carried out with either 3.75 or 7.5 pmol 80S couples to show that ribosomes were the limiting component in the assay.

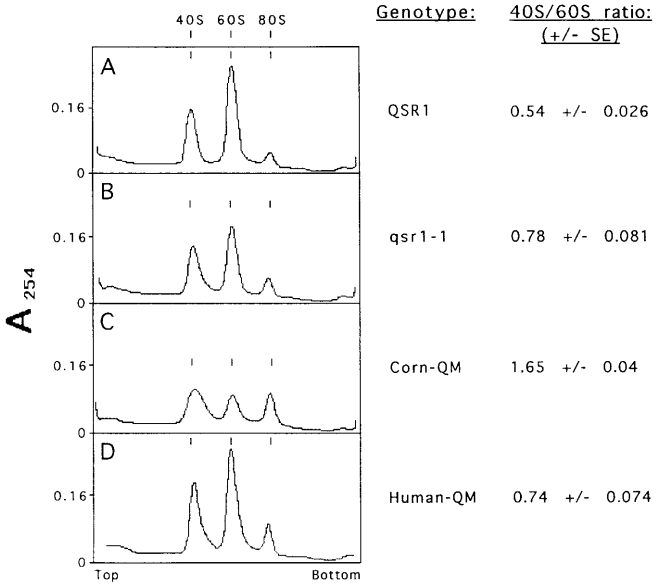

Figure 5. Mutations in *QSR1* result in salt-labile 60S subunits. Purified 80S couples from the yeast strains described in Figure 2 were dissociated in buffer containing 0.5 M KCl and centrifuged through 10–40% sucrose gradients also containing 0.5 M KCl. Subunit ratios were calculated as described in Materials and Methods. Error bars indicate the standard error.

distribute RNA and protein throughout the gradient and result in small changes in the baseline 254 nm absorbance.

Examination of the 80S peaks that are resistant to 0.5 M KCl dissociation reveals that they are more abundant in the strains containing heterologous ribosomes (compare Fig. 5C and D with A and B). 80S couples that are resistant to dissociation with 0.5 M KCl are engaged in translation (28), but are unable to terminate without puromycin treatment (29). These engaged couples are unlikely to participate in poly(U) translation, consequently, they should be excluded when quantitating translation rates.

Treatment of ribosomal subunits with 0.5 M KCl is routinely used to strip away contaminating proteins during purification (23,29,30). Since some 60S subunits from these mutant and heterologous strains are degraded by this treatment, it seems likely that these labile subunits may not be active in elongation assays. To assess the impact of potentially non-functional 60S subunits and 80S ribosomes in the poly(U) translation assays, translation rates were normalized to 80S couples and to free 60S subunits that were stable in velocity gradients containing 0.5 M KCl. Large ribosomal subunits that are resistant to 0.5 M KCl were quantitated by determining the area under the 60S peak, using the 40S peak areas as an internal control to normalize 60S quantities between samples. As shown in Figure 6, normalization to 80S shows the diminished translation rates seen in Figure 4, while the rates for the mutant and heterologous strains normalized to stable 60S subunits vary only slightly from the wild-type rate.

Taken together, this evaluation of 60S subunits containing mutant qsr1-1p or QM proteins suggests that these proteins do not greatly affect the elongation rates of functional 60S subunits. These experiments demonstrate that the heterologous genes encode proteins that are capable of functioning almost as well as the wild-type in translation. However, it appears that these proteins are also defective in assembly, like the temperature-sensitive alleles of *QSR1*, and that these proteins cause subunits to become unstable and lose activity.

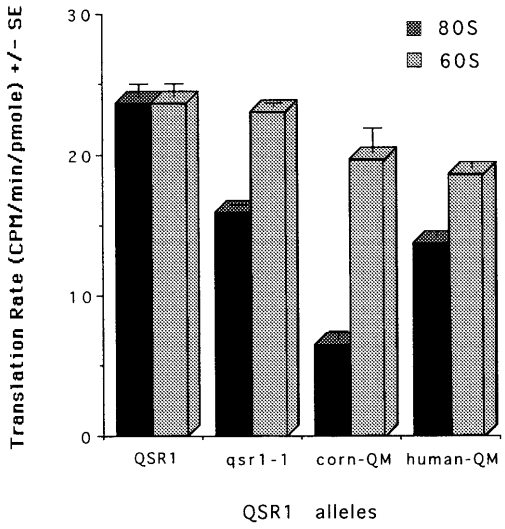

Figure 6. Translation rates of yeast strains carrying the *qsr1-1* allele and the corn and human genes are similar to those of the wild-type strain if normalized to 0.5 M KCl-resistant 60S subunits. Translation rates of poly(U) templates were normalized between *QSR1* alleles by expressing the activity as c.p.m./min pmol 80S ribosomes or 0.5 M KCl-resistant 60S subunits. Error bars indicate the standard error.

DISCUSSION

We have shown that the yeast *QSR1* gene is functionally equivalent to homologs from two separate kingdoms; animals and plants. This is consistent with the high degree of sequence conservation of this protein between these species. The rate of divergence of the nucleotide sequence of the *QSR1*/*QM* genes has been determined to be 1% over 22 million years, a rate that is slower than histone genes H2A/H2B and H1 (2). Interestingly, other ribosomal protein genes have been reported to have similar levels of conservation (3), however, there is only one example of heterologous complementation of a yeast ribosomal protein gene in the literature (4).

One possible explanation for the infrequent demonstration of functional equivalence of ribosomal proteins (J.Warner, personal communication) could be the structure of higher eukaryotic ribosome protein transcripts. In higher eukaryotes expression is controlled at the level of translation by an AU-rich 5′-untranslated region (31). In yeast ribosomal protein gene expression is controlled primarily at the level of transcription (30), although other mechanisms such as splicing also play an important role (6). It is conceivable that expression of ribosomal proteins from higher eukaryotes in yeast is impaired by the 5′-untranslated region. Attempts to complement the *qsr1*-Δ*1* deletion with human or corn genes were unsuccessful when placed under control of the inducible GAL or constitutive ADH promoters (results not shown). Both of these constructs expressed the 5′- and 3′-untranslated regions of the human or corn *QM* genes. In the constructs used here that were successful the human and corn open reading frames were placed completely within the context of the 5′- and 3′-untranslated regions of *QSR1*. These heterologous constructs were also under the control of the *QSR1* promoter, which contains the consensus sequences for the ribosomal protein gene box and a d(A:T)-rich region (1,17), which are important in regulating ribosomal protein gene expression in yeast (30). Taken together, these observations suggest that the lack of heterologous complementation of yeast ribosomal protein genes may reflect improper expression, rather than insufficient conservation of structure.

This study also reveals an important role for Qsr1p in maintaining the structure of 60S ribosomal subunits. A previous investigation of Qsr1p function took advantage of temperature-sensitive mutant alleles and conditional expression to determine a role for Qsr1p in subunit joining to form inactive 80S couples and subunit joining in the late stages of translational initiation (14). Those studies revealed that inactivation of *qsr1-24* at the non-permissive temperature resulted in half-mer polyribosomes, accompanied by accumulation of 60S subunits that were unable to participate in this step of initiation. Closer examination of the accumulated 60S subunits revealed that they were missing the mutant qsr1-24p. Further investigations of a regulatable *GAL-QSR1* strain in which expression of *QSR1* was repressed has revealed that these cells display the same phenotype of half-mers and inactive 60S subunits that lack Qsr1p (14). These experiments would seem to indicate that the temperature-sensitive lesion is at the level of Qsr1p assembly onto 60S subunits, since blocking expression of *QSR1* has the same effect as shifting the cells to the non-permissive temperature. The heterologously complemented strains used in these studies also display the same phenotype of half-mer polyribosomes, suggesting that they are likely to be defective in this process as well.

Interestingly, Qsr1p has recently been determined to be synonymous with L7 in the nomenclature of Zinker and Warner (32,33) or rpL10 in the new unified nomenclature (34). This protein has been shown to be one of the last ribosomal proteins assembled onto 60S subunits (35). This process of assembly has been demonstrated to involve replacement of rpL10 with a newer copy of the protein on previously functional ribosomes (32,35,36). Since this assembly occurs in the cytoplasm after processing of rRNA and assembly of most ribosomal proteins, it is not surprising that *qsr1* mutants show an increase in free 60S subunits that are deficient for this protein.

In previous studies the function of Qsr1p has only been examined before ribosomes are engaged in translation. The results presented above indicate that Qsr1p is unlikely to participate in any of the catalytic steps or in binding of tRNA or elongation factors. This conclusion is drawn from the fact that the constitutive mutant forms of Qsr1p used here have very similar translation rates as wild-type Qsr1p. These results also show that the heterologously complementing genes encode proteins that can function at nearly wild-type levels in translation, provided that they are properly assembled onto the 60S subunit. Western blot analysis demonstrates that the human and corn *QM*-encoded proteins are assembled into hybrid ribosomes. *In vitro* translation experiments using purified ribosomes establishes that heterologous complementation of the role of Qsr1p in translation does not occur from *QM*-encoded proteins functioning somewhere outside the 60S subunit. However, these heterologously expressed proteins render the subunits unstable and prone to inactivation through some, as yet undetermined, mechanism. It is also not known whether this loss of function occurs in ribosomes containing temperature-sensitive forms of yeast Qsr1p or whether this loss of function occurs *in vivo*.

The human and corn homologs of *QSR1* encode proteins that can be viewed as highly mutated forms of the yeast protein. The

fact that the human and corn proteins have minimal effects on translation in active stable 60S subunits suggests that Qsr1p may not participate in any ribosome functions which occur after translation initiation. This would suggest that the primary function of Qsr1p is to regulate activity of the 60S subunit in translational initiation by blocking subunit joining in its absence (14) or by maintaining ribosomal subunits in an active state. Qsr1p is one of the last ribosomal proteins to be assembled onto 60S subunits, since a 60S sized particle is assembled in its absence (14). Additionally, a putative cytosolic assembly factor for Qsr1p has already been described (15). Late stage cytoplasmic assembly of proteins onto the 60S subunit is not unprecedented, as it has been observed in at least two other laboratories (32,33). It remains to be established whether Qsr1p has a direct role in subunit joining and regulates translational initiation by rendering 60S subunits inactive in its absence or whether Qsr1p stabilizes other ribosomal proteins on the 60S subunit that mediate subunit joining and in this manner indirectly exerts control on this process.

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