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Hypothesis

Exchangeability of Qsr1p, a large ribosomal subunit protein required for subunit joining, suggests a novel translational regulatory mechanism

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Abstract **Qsr1p is a 60S ribosomal subunit protein that is necessary for joining of large and small ribosomal subunits and is also one of the last proteins assembled onto the 60S ribosomal subunit in the cytoplasm. The finding that Qsr1p is identical to L7, a protein previously shown to cycle on and off large ribosomal subunits in the cytoplasm, suggests that the addition of Qsr1p onto the 60S ribosomal subunit could be utilized as a translational regulatory mechanism by limiting the supply of functional 60S subunits.**

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Key words: Ribosomal biogenesis; Translation; Ribosome; Two-dimensional polyacrylamide gel electrophoresis; Mitochondrion; *Saccharomyces cerevisiae*

1. Introduction

The *QSR1* gene in *Saccharomyces cerevisiae* encodes a large ribosomal subunit protein [1]. *QSR1* was identified through a synthetic lethal relationship with *QCR6*, the nuclear encoded gene for subunit 6 of the mitochondrial cytochrome bc_1 complex [2]. The exact manner in which *QCR6* suppresses the loss of function of the *qsr1-1* allele is not known. However, analysis of the *qsr1-1* and *qsr1(ts)* mutants indicates that mutant forms of Qsr1p are defective in assembling onto the 60S ribosomal subunit [3]. This implies that Qcr6p may aid in assembly of Qsr1p onto the 60S subunit.

The assembly of ribosomal subunits is a complex process involving rRNA modifications in the nucleolus [4]. This is also where most ribosomal subunit proteins are assembled onto the rRNA [5,6]. However, it has been shown that ribosomal subunits that reach the cytoplasm are relatively slow to become incorporated into polysomes [7], suggesting that additional modifications take place in the cytoplasm. Examination of radiolabelled proteins that are incorporated into previously existing ribosomes has indicated that there are at least three proteins that are exchanged off and on mature 60S ribosomal subunits in the cytoplasm [8,9]. Assembly studies in which the order of addition of ribosomal proteins to the subunits was investigated have also indicated that some proteins are added to 60S ribosomal subunits in the cytoplasm [5]. In these studies of assembly the large ribosomal subunit protein L7 was identified as a protein that is added in the cytoplasm.

In previous studies we have shown that 60S sized ribosomes are able to assemble without Qsr1p [3]. We also showed that 60S subunits depleted of Qsr1p are unable to couple with 40S subunits in subunit joining assays and exhibit a defect in translational initiation [3]. A cytoplasmic protein essential for viability, Sqt1p, has also been shown to interact biochemically and genetically with Qsr1p [10], and the loss of function resulting from depletion of Sqt1p results in the accumulation of 60S sized subunits which lack Qsr1p [10]. Here we examine the implications of Qsr1p function in light of the finding that it is L7, a known exchangeable protein that is added to the large ribosomal subunit in the cytoplasm. We also discuss the means by which Qsr1p exchange could be used as a form of translational control.

2. Qsr1p and L7 are synonymous

Qsr1p is a stoichiometric component of the 60S subunit [1] and its size and isoelectric point are typical of ribosomal proteins [2,6]. For these reasons it seemed likely that Qsr1p has been revealed, although otherwise not characterized, in previous two-dimensional gel analyses [9,11].

Two-dimensional gel electrophoresis was carried out in order to correlate the identity of Qsr1p with previously described 60S ribosomal proteins, using a method [12] that has been modified for studying yeast ribosomal proteins [13,14]. A Coomassie stained gel of 60S ribosomal subunit proteins from wild-type yeast is shown in Fig. 1A. A duplicate gel was transferred to nitrocellulose and probed with antibodies to Qsr1p and another ribosomal protein, known as L1 in the nomenclature described by Zinker and Warner [9]. L1 and Qsr1p spots were distinguished from one another by initially probing the same blot with both antibodies independently (results not shown), after which probing with both antibodies resulted in the Western blot shown in Fig. 1B.

From the Western blot of the two-dimensional gel the spot corresponding to L7 appeared to be the best candidate for Qsr1p. N-terminal peptide sequence analysis was performed on PVDF membranes that were excised from blots containing L7 [15], and the resulting sequence is compared to the predicted N-terminal sequence of Qsr1p in Fig. 1C. Exclusive of the N-terminal methionine, 11 of the 12 amino acids are identical, and a search of the *Saccharomyces* Genome Database gave an unambiguous match of this peptide sequence with Qsr1p.

3. Exchangeable ribosomal proteins

Qsr1p would be designated L7 in the nomenclature of

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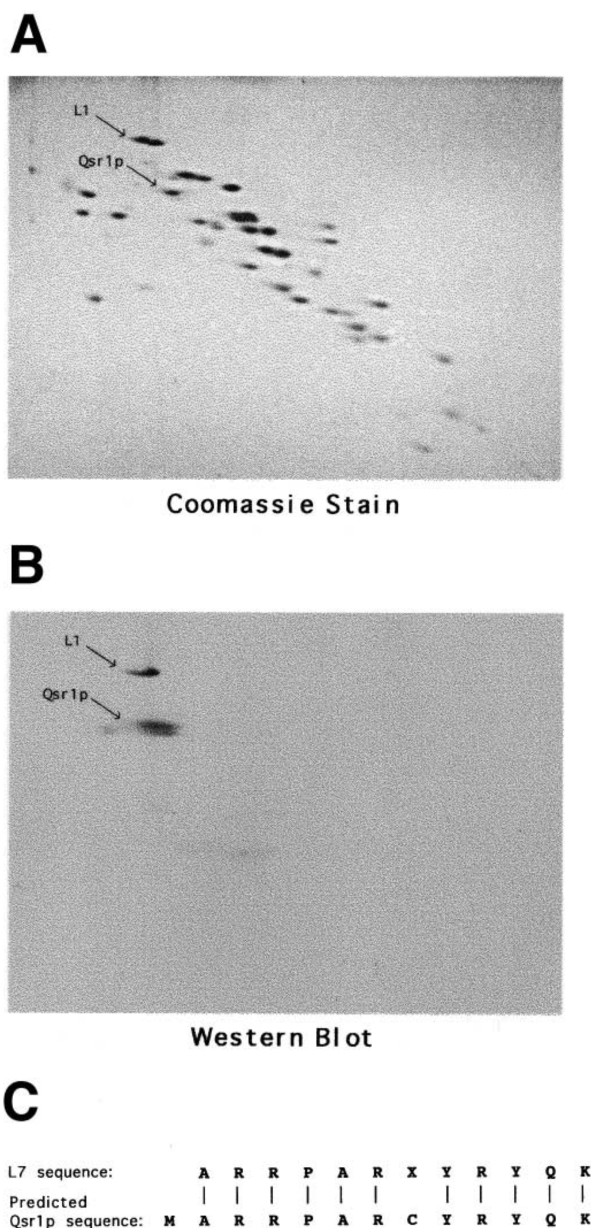

Fig. 1. Qsr1p corresponds to ribosomal protein L7. Two-dimensional Kaltschimdt-Wittman gels [12] using the modifications described for yeast ribosomal proteins [13,14] were run to analyze proteins from wild-type 60S subunits. The Coomassie stained gel (A) shows basic 60S proteins. The corresponding Western blot (B) was probed with antibodies to ribosomal protein L1 [2] and Qsr1p [3] and shows that Qsr1p migrates similar to the 60S subunit protein referred to as L7 [9]. The amino-terminal sequence of the protein spot corresponding to L7, excised from a PVDF membrane and subjected to N-terminal microsequence analysis [15], is compared with the predicted Qsr1p sequence (C).

Zinker and Warner [9], YL7 in the ribosomal protein nomenclature of Otaka and Osawa [16], and L9 in that of Kruiswijk et al. [11]. Zinker and Warner [9] previously reported that Qsr1p (sic L7) is one of three exchangeable ribosomal proteins. Exchangeability implies that a newly synthesized protein can replace an older copy of itself on a mature ribosome. Kruiswijk and coworkers identified Qsr1p (L9 in their nomenclature) as one of the last proteins to be assembled onto newly synthesized 60S ribosomal subunits in the cytoplasm [5]. These authors also reported that the radiolabelling and incorporation rate of Qsr1p (L9) onto 60S subunits greatly exceeded the rate of incorporation of other proteins and could only reconcile this finding by assuming that Qsr1p is being exchanged for another unlabelled Qsr1p on a mature 60S subunit [5].

Only one of the three exchangeable proteins on the 60S ribosomal subunit reported by Zinker and Warner [9] has been identified. That protein is a member of the family of proteins called the P-proteins that form the stalk structure on the large ribosomal subunit [17]. Exchange of the P-proteins on and off the 60S subunit appears to be regulated by phosphorylation [17]. From deleting all four cognate genes it has been determined that the P-proteins function in translational elongation and that ribosomes which lack these proteins preferentially translate different messages than wild-type ribosomes [18]. The identity of the third exchangeable protein described by Zinker and Warner is unknown. It has been proposed to be the 5S rRNA binding protein encoded by *RPL1* [19] or the P_0 protein that is also involved in forming the stalk structure [17].

4. Assembly of Qsr1p onto large ribosomal subunits could be a point of translational control

Because Qsr1p is required for subunit joining and initiation [3] and is here identified as a cytoplasmically exchangeable protein, we speculate that this could be exploited as a means of translational control. Down regulation of *QSR1* from a regulatable *GAL* promoter has shown that a decrease in amount of Qsr1p blocks translational initiation [3]. Exchangeability thus presents a means by which a cell could block the incorporation of Qsr1p onto 60S ribosomal subunits and regulate bulk translational initiation by limiting the supply of functional 60S subunits as outlined in Fig. 2.

The *QSR1* promoter appears to have the same 5′ elements that are present in most yeast ribosomal protein genes [1,2], and they have been shown to be necessary for *QSR1* expression [2]. This would seem to indicate that yeasts are unable to differentially regulate *QSR1* expression at the transcriptional level. However, the *QSR1* homologs in humans, maize, mice, and tobacco were all cloned based on their differential expression between growing and non-growing or differentiated cells [20–23]. In these studies no other transcripts encoding ribosomal proteins have been reported to be differentially expressed, suggesting that some level of mRNA regulation is occurring. This indicates that in yeast the supply of Qsr1p for assembly onto 60S subunits would need to be regulated through some form of mRNA stability, translational regulation mechanism, or a modification of the assembly process. Additionally, Qsr1p does not appear to be phosphorylated [9], indicating that Qsr1p exchange is controlled differently than the P-proteins.

Recently we described a putative assembly factor, Sqt1p, which is cytoplasmically localized and which appears to be required for assembly of Qsr1p onto 60S subunits [10]. Sqt1p loosely associates with ribosomes, interacts with Qsr1p genetically and biochemically, and the phenotype of cells in which Sqt1p has been depleted is like that resulting from Qsr1p depletion [10]. Thus, Sqt1p, either alone or in combination with other factors involved in the assembly proc-

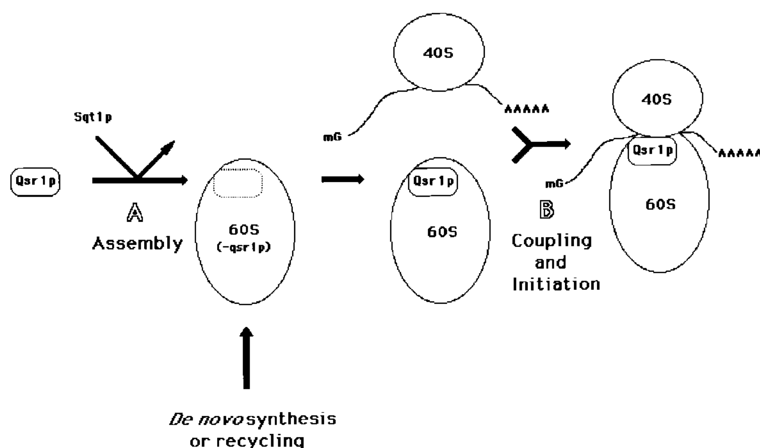

Fig. 2. Proposed role for Qsr1p and Sqt1p in ribosomal biogenesis and translational regulation. 60S subunits lacking Qsr1p (−qsr1p) and incapable of subunit joining [10] are derived either from recently synthesized subunits assembled in the nucleolus or from recycling of mature 60S subunits that have lost Qsr1p. In step (A) Qsr1p is added to inactive 60S subunits in a Sqt1p dependent manner [3]. In step (B) 60S subunits containing Qsr1p are capable of joining with 40S subunits and participating in translational initiation.

ess, may control the assembly of Qsr1p onto the 60S subunit in yeast (Fig. 2).

In conclusion, it should also be mentioned that a second mutant, *qsr2-1*, that is synthetically lethal with *QCR6* has been described [2]. This mutant also displays a half-mer polyribosome phenotype like that seen in *qsr1* mutants [3] and cells in which Sqt1p is depleted by down regulation ([10], D. Eisinger, unpublished observation). This suggests that the *QSR2* gene product is yet another uncharacterized ribosomal protein involved in subunit joining, much like Qsr1p, or that it is another factor required for the assembly of Qsr1p onto 60S subunits. The fact that *QCR6* suppresses recessive mutations in two different genes that lead to the same mutant phenotype seems to indicate that the genetic relationship between *QCR6* and the *QSR* genes is specific. Whether this relationship is revealing a novel form of communication from mitochondria to the translational machinery will need to await further experimentation.

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