Functional Redundancy in the Nonspecific RNA Binding Domain of a Class I tRNA Synthetase*

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The sequence of a 228-amino acid nonspecific RNA binding domain appended to the N terminus of a eukaryote tRNA synthetase is shown here to have two lysine-rich clusters (LRCs) that are functionally significant in vivo and in vitro. These two LRCs have unrelated sequences and are separated by a spacer of over 100 amino acids. By using a sensitive test for function in vivo, each LRC is shown to be sufficient in the absence of the other. This sufficiency requires fusion of the spacer to either of the LRCs. Experiments in vitro confirmed that the LRCs are each important for RNA binding. Thus, this nonspecific RNA binding domain has two dissimilar lysine-rich sequence elements that are functionally redundant. Further experiments suggest that this redundancy is not used to dock two molecules of RNA but rather to enhance the overall affinity for a single RNA molecule.

Although high specificity is required for biological transactions involving RNA or DNA, increasing evidence shows that nonspecific protein-RNA contacts have an essential role in enhancing the stability of specific protein-nucleic acid complexes (1–5). These nonspecific interactions provide a framework upon which specific interactions are superimposed. The specific component adds sufficient incremental interactions to achieve fine discrimination between similar targets and generate a biological response in the appropriate concentration range.

In the case of transactions involving transfer RNAs, the role of nonspecific RNA binding domains in the yeast Saccharomyces cerevisiae has been demonstrated with the protein Arc1p (6) and with a particular domain of glutaminyl-tRNA synthetase (7, 8). Arc1p has a C-terminal domain that exhibits nonspecific tRNA binding activity. A second domain enables Arc1p to dock onto glutamyl- and methionyl-tRNA synthetases and thereby bring a nonspecific tRNA binding domain to these proteins. In case of yeast methionyl-tRNA synthetase, the docking of Arc1p enhances the aminoacylation activity (6). Other experiments showed that an artificial fusion of the RNA-binding segment of Arc1p with an Escherichia coli tRNA synthetase achieved a similar effect (8).

Yeast glutaminyl-tRNA synthetase has an N-terminal appended domain (AdScQRS) of 228 amino acids that has RNA-binding activity directed toward diverse sequences and structures including single-stranded oligodeoxyacetic acid, unfractrated yeast tRNA, and an RNA pseudoknot (8). Significantly, this activity imparts function in yeast on the heterologous E. coli enzyme that is normally inactive on yeast tRNA (7). In particular, fusion of AdScQRS to E. coli glutaminyl-tRNA synthetase (to give AdScQRS-EcQRS) yields a hybrid protein with appreciable aminoacylation activity in vitro using yeast tRNA as a substrate. This gain in specific aminoacylation activity is correlated with a gain in affinity for yeast tRNA (7, 8). In addition, although EcQRS cannot sustain growth of a yeast strain with a knockout allele of the gene for cytoplasmic glutaminyl-tRNA synthetase, expression of the AdScQRS-EcQRS fusion protein rescues growth (7, 8). These experiments demonstrate a context in which the AdScQRS nonspecific RNA binding domain provides an essential function. Whether the appended domains seen in certain other yeast tRNA synthetases, glutamyl-, isoleucyl-, lysyl-, methionyl-, and valyl-tRNA synthetases (9), also have nonspecific RNA binding activity is not yet known.

Little is known about the protein determinants for nonspecific RNA binding in these systems or about how these determinants are organized in their respective sequences. Interestingly, AdScQRS and the appended domains of the aforementioned other yeast tRNA synthetases are lysine-rich (9). Because the positively charged lysine side chain is used for nonspecific phosphate backbone contacts in protein-nucleic acid complexes, we imagined that at least some of the lysine residues might be important for nonspecific RNA interactions. Interestingly, two lysine clusters at opposite ends of the sequence of AdScQRS stand out as obvious candidates for further investigation. With these considerations in mind, we set out to manipulate AdScQRS by deletion and point mutations and thereby study the consequences in vivo and in vitro of these manipulations. To our surprise, this analysis demonstrated an unusual functional redundancy in the organization of structural elements needed for nonspecific RNA-binding interactions. This redundancy may have several implications for the design and evolution of proteins involved in transactions with RNA.

MATERIALS AND METHODS

Complementation—An S. cerevisiae strain (EFW6) with a partial deletion of GLN4 was constructed as described previously (7). Briefly, an 835-base pair EcoRI fragment (encoding residues 384–662) of gln4 was deleted and replaced by TRP1, using standard genetic methods with a ura3- strain. The growth of EFW6 gln4Δ:TRP1 strain was maintained by plasmid pEFW111 that contains GLN4 and the selectable marker URA3. The complementation assay was performed by introducing into EFW6 a second plasmid containing a test gene, such as an E. coli glnS derivative and LEU2 selectable marker and testing the
ability of EF68 to grow on 5-fluoroorotic acid (5-FOA) plates. The transformant evicts the maintenance plasmid with the URA3 marker in the presence of 5-FOA. Thus, only an enzyme with functional GlnS activity encoded by the second plasmid (with the LEU2 marker) can rescue the growth defect.

Deletion and Mutational Analyses—For constructing an AdS<sup>CQRS</sup>-E. coli glnS fusion in a yeast shuttle vector, an NdeI-NdeI fragment containing the coding sequence for residues 1–228 of yeast cytoplasmic glutaminyl-tRNA synthetase was amplified from a clone that contains the entire sequence of GLN4, the yeast gene for cytoplasmic glutaminyl-tRNA synthetase (10). This NdeI segment was subsequently cloned in-frame into the NdeI site at the start of the reading frame for E. coli glnS. (The coding sequence for the C terminus of E. coli glnS was tagged previously (7) with a sequence encoding the 12CA5 epitope.) The resulting DNA containing the coding sequence for AdS<sup>CQRS</sup>-E. coli glnS was subsequently used as a template for mutagenesis (11). Polymerase chain reaction amplification and fusion of the NdeI-NdeI DNA segments encoding portions of AdS<sup>CQRS</sup> to the coding region of E. coli glnS followed a similar approach to that for creating the AdS<sup>CQRS</sup>-glnS fusion. Expression in yeast of E. coli glutaminyl-tRNA synthetase as well as the fusion proteins was monitored by immunoblotting with the anti-12CA5 antibody (12) (Roche Molecular Biochemicals).

Expression and Purification of AdS<sup>CQRS</sup> and Its Derivatives—For purification of the wild-type, truncated, or mutated yeast AdS<sup>CQRS</sup> constructions were made in the E. coli expression vector pET-21b (Novagen, Madison, WI). Briefly, DNA fragments containing sequences encoding residues 1–228, 1–106, and 107–228 of GLN4 were polymerase chain reaction-amplified and cloned independently into the NdeI/XhoI site of pET-21b. The resulting DNA containing the full-length AdS<sup>CQRS</sup> was subsequently used as a template for mutagenesis at Lys-19/Lys-29, Lys-188/Lys-189, and Lys-20, Lys-20/Lys-29, Lys-188/Lys-189, Lys-189/Lys-190, Lys-190/Lys-191, and Lys-191/Lys-192. The density of the buffer (50 mM Tris, 50 mM boric acid, and 5 mM MgCl<sub>2</sub>) was previously determined to be 1.0016 g/ml (15). The molar extinction coefficient (ε) of AdS<sup>CQRS</sup> at 280 nm was calculated using the program Sednterp (17) as 19,940, whereas (ε) for tRNA<sub>Met</sub> was estimated at 726,700 M<sup>-1</sup> cm<sup>-1</sup> (17).

RESULTS

Strategy for Mapping Determinants for RNA Interactions in AdS<sup>CQRS</sup>—We took advantage of the previously described observation that E. coli glutaminyl-tRNA synthetase poorly acylates yeast tRNA<sub>Gln</sub> and that the acylation deficiency can be corrected by fusion of AdS<sup>CQRS</sup> to the N terminus of the E. coli enzyme. Interestingly, the S. cerevisiae and E. coli enzyme are close homologs, sharing 40% sequence identity over the entire length of the 551-amino acid sequence of the E. coli protein (Fig. 1) (10). The greater length of the yeast synthetase (809 amino acids) is mostly due to the 228 amino acids of AdS<sup>CQRS</sup> that are not present in E. coli glutaminyl-tRNA synthetase. Given the close similarity of the sequences of the main bodies of the enzymes, we imagined that many of the specific contacts between S. cerevisiae glutaminyl-tRNA synthetase and tRNA<sub>Gln</sub> are also possible with the E. coli synthetase. However, the “fit” of S. cerevisiae tRNA<sub>Gln</sub> on E. coli glutaminyl-tRNA synthetase is not sufficiently precise to ensure formation of an active complex. Thus, the demonstrated nonspecific RNA binding activity of AdS<sup>CQRS</sup> provided sufficient stability so as to overcome subtle steric conflicts that might occur in the heterologous complex.

Thirty-three lysines are present in the 228-amino acid sequence of AdS<sup>CQRS</sup>. (In contrast, there are only 7 arginines.) The lysines are distributed throughout the sequence, but two regions show an unusual concentration. These regions are located near the N- and C-terminal ends, respectively, of AdS<sup>CQRS</sup> (Fig. 1). The N-terminal cluster of 6 lysine residues is located between Lys-19 and Lys-29. The C-terminal cluster of 7 lysines is between Lys-188 and Lys-198. Thus, these two lysine-rich clusters (LRCs) contain 40% of all lysines in AdS<sup>CQRS</sup>.

Our strategy was to make deletion and point mutations that centered on the role of these LRCs. For assessing function in vivo, we made fusions with E. coli glutaminyl-tRNA synthetase of fragments bearing deletions and point mutations in AdS<sup>CQRS</sup>, and we tested the ability of the fusion proteins to rescue growth of a yeast strain carrying a null allele of the gene for cytoplasmic glutaminyl-tRNA synthetase.
mic glutaminyl-tRNA synthetase. For in vitro analysis, we relied on polyacrylamide co-electrophoresis to investigate effects of mutations on RNA binding. From this analysis, we were able to gain significant insight into the organization of determinants for the RNA binding activity of AdScQRS.

Deletions in AdScQRS That Do and Do Not Rescue Growth—Initial experiments focused on investigating whether either of the LRCs played a role in determining the ability of the fusion of AdScQRS to EcQRS to complement the yeast strain bearing the null allele of the gene for glutaminyl-tRNA synthetase. Seven different deletions in AdScQRS were constructed and fused to the N terminus of EcQRS. The results of individual complementation assays are shown in Fig. 2A, and a schematic summary of the results is given in Fig. 2B.

Fusions that joined either the N- or C-terminal LRC alone to EcQRS failed to complement. These fusions included AdScQRS-(1–42)-EcQRS and AdScQRS-(180–228)-EcQRS. These fusions used less than 50 residues from AdScQRS. Longer fusions (AdScQRS-(1–106) and AdScQRS-(107–228)) that extended the tested portions of AdScQRS to over 100 residues also failed to complement the null strain. Finally, fusion of the bulk of the spacer between the two LRCs also gave a protein (AdScQRS-(43–179)-EcQRS) that did not complement the null strain. By using Western blot analysis with antibodies directed against the 12CA5 tag of EcQRS, we established that each of the defective fusion proteins was expressed and accumulated in yeast (data not shown). Thus, the failure to complement was not due to lack of stable production of the mutant proteins.

In contrast, two fusion constructions were active in vivo. These constructs were AdScQRS-(1–179)-EcQRS and AdScQRS-(43–228)-EcQRS (Fig. 2, A and B). Each of these includes only one LRC but also encodes the spacer between the two LRCs. In one instance (AdScQRS-(1–179)-EcQRS), the spacer is joined to the C-terminal side of the LRC, whereas in the other (AdScQRS-(43–228)-EcQRS), it is joined to the N-terminal side. Empirically, there was no difference in the ability of either of these constructs to complement the null allele.

Point Mutations and Combinations of Point Mutations in the LRCs—In each of the two LRCs, contiguous lysines are present in two groupings. In the N-terminal LRC, these are Lys-19, Lys-20 and Lys-28, Lys-29. In the C-terminal LRC, the groupings include Lys-188, Lys-189, Lys-190 and Lys-196, Lys-197, Lys-198. We made tandem Lys to Ala substitutions in each of these groups to give, separately, proteins with the following mutations in AdScQRS: K19A/K20A, K28A/K29A, K188A/K189A, and K196A/K197A. Each of these four mutant proteins was fused to EcQRS, and the four resultant mutant fusion proteins were expressed in S. cerevisiae and tested for their ability to complement the null strain. None of these four failed to complement the defective yeast strain (Fig. 3A).

We next constructed mutants of AdScQRS that had paired point substitutions in each of the LRCs. The rationale was that although mutants such as the K19A/K20A fusion protein were active in vivo, placement of an additional mutation in the same or the other LRC might be sufficient to cause inactivation. Thus, we visualized a “matrix” where we combined double mutations to give fusion proteins with four Lys to Ala substitutions.

The matrix designed is summarized in Fig. 3B, and the data supporting the entries in the matrix are reported in Fig. 3A.
Two constructs failed to complement the null strain. Each of these (K19A/K20A/K188A/K189A and K28A/K29A/K188A/K189A) combined a double Lys to Ala replacement in the N-terminal LRC with a K188A/K189A substitution in the C-terminal motif. These results demonstrated the importance of Lys-188/Lys-189 relative to Lys-196/Lys-197 in the C-terminal LRC. They also suggest a functional role for both Lys-19/Lys-20 and Lys-28/Lys-29 in the N-terminal LRC.

In contrast to the inactivation seen with fusion proteins having substitutions in both LRCs, those constructs with four replacements in the same LRC (K19A/K20A/K28A/K29A and K188A/K189AK196AK197A) were active. Thus, mutations are required in both LRCs in order to inactivate AdScQRS. This result supports the conclusion reached in the experiments with the deletion proteins (Fig. 2), namely that only one LRC is required for function in vivo.

**In Vitro Analysis of RNA Binding by Wild-type and Mutant AdScQRS**—To correlate the complementation results shown in Figs. 2 and 3 with a biochemical phenotype, we investigated RNA binding with a transcript of *S. cerevisiae* tRNA<sup>Gln</sup> as the RNA ligand. Affinity co-electrophoresis was used to assay for RNA binding (13, 14). With this assay, a gradient of each of the tested fusion proteins was established in the horizontal direction across the gel. Radioactively labeled RNA was then electrophoresed through that gradient. The binding of [32P]tRNA to the protein results in retardation of the tRNA, with retardation proportional to the amount of bound RNA. The results are shown in Fig. 3B. For example, the wild-type protein (WT) binds RNA strongly, while the K19A/K20A and K188A/K189A constructs bind more weakly, and the K196A/K197A construct does not bind at all.

![Fig. 3](http://www.jbc.org/)

**Fig. 3. Complementation of the GLN4::TRP1 knockout strain EFW6 by various fusions of the E. coli enzyme with mutant AdScQRS proteins.** A, double lysine to alanine substitutions were made in each (upper panel) or both (lower panel) lysine-rich clusters of AdScQRS that had been fused to the E. coli glutamine enzyme. B, a schematic matrix summarizing the complementation results shown in A. WT, wild type.
The LRCs of AdScQRS contain important RNA binding determinants. The purified wild-type, truncated, and mutated AdScQRS proteins were tested individually for their interaction with a yeast tRNAfMet transcript. The polycrystalline affinity co-electrophoresis gels have a protein gradient that increases in 3-fold increments from left to right, as indicated at the bottom of each panel. A, AdScQRS-(1–106) (left), AdScQRS(1–106) (middle), and AdScQRS(107–228) (right) bound to tRNAfMet. B, AdScQRS(K19A/K20A) (left), AdScQRS(K188A/K189A) (middle), and AdScQRS(K19A/K20A/K188A/K189A) (right) bound to tRNAfMet. In all panels, the tRNA substrate moved in the direction indicated by the arrow.

RNA Binding Domain

We tested wild-type AdScQRS, AdScQRS-(1–106), AdScQRS-(1–106), AdScQRS(1–106), AdScQRS(1–228), AdScQRS(107–228), AdScQRS(K19A/K20A), AdScQRS(K188A/K189A), and AdScQRS(K19A/K20A/K188A/K189A) in the RNA binding assay. The binding of wild-type AdScQRS was readily demonstrated, and the apparent Kd value was similar to that (0.6 µM) previously measured for the binding of AdScQRS to unfraccionated yeast tRNA (Fig. 4A). In contrast, the apparent Kd value for the complex of AdScQRS-(1–106) and AdScQRS(107–228) with yeast tRNAfMet was raised about 10-fold relative to the wild-type protein. This elevation in Kd values for these two deletion proteins correlates with the failure of each of them to confer the complementation phenotype when fused to EcQRS (Fig. 2). In the case of the AdScQRS proteins harboring mutations in either or both of the LRCs, AdScQRS(K19A/K20A) and AdScQRS(K188A/K189A) each had a small but significant reduction in affinity for tRNAfMet (Fig. 4B). The apparent Kd value of each was raised by roughly 3-fold. However, with mutations in both LRCs(AdScQRS(K19A/K20A/K188A/K189A)), the apparent Kd value was raised about 10-fold, and was similar to that seen with the deletion proteins. Thus, these results also correlate with the in vivo complementation analysis, where the AdScQRS(K19A/K20A) and AdScQRS(K188A/K189A) fusion proteins were active, and the fusion protein with substitutions in both LRCs was inactive (Fig. 2).

Stoichiometry of the AdScQRS-tRNA Complex—We considered the possibility that each LCR forms a separate RNA-binding site so that two molecules of RNA were bound per AdScQRS. The affinity co-electrophoresis studies (Fig. 4) are not definitive in this regard, because a species with one bound RNA molecule is difficult to deconvolute from a species that has two bound RNAs. To address this issue more rigorously, sedimentation equilibrium analysis of a mixture of AdScQRS and tRNAfMet was performed at various speeds and concentrations. (For these experiments, we mostly used E. coli tRNAfMet because it was more easily obtained in quantities sufficient for study by ultracentrifugation. Our results showed that AdScQRS had approximately the same affinity for tRNAfMet and tRNAfMet (data not shown).) Due to the difference in extinction coefficients between tRNA and AdScQRS (the extinction coefficient of tRNAfMet is significantly larger than that of AdScQRS), the contribution to the total absorbance at 260 nm by AdScQRS is negligible and thus allows us to follow the formation of the tRNA-AdScQRS complex by fitting data to the size of the tRNA species (16). Parallel experiments were performed to estimate the molecular masses of the free tRNAfMet, free AdScQRS, and the AdScQRS-tRNA complex. For example, free tRNAfMet and AdScQRS have measured molecular masses of 24.1, and 27.7 kDa, respectively. A 1:1 molar ratio of tRNAfMet and AdScQRS at a concentration of 500 nM was spun at 12,000 rpm at 20 °C until equilibrium. The resulting data can be fit to a two-species system corresponding to the 27% free tRNAfMet (24.1 kDa) and 73% 1:1 tRNAfMet-AdScQRS complex (48.7 kDa) (Fig. 5). The resulting x^2 error was estimated at 5.589 x 10^-4. A 4:1 molar ratio of protein:tRNA with the tRNA at 500 nM was indicative of one species in solution with a molecular mass of 48.7 kDa with a x^2 error of 4.124 x 10^-4. This corresponded to a 1:1 protein-tRNA complex (Fig. 5). Similar results were obtained at 10,000 rpm.

We next used a traditional gel-mobility shift assay to confirm this conclusion. We imagined that if tRNA preferentially binds AdScQRS in a 2:1 ratio in the assay, then two species of complexes (1:1 and 2:1) would be observed at concentrations of AdScQRS close to the apparent Kd (roughly 0.6 µM). However, only one species of AdScQRS-tRNA complex was detected at all tested concentrations of AdScQRS. That is, the same...
AdScQRS–tRNA complex was present at high as at low concentrations of AdScQRS (data not shown).

**DISCUSSION**

Like yeast glutaminyl-tRNA synthetase, many eukaryote cytoplasmic class I tRNA synthetases have an extra domain appended to the N- or C-terminal end of the catalytic body (9). This extra domain is not found in their *E. coli* counterparts. In higher eukaryotes some of these appended domains are believed to be involved in forming a multienzyme assembly (9, 18), a complex that could potentially enhance the overall efficiency of protein synthesis. These appended domains in higher eukaryotes may also have specific cytokine and nonspecific RNA binding activities (19, 20). In the yeast *S. cerevisiae*, each of at least six tRNA synthetases have an appended domain. However, the existence of a multisynthetase complex in yeast is controversial (9), and other potential roles are just now being determined. These roles center around the ability of these domains to enhance formation of specific synthetase-tRNA complexes (6–8).

Our previous data showed that the *E. coli* glutamine enzyme poorly acylates yeast tRNA\(^{\text{Glu}}\) and thus fails to rescue a yeast knockout allele of the gene for cytoplasmic glutaminyl-tRNA synthetase (7, 8). Fusing AdScQRS or an unrelated RNA binding domain to the *E. coli* enzyme raised the affinity of the *E. coli* enzyme for yeast tRNA so that the aminoacylation rate can sustain the growth of the yeast strain harboring a GLN4-disrupted allele (7, 8). Thus, an increase in tRNA binding affinity of the *E. coli* enzyme seemed to be all that was needed to boost the enzyme into a functional yeast enzyme. With this in mind, we attempted to rescue the yeast null allele with a construct that highly expressed the *E. coli* enzyme in vivo. To this end, the DNA coding sequence for the *E. coli* glutamine enzyme was cloned in a high copy number plasmid under the control of the constitutive alcohol dehydrogenase promoter and tested for its complementing activity. However, this construct failed to rescue growth of the yeast knockout strain (data not shown). Considering that the apparent *K_\text{d}* value of the *E. coli* enzyme for yeast tRNA\(^{\text{Glu}}\) is lowered about 400-fold by the fusion of AdScQRS (8), this lowering of the *K_\text{d}* is probably more than what can be compensated for by overproduction of the unfused *E. coli* enzyme.

Recently, we showed that fusion of the nonspecific RNA-binding protein Arc1p to *E. coli* GlnRS also activated aminoacylation of yeast tRNA\(^{\text{Glu}}\) in vivo (8). To extend further this finding, we showed that the appended domain of yeast glutamyl-tRNA synthetase (GluRS) can activate aminoacylation by *E. coli* GlnRS. For example, fusing the appended domain of the yeast glutamate enzyme to the *E. coli* GlnRS enabled the AdScERS-EcQRS fusion enzyme to complement a yeast knockout allele of the gene for cytoplasmic GlnRS (data not shown). Thus, like AdScQRS, the appended domain of the yeast GluRS (AdScERS) is probably a RNA-binding protein. Unlike AdScQRS, however, no lysine clusters have been found for AdScERS that are similar to those found in AdScQRS. Instead, the lysine residues of AdScERS are more evenly distributed throughout the entire sequence (data not shown). Similarly, Arc1p is not related in sequence to AdScQRS or AdScERS. Thus, it is not likely that AdScQRS, AdScERS, and Arc1p contact tRNA in exactly the same way. These considerations suggest that the main contribution of these domains is to increase the lifetime of the respective complex with tRNA, so that sufficient time is allowed for a conformational change that leads to a productive aminoacylation. Indeed, early work demonstrated a conformational change associated with a cognate but not a non-cognate synthetase-tRNA complex (21, 22).

In contrast to most other RNA-binding proteins, AdScQRS is unique in the way that it binds to a wide variety of RNA substrates with a similar affinity (roughly 0.6–2 \(\mu\text{M}\)) (8). Given the nonspecificity and modest affinity, we imagine that the AdScQRS–nucleic acid complexes derive much of their binding energy from electrostatic interactions. Indeed, our mutational analyses demonstrated the importance of lysine residues within the two LRCs of AdScQRS (8). Conceivably, these two LRCs are involved in direct contact with nucleic acids. Because deletions and mutations in either LRC can be tolerated as long as the linker between the two LRCs is intact, we believe these LRCs are functionally redundant. However, despite the presence of the two LRCs in one AdScQRS, the strict requirement for
the linker (with either LRC) explains why the stoichiometry is limited to 1:1 (tRNA:AdScQRS). Our data are not clear on whether the essential spacer that connects the two LRCs plays a direct role in making RNA contacts or whether its role is indirect.

Several RNA-binding motifs have been identified in proteins involved in diverse cellular functions. These include the ribonucleoprotein motif, arginine-rich motif, RGG box, and dsRNA-binding motif, among others (23–25). The particular RNA-binding domain reported here seems to be the only one with lysine-rich clusters and thus might represent a novel class of RNA binding domains. The existing data suggest the possibility that AdScQRS has a general structural arrangement that is similar to the lysine/arginine-rich dsRNA binding domain of protein kinase PKR (26). The NMR structure of the dsRNA binding domain of the free protein revealed a dumb-bell shape comprising two tandem RNA-binding motifs connected by a flexible linker (27). Like AdScQRS, these RNA-binding elements are used to dock a single RNA molecule in a sequence-independent manner (28). Unlike AdScQRS, the RNA-binding elements of the dsRNA binding domain of protein kinase PKR are highly conserved (among eukaryotes) and bind exclusively to dsRNA (29).

In contrast, AdScQRS is found in fungal organisms, and counterparts in higher eukaryotes have yet to be identified.

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