Arc1p Organizes the Yeast Aminoacyl-tRNA Synthetase Complex and Stabilizes Its Interaction with the Cognate tRNAs*

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Eukaryotic aminoacyl-tRNA synthetases, in contrast to their prokaryotic counterparts, are often part of high molecular weight complexes. In yeast, two enzymes, the methionyl- and glutamyl-tRNA synthetases associate in vivo with the tRNA-binding protein Arc1p. To study the assembly and function of this complex, we have reconstituted it in vitro from individually purified recombinant proteins. Our results show that Arc1p can readily bind to either or both of the two enzymes, mediating the formation of the respective binary or ternary complexes. Under competition conditions, Arc1p alone exhibits broad specificity and interacts with a defined set of tRNA species. Nevertheless, the in vitro reconstituted Arc1p-containing enzyme complexes can bind only to their cognate tRNAs and tighter than the corresponding monomeric enzymes. These results demonstrate that the organization of aminoacyl-tRNA synthetases with general tRNA-binding proteins into multimeric complexes can stimulate their catalytic efficiency and, therefore, offer a significant advantage to the eukaryotic cell.

The function of aminoacyl-tRNA synthetases establishes the faithful translation of the genetic code, since these enzymes catalyze the coupling of tRNAs to their cognate amino acids (1, 2). The 20 different aminoacyl-tRNA synthetases, one for each amino acid, can be assigned to two classes (I and II), depending on the sequence and structure of their conserved catalytic domains (3, 4). The enzymes also contain additional idiosyncratic domains that are attached to or inserted in the class-defining catalytic core and are responsible for binding the cognate tRNAs (5). Enzymes from yeast and higher eukaryotes are further characterized in comparison to the corresponding prokaryotic enzymes by the addition of extensions to the N or C termini, which are often dispensable for activity (6–10). However, more recent data suggest that, in several cases, these appended domains can bind nonspecifically to RNA and, therefore, may facilitate the association of the synthetases with their cognate tRNAs (10–16). An alternative function of the eukaryote-specific appendices may be protein interactions that lead to the assembly of multisynthetase complexes (17–20), although the catalytic domains may also contribute to the formation of these complexes (20). Higher eukaryotes indeed contain a supramolecular multienzyme complex comprised of nine aminoacyl-tRNA synthetases and three nonenzymatic polypeptides of 43, 38, and 18 kDa called p43 (pro-EMAPII), p38, and p18, respectively (21–24). The function of this complex is still unclear. It is possible that the association of the synthetases with the other components of the complex may modulate their activity, as has been shown in the case of human ArgRS, whose activity can be stimulated by the interaction with p43 (25).

We previously identified in yeast a smaller complex of aminoacyl-tRNA synthetases consisting of two class I enzymes, methionyl-tRNA (MetRS)1 and glutamyl-tRNA (GluRS) synthetases, and the protein Arc1p, which is the yeast homologue of p43 (26, 27). Subsequently, it was shown that Arc1p associates with the two enzymes of the complex through its N-terminal domain, whereas its C-terminal part harbors a tRNA binding domain (TRBD) (28). The biggest part of the TRBD is conserved between yeast Arc1p and mammalian p43 and is also found fused at the C-terminal end of the catalytic domain of the human tyrosyl-tRNA synthetase (29). Human TyrRS as well as p43 can be targets of proteolytic enzymes during apoptosis (30–33). This results in the release of the corresponding TRBDs, which can act as EMAPII (endothelial monocyte-activating polypeptide)-like cytokines, possibly linking the progression of apoptosis to the inhibition of protein translation (reviewed in Ref. 34). Recently, the structure of the TRBD of p43 has been solved, showing that it adopts an OB fold, an oligonucleotide binding structural motif also found in the anticodon binding domains of class IIb aminoacyl-tRNA synthetases (35). Part of the TRBD of Arc1p is also conserved in prokaryotes as a domain of two synthetases, MetRS and PheRS (26), or as an independent polypeptide, Trbp111 (36). Trbp111 has indeed been characterized as a structure-specific tRNA-binding protein, which may stabilize the L-shape of tRNA (36).

In yeast, the presence of TRBD in Arc1p is necessary for the stimulation of the catalytic efficiency of MetRS by increasing its apparent affinity for tRNA^Met (28). Arc1p is required for optimal cell growth and is essential for viability in the absence of the tRNA nuclear export factor Los1p (26). Indeed, tRNA aminoacylation has been shown to be required for efficient nuclear tRNA export in yeast (37, 38).

To understand the molecular details of the function of Arc1p in tRNA aminoacylation, we attempted the in vitro reconstitution of its complex with tRNA and/or the two aminoacyl-tRNA synthetases. Our results show that the formation of the Arc1p-MetRS-GluRS complex can occur in vitro, requiring no other factors. Furthermore, although Arc1p has a broad specificity for tRNAs, its association with the synthetases facilitates the interaction exclusively with the corresponding cog-

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1 The abbreviations used are: MetRS, methionyl-tRNA synthetase; GluRS, glutamyl-tRNA synthetase; TRBD, tRNA binding domain; ORF, open reading frame; PAGE, polyacrylamide gel electrophoresis.
nate tRNAs and leads to stimulation of aminocacylation efficiency. These data can explain why eukaryotic aminocacyl-tRNA synthetases are organized into multimeric complexes containing additional tRNA-binding proteins of broad specificity.

**EXPERIMENTAL PROCEDURES**

**Construction of Plasmids Expressing His<sub>si</sub>-MetRS and His<sub>si</sub>-GluRS**—To overproduce eight histidine-tagged MetRS and GluRS in yeast, the vector pEMBLyex4, with the selectable markers URA3 and the poorly expressed lou2-d allele of LEU2, which increases the copy number of the plasmid under leucine-selecting conditions (29, 40), was modified to create vector pEMBLyex4-His<sub>si</sub>. A small DNA piece, constructed by annealing two complementary synthetic oligonucleotides, was inserted at the BamHI site at the 5’ end of the polylinker of pEMBLyex4, adding the sequence 5’-GATCCAGTCACACACACACACCACCGAACCTGGGG-3’. This codes for the start methionine followed by eight histidines and contains a XhoI restriction site at the 3’ end (underlined) to facilitate the selection of the correctly ligated constructs. Only the BamHI site at the 3’ end of the new sequence was regenerated. The ORF of MetRS was amplified by polymerase chain reaction using as template plasmid pUN100-MES1 (26) and primers that created a BamHI restriction site before the second codon and a PstI restriction site in the 3’-untranslated region of the gene. The ORF was then cloned into pEMBLyex4-His<sub>si</sub>, previously cut with BamHI/PstI, which created a coding sequence for an in-frame fusion protein of eight histidine residues joined by a spacer Leu-Glu-Gly tripeptide to the amino acid immediately after the start methionine of the original MetRS ORF (plasmid pEMBLyex4-His<sub>si</sub>-MetRS). The ORF of GluRS was amplified by polymerase chain reaction using as template yeast genomic DNA and primers that created a XhoI restriction site before the second codon and a PstI restriction site in the 3’-untranslated region of the gene. The ORF was then also cloned into pEMBLyex4-His<sub>si</sub> previously cut with XhoI/PstI, which created a coding sequence for a fusion protein containing eight histidine residues joined by a spacer Leu-Glu-Gly-Ser-Ser-Arg hexapeptide to the amino acid immediately after the start methionine of the original GluRS ORF (plasmid pEMBLyex4-His<sub>si</sub>-GluRS). The inserts containing the fusion proteins were sequenced, and no mutations could be found.

**Protein Purification**—Purification of recombinant epitope (His<sub>8</sub>)-tagged Arc1p and Arc1p deletion mutants or Arc1p domains from *Escherichia coli* and untagged full-length MetRS from an overproducing yeast strain was performed as described previously (26, 28, 41). The epitope-tagged His<sub>8</sub>-MetRS and His<sub>8</sub>-GluRS were purified as follows: plasmids pEMBLyex4-His<sub>8</sub>-MetRS and pEMBLyex4-His<sub>8</sub>-GluRS were transformed to haploid RS453 yeast cells. Cultures of the transformed yeast cells were grown in SDC-leu medium to an OD<sub>600</sub> of 0.5 and 1. The cells were then collected by centrifugation, re-suspended in 1 liter of galactose containing SGC-leu minimal medium at an OD<sub>600</sub> of 0.4, and grown for 14 h to an OD<sub>600</sub> of ~1.7. The cells were then collected by centrifugation, washed with Tris-hCl, pH 7.8, 10 mM MgCl<sub>2</sub>, 5 mM dithiothreitol, 1.2 mg/ml bovine serum albumin, 2 mM ATP, 6 mg/ml total yeast tRNA (Roche Molecular Biochemicals), and 0.1 mM L-[1-14C]glutamic acid (specific activity 56 mCi/mmol), and the reaction mixture was incubated at 25 °C. At various time intervals the reaction mixture was collected by centrifugation at 27,000 × g for 30 min, and the supernatant was applied onto a nickel nitrilotriacetic acid resin (Qiagen, Hilden, Germany) column (bed volume: 0.5 ml) that was then washed with yeast extract buffer until no more protein could be detected in the eluate. Bound proteins were then eluted by 4 ml of LB containing 250 mM imidazole, dialyzed against MonoQ buffer (20 mM Tris-Cl, pH 8.0, 5 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, 10% glycerol), loaded onto a MonoQ HR 5/5 column (Amersham Pharmacia Biotech) equilibrated in the same buffer and, eluted with a NaCl gradient. His<sub>8</sub>-MetRS was eluted with ~230 mM NaCl, whereas His<sub>8</sub>-GluRS was eluted with ~130 mM NaCl. The peak fractions were frozen in liquid nitrogen and kept at -80 °C. In all purification steps the identity of the purified proteins bands was verified by Western blotting using anti-MetRS polyclonal antibodies for the detection of MetRS and monoclonal antibodies against the histidine tag (BABC Bio, Berkeley, CA) for the detection of His<sub>8</sub>-GluRS.

**Binding Assays and Gel Filtration**—Protein-protein and protein-tRNA binding reactions were performed by mixing the individual proteins, yeast tRNA<sub>ytm</sub> (Sigma) or total yeast tRNA (Roche Molecular Biochemicals) in a total volume of 150–250 μl of binding buffer (20 mM HEPES, pH 7.4, 150 mM NaCl, 5 mM MgCl<sub>2</sub>, 10% glycerol) and incubating at 30 °C for 30 min. After the end of the incubation, the mixture was spun at 14,000 rpm on an Eppendorf tabletop centrifuge for 15 min at 4 °C, and 100–200 μl of the supernatant were loaded onto a Superdex 200 HR 10/30 column (Amersham Pharmacia Biotech). The column was eluted with binding buffer at a flow rate of 0.4 ml/min, and 0.5-ml fractions were collected. The fractions that exhibited detectable absorption at 280 nm were analyzed by SDS-PAGE and, when appropriate, by RNA extraction followed by electrophoresis on 10% urea-polyacrylamide gels followed by ethidium bromide staining. Only the relevant parts of the gels are shown, and the positions of Arc1p, tRNA, and 5 S rRNA are indicated. In A–C, the arrows indicate the muscle masses that correspond to the elution volume of the absorption peaks. In D and E, the brackets indicate the fractions that would contain Arc1p (Pool A) and the ones containing free tRNA (Pool B).

![Fig. 1. Reconstitution of a stable Arc1p-tRNA complex and separation by gel filtration.](http://www.jbc.org/)}
The filters were washed three times with cold trichloroacetic acid containing 1 mM glutamic acid and three times with 70% ethanol, dried, and counted for radioactivity in scintillation liquid.

Miscellaneous Procedures—Protein concentrations were determined using the protein assay reagent from Bio-Rad. SDS-PAGE, DNA manipulations (restriction digests, ligations, polymerase chain reaction amplifications, etc.), RNA extraction, and Northern analysis were performed according to standard protocols. Detection of tRNAs on Northern blots was done using end-labeled synthetic DNA oligonucleotides complementary to tRNA-specific sequences. The sequences of the oligonucleotides used for polymerase chain reaction or Northern are available upon request.

RESULTS

Arc1p Binds Preferentially to a Subset of tRNA Species—To reconstitute and isolate Arc1p complexes with tRNA or aminoacyl-tRNA synthetases, we chose to use gel filtration as a method for separating the bound from the free complex components. We first analyzed the migration of recombinant Arc1p on a Superdex 200 gel filtration column. According to its amino acid sequence, Arc1p has a predicted molecular mass of 42 kDa. However, recombinant Arc1p was eluted from the column as a symmetrical peak with an apparent molecular mass of 90 kDa (Fig. 1A). To find out which part of Arc1p may be responsible for this aberrant migration, we analyzed individual Arc1p domains or Arc1p deletion mutants. The results show that the N- and C-terminal domains of Arc1p behaved as monomers (Table I). The Arc1-DM mutant, lacking the middle (M) domain, migrated with an apparent molecular mass close to the theoretical one. In contrast, the mutants Arc1-DN and Arc1-DC, both

![Diagram](https://example.com/diagram.png)

**FIG. 2.** Arc1p binds to a subset of tRNA species. A, the column fractions of the experiments shown in Fig. 1D (right panels) and 1E (left panels) were analyzed by Northern using oligonucleotide probes directed against the indicated major tRNA isoacceptors. Only the relevant parts of the autoradiograms are shown. In the right panels of Fig. 1, fractions 17–19 (Pool A) contain tRNAs bound to Arc1p, whereas fractions 20–23 (Pool B) contain free tRNAs. Note that in the absence of Arc1p (left panels), the Pool A fractions do not contain any tRNA. B, schematic representation of the three-dimensional structure yeast tRNA$^\text{Phe}$. Positions shown in gray contain the invariable bases. Positions shown in black contain the bases that are conserved in all the tRNA species that interact strongly with Arc1p.

| Protein or complex | Predicted molecular mass (kDa) | Apparent molecular mass (kDa) |
|-------------------|-------------------------------|------------------------------|
| Arc1p             | 43                            | 85                           |
| Arc1-N (1–133)$^a$ | 17                            | 19.5                         |
| Arc1-ΔN (123–376)$^a$ | 30                           | 65                           |
| Arc1-C (199–376)$^a$ | 22                           | 24                           |
| Arc1-ΔC (1–211)$^a$ | 25.5                         | 55                           |
| Arc1-ΔM (1–133 + 199–376)$^a$ | 36.5 | 49                           |
| Arc1p + tRNA$^\text{Phe}$ | 68                    | 91                           |
| Arc1-ΔN + tRNA$^\text{Phe}$ | 55                    | 90                           |
| MetRS             | 85.5                          | 89.5                         |
| Arc1p + MetRS     | 125.5                         | 219                          |
| Arc1-N + MetRS    | 102.5                         | 132                          |
| Arc1p + MetRS + tRNA | 153.5                 | 212                          |
| His$_8$-GluRS     | 84.5                          | 92                           |
| Arc1p + His$_8$-GluRS | 127.5                | 210                          |
| Arc1-N + His$_8$-GluRS | 101.5               | 120                          |
| Arc1p + His$_8$-GluRS + tRNA | 152.5           | 200                          |
| Arc1p + His$_8$-MetRS + His$_8$-GluRS | 214.5 | 335                         |
| Arc1-N + His$_8$-MetRS + His$_8$-GluRS | 188.5 | 270                        |
| Arc1p + His$_8$-MetRS + His$_8$-GluRS + tRNA | 239.5 | 324                        |

$^a$ The numbers in parenthesis indicate the amino acids that constitute the Arc1p domains.
containing the M domain, displayed a much higher apparent molecular mass than predicted. Therefore, the aberrant migration of Arc1p must be due to the presence of the M domain, which may cause formation of dimers (see also “Discussion”).

To isolate a stable Arc1p:tRNA complex, recombinant Arc1p was incubated with yeast tRNA^{Phe}, and the mixture was then loaded on the Superdex 200 column. Elution revealed two peaks (Fig. 1B), as compared with one peak when tRNA was analyzed alone (Fig. 1C). Protein and RNA electrophoresis demonstrated that the 35-kDa peak contained only tRNA^{Phe}, whereas the 90-kDa peak contained both Arc1p and tRNA^{Phe}. Therefore, a significant amount of tRNA was bound to Arc1p. We then went on to investigate the specificity of Arc1p by challenging it with a population of different tRNAs. Arc1p was incubated with total yeast tRNA, and the mixture was then loaded onto the column (Fig. 1D). As a control, total yeast tRNA was also analyzed in the absence of Arc1p (Fig. 1E). The Arc1p:tRNA mixture gave two peaks, one corresponding to free tRNAs (Pool B) and one corresponding to Arc1p and the Arc1p:tRNA complexes (Pool A). RNA extraction followed by electrophoresis revealed the presence of several tRNA species in this latter peak. To identify the tRNAs that associate with Arc1p, we performed extensive Northern analysis using probes against 16 different major isoacceptor tRNAs. As can be seen in Fig. 2A, these tRNAs can be divided in three groups. The first group (“strong binders”) contains tRNAs that bind significantly to Arc1p and includes tRNA^{Glu}, elongator tRNA^{Met}, tRNA^{Phe}, tRNA^{Asp}, and tRNA^{Arg}. In the second group (“weak binders”), the amount of bound tRNA represents only a small fraction of the total tRNA. Finally, in the third group (“nonbinders”), no tRNA could be detected co-merging with Arc1p.

To confirm these results and to check whether the N-terminal domain of Arc1p plays a role in tRNA binding specificity, we repeated the analysis using the Arc1-ΔN mutant form. We obtained exactly the same results (data not shown), showing that the relative affinity of Arc1p for the tRNA species is determined solely by the TRBD that comprises the middle (M) and C-terminal (C) parts of Arc1p. The TRBD recognizes structural features that are common to all tRNAs. However, under competition conditions, which better mimic the in vivo situation, the TRBD binds preferentially to a subset of tRNA species, i.e. it exhibits broad specificity. It is possible that the tRNA species that binds strongly to Arc1p contains certain elements that facilitate the TRBD-tRNA interaction. Indeed, examination of the sequences of these tRNAs revealed a combination of nucleotides in certain positions that is unique for the strong binders. Some of these nucleotides, which include C^5, G^{12}, C^{20}, C^{22}, A^{44}, G^{45}, G^{51}, G^{57}, C^{63}, and G^{71} (highlighted in Fig. 2B) may actually act as positive determinants for the interaction with Arc1p (see also “Discussion”).

**The Arc1p:MetRS Complex Binds Specifically to Elongator tRNA^{Met}**—We then tried to reconstitute the Arc1p:MetRS complex to test its tRNA binding abilities compared with monomeric MetRS. First, purified MetRS was applied to the gel filtration column. The elution profile shows that MetRS migrated with an apparent molecular mass of 90 kDa, confirming its monomeric nature (Fig. 3A). When MetRS was pre-mixed with recombinant Arc1p, they were both eluted together as a heterodimer (Fig. 3A). This shows that Arc1p and MetRS can readily associate in vitro, forming a stable complex. The size of this complex as well as its composition as analyzed by SDS-PAGE strongly suggest that it contains equimolar amounts of Arc1p and MetRS. Previous in vivo data suggest that the Arc1p-MetRS interaction is mediated by the N domain of Arc1p (28). To also show that in vitro, we used the N domain instead of full-length Arc1p. As can be shown in Fig. 3B, Arc1-N and MetRS associate readily to form a 132-kDa complex (the second peak with a molecular mass of 20 kDa corresponds to free Arc1-N, which was added in excess).

We then tested both monomeric MetRS and the Arc1p:MetRS complex for binding to tRNA by incubating them with total yeast tRNA. Analysis of the MetRS-tRNA mixture by gel fil-
Fig. 4. The association of Arc1p with MetRS stimulates binding of the cognate tRNA. A, a sample (100 µl) containing 65 µg of purified MetRS pre-incubated with 100 µg of total yeast tRNA (left panels) or 65 µg of purified MetRS pre-incubated with 35 µg of recombinant Arc1p and 300 µg of total yeast tRNA (right panels) was chromatographed through a Superdex 200 column. 60 µl of each column fraction were analyzed for RNA as in Fig. 1. B, the column fractions of the experiments shown in A were analyzed by Northern blot using oligonucleotide probes directed against the indicated major tRNA isoacceptors.

Overexpression and Purification of Histidine-tagged GluRS and MetRS from Yeast—To reconstitute the ternary Arc1p-MetRS-GluRS complex, we needed to produce GluRS, which had never been purified before from yeast, as well as additional amounts of MetRS. To facilitate purification, the two enzymes were tagged at their N termini with a stretch of eight histidines and overexpressed in yeast cells. Soluble cell lysates were passed through a nickel nitrilotriacetic acid column, and elution by 250 mM imidazole led to the efficient recovery of His8-MetRS or His8-GluRS (Fig. 5). To ensure removal of minor contaminants, the proteins were further purified by ion exchange chromatography on a MonoQ column (Fig. 5). The purification scheme proved to be very efficient since ~1.2 mg of purified tagged MetRS or GluRS could be recovered from a 1-liter culture of the corresponding overexpressing yeast cells.

Reconstitution of the Ternary Arc1p-MetRS-GluRS Complex—Purified His8-MetRS and His8-GluRS were loaded onto the gel filtration column alone or after being mixed and incubated with one another or with roughly equimolar amounts of Arc1p. The results of these experiments are summarized in Fig. 6A and Table I. When applied alone, both enzymes were eluted from the column as monomers. When incubated together, His8-MetRS and His8-GluRS remained monomeric, showing that no direct interaction can take place between them in vitro. However, incubation with Arc1p resulted in the formation of stable dimeric and stoichiometric complexes, both, in the case of His8-
MetRS and His8-GluRS. Finally, incubation of both enzymes together with Arc1p led to the assembly of all three components into a ternary complex with an apparent stoichiometry of 1:1:1. As in the case for the Arc1p-MetRS complex, the N domain of Arc1p was sufficient to convey association with His8-GluRS (Fig. 6B and Table I). Moreover, the 20-kDa N domain of Arc1p could accommodate the interactions with both aminoacyl-tRNA synthetases (Fig. 6B). Therefore, the N domain of Arc1p is the central component of the Arc1p-synthetase complex and must contain binding sites for both MetRS and GluRS.

Arc1p Enhances the Interaction between GluRS and the Cognate tRNA<sub>Glu</sub> and Stimulates Its Aminoacylation Efficiency—To test the effect of Arc1p on GluRS, monomeric His<sub>8</sub>-GluRS or the in vitro reconstituted Arc1p-His<sub>8</sub>-GluRS binary complex was incubated with total yeast tRNA and applied to the gel filtration column. As shown in Fig. 7A, a single tRNA species bound to and coeluted with His<sub>8</sub>-GluRS (Pool I). Northern analysis (Fig. 7B) demonstrated that this tRNA species corresponds to the cognate tRNA<sub>Glu</sub>. As estimated by scanning the autoradiograms, almost 40% of the tRNA<sup>Glu</sup> in the total yeast tRNA mixture shifted to fractions containing His<sub>8</sub>-GluRS, showing that, unlike MetRS, monomeric His<sub>8</sub>-GluRS is able to form a stable complex with its cognate tRNA. The stability of this complex was significantly increased in the presence of Arc1p. More than 80% of the total tRNA<sup>Glu</sup> shifted to the fractions (Pools I-III) containing the Arc1p-His<sub>8</sub>-GluRS complex (Fig. 7B). Noncognate tRNAs were not enriched in these fractions. The fact that Arc1p can stimulate the binding of tRNA<sup>Glu</sup> to GluRS suggests that Arc1p can also modulate the aminoacylation efficiency of GluRS. To test this, we performed aminoacylation assays using as enzyme source monomeric His<sub>8</sub>-GluRS or equimolar amounts of the pre-formed Arc1p-His<sub>8</sub>-GluRS complex. As shown in Fig. 8, the aminoacylation activity of the complex was more than 2-fold higher than that of monomeric His<sub>8</sub>-GluRS. When instead of using the pre-formed complex, Arc1p was simply mixed with His<sub>8</sub>-GluRS before the assay, a stimulation of the activity was also observed. However, this stimulation was lower, probably due to the fact that not all of His<sub>8</sub>-GluRS associated with Arc1p under the conditions of the assay. From these results we can conclude that Arc1p can stimulate the catalytic efficiency of both the aminoacyl-tRNA synthetases it associates with.

The Ternary Arc1p-MetRS-GluRS Complex Can Select and Bind Efficiently to Both Cognate tRNAs—We have shown so far that the binary Arc1p-MetRS and Arc1p-GluRS complexes are capable of interacting with their corresponding cognate tRNAs more efficiently than the respective monomeric enzymes. We then tested if this is also true for the Arc1p-MetRS-GluRS ternary complex. As shown in Fig. 9, incubation of the ternary complex with total yeast tRNA followed by gel filtration resulted in a dramatic shift of both elongator tRNA<sub>Met</sub> and tRNA<sup>Glu</sup> from the pool of the free tRNA to the fractions containing the protein complex. This suggests a very efficient tRNA selection and binding. The initiator tRNA<sub>Met</sub>, in contrast, was only very weakly bound, whereas the noncognate tRNA<sup>Glu</sup> was completely absent from the fractions containing the complex. The exact comigration of the two tRNAs that bind to the complex might suggest that two tRNA molecules, one for Glu and the other for Met, can simultaneously bind to the ternary complex in an Arc1p-dependent way. However, because the binding of the tRNAs does not cause a significant shift in the molecular mass of the complex (see Table I), it is also possible that two comigrating complex populations are formed, one containing only tRNA<sub>Met</sub> and the other only tRNA<sup>Glu</sup>. In either case, these results show clearly that association of the two enzymes with Arc1p offers them a strong advantage in terms of specific tRNA binding.

**DISCUSSION**

Our previous identification of a stable in vivo complex between Arc1p and two aminoacyl-tRNA synthetases in yeast, GluRS and MetRS (26, 28), and its implication in both aminoacylation and tRNA intracellular transport raised a number of questions. Is this complex important for the folding and stability of its protein components? Are other factors such as protein chaperones and tRNA required for the efficient assembly of the complex? How can the apparent nonspecific tRNA binding properties of Arc1p be reconciled with the highly specific synthetase-cognate tRNA interaction? Finally, how does association with Arc1p affect the activity of the enzymes? To address these questions, we first had to purify the individual components, Arc1p, MetRS, and GluRS. Arc1p was produced in *E. coli* (28). The purification of yeast GluRS, which has never been tried successfully before, was achieved by combining epitope
In our results that Arc1p or even its N-terminal domain alone can associate readily with either or both of GluRS- and MetRS-forming stable binary or ternary complexes, respectively. Furthermore, we have shown that under competition conditions, when Arc1p is challenged with a mixture of different tRNAs, it exhibits a certain degree of specificity and binds strongly to a limited number of tRNA species. Therefore, the presence of Arc1p in the complex may mediate the pre-selection of a certain class of tRNA species with common sequence elements, thus favoring the final selection of the cognate tRNAs by the synthetases. Indeed, our binding experiments have shown that only the cognate tRNAs can bind tightly to the enzymes when in complex with Arc1p, so their binding specificity is not at all compromised while their binding affinity is significantly enhanced. This cooperation between Arc1p and the synthetases in tRNA binding may not only be due to the addition of the corresponding affinities but may also reflect the stabilization of favorable conformational states upon complex formation. In either case, the properties of Arc1p demonstrate a general principle that has recently emerged in the field of aminoacyl-tRNA synthetases: nonspecific tRNA binding domains (that recognize structural features of the tRNA) can facilitate the specific interactions between a synthetase and the cognate tRNA.

The function of these, usually nonessential, nonspecific tRNA binding domains has been established in a number of cases. Yeast glutaminyl-tRNA synthetase contains an N-terminally appended noncatalytic domain that has general RNA binding properties (14, 15). Fusion of this sequence to the E. coli glutaminyl-tRNA synthetase, which lacks a similar domain, renders the bacterial enzyme capable of substituting yeast glutaminyl-tRNA synthetase both in vitro and in vivo (13). Yeast aspartyl-tRNA synthetase also contains an N-terminal extension that can bind to tRNA (16). This domain is not found in the prokaryotic homologue but is conserved in other eukaryotic class IIB synthetases. In the mammalian bifunctional glutamylprolyl synthetase, the linker sequence between the two catalytic domains contains repeats of a 50-amino acid long motif, which is also conserved in the appended domains of six other metazoan synthetases (12). This motif forms an antiparallel coiled-coil and has general RNA binding capacity (12). In all these cases, the nonspecific RNA binding domains always serve as cis-acting cofactors since they are part of the same polypeptide as the catalytic core. This is in contrast to the TRBD of Arc1p that appears to act only in trans in yeast, although it may act both in trans (as part of the noncatalytic multisynthetase complex component p43) or in cis (connected to the catalytic domain of TyrRS) in human.

A reason for this inter-molecular (trans) mode of action may simply be economy; Arc1p as part of the Arc1p-MetRS-GluRS complex can simultaneously “serve” two synthetases, whereas p43 is in contact with almost all the enzymes of the multisynthetase complex (42) and can potentially modulate the activities of several of them. Although MetRS and GluRS were the only enzymes that could be coisolated with Arc1p by affinity purification (26), it is still possible that Arc1p may interact with additional synthetases, forming weaker and therefore not easily detectable complexes. This could explain the preferential binding of Arc1p not only to tRNA\(^{\text{Glu}}\) and tRNA\(^{\text{Met}}\) but also to tRNA\(^{\text{Phe}}\), tRNA\(^{\text{Leu}}\), and tRNA\(^{\text{Arg}}\) (Fig. 2A). When the tRNA sequences are compared, a combination of bases can be found that is unique for these five tRNAs (shown in Fig. 2B). Most of these elements are located close to the central core of the tRNA structure and, together with the invariant bases, may constitute positive determinants for the interaction with Arc1p, which requires the L-shape of the tRNA (26).
It is not clear from our data if Arc1p is a monomer in solution or if it forms dimers. Our gel filtration experiments show an aberrant migration of recombinant Arc1p, which is clearly due to the presence of the middle (M) domain (Table I). The most likely explanation is that this domain triggers dimerization of Arc1p. However, we were not able to detect an Arc1p homodimer in vivo when a protein A-tagged version of Arc1p was expressed in yeast cells containing also endogenous Arc1p.² It may also be possible that the largely unstructured and positively charged M domain (26), which lies between the two

² G. Simos, unpublished observations.
globular N and C domains, causes the Arc1p monomer to display a larger Stokes radius that predicted. Another interesting possibility that could reconcile our data is that when Arc1p is alone in solution, a homodimer is formed, which, however, dissociates upon binding of tRNA or the aminoacyl-tRNA synthetases. Indeed, the Arc1p-tRNA complex displays a similar apparent molecular mass as Arc1p (Fig. 1B), and the Arc1p-synthetase complexes are stoichiometric. Certainly, additional biophysical experiments such as sedimentation or light-scattering analyses are required to establish unequivocally the monomeric or dimeric nature of recombinant Arc1p in solution.

Arc1p not only affects the binding affinity of MetRS and GluRS for their cognate tRNAs but also modulates their catalytic efficiency. We have previously shown that when Arc1p is bound to MetRS, it increases almost 500-fold its catalytic efficiency. We have previously shown that when Arc1p is bound to MetRS, it increases almost 500-fold its catalytic efficiency. We have previously shown that when Arc1p is bound to MetRS, it increases almost 500-fold its catalytic efficiency. We have previously shown that when Arc1p is bound to MetRS, it increases almost 500-fold its catalytic efficiency. We have previously shown that when Arc1p is bound to MetRS, it increases almost 500-fold its catalytic efficiency. We have previously shown that when Arc1p is bound to MetRS, it increases almost 500-fold its catalytic efficiency. We have previously shown that when Arc1p is bound to MetRS, it increases almost 500-fold its catalytic efficiency. We have previously shown that when Arc1p is bound to MetRS, it increases almost 500-fold its catalytic efficiency. We have previously shown that when Arc1p is bound to MetRS, it increases almost 500-fold its catalytic efficiency. We have previously shown that when Arc1p is bound to MetRS, it increases almost 500-fold its catalytic efficiency.

Therefore, both enzymes that associate with Arc1p in yeast obtain a catalytic advantage. An additional feature of the TRBD of Arc1p is that it can remain functional when it is artificially transplanted to the catalytic domain of a synthetase other than MetRS or GluRS. Previous experiments show that the fusion of the TRBD to the E. coli glutaminyl-tRNA synthetase allows the enzyme to aminoacylate the heterologous yeast tRNA<sup>Glu</sup><sub>His</sub>, whereas fusion of the TRBD to a fragment of E. coli alanine-tRNA synthetase AlaRS stimulates its activity toward a micro-helix (14, 43). In both these cases, the fusion proteins must be exploiting the general RNA binding properties of the TRBD.

Taking into account the results discussed above, the following question can be raised. Why do the eukaryotic aminoacyl-tRNA synthetases need the higher affinity for tRNA, which is provided by the cis- or trans-acting nonspecific tRNA binding domains? The answer to this question may lie in the fact that the eukaryotic cell is highly compartmentalized. Recent observations indicate that aminoacyl-tRNA synthetases may also be found inside the nucleus where they charge the newly synthesized mature tRNAs (35, 44). Indeed, aminoacylation of tRNAs facilitates their nuclear export both in yeast and higher eukaryotes (37, 38, 44). Experiments based on fluorescent in situ hybridization show that the concentration of mature tRNAs inside the yeast nucleus is significantly lower than in the cytoplasm (37, 47). Therefore, the nuclear aminoacyl-tRNA synthetases have to work efficiently with low concentrations of tRNA, and in this case, a high tRNA affinity becomes indispensable. The additional affinity for tRNA may actually also be required to facilitate the release of mature tRNA from the nuclear tRNA-processing and modification machinery and, thus, enhance the efficiency of transport into the cytoplasm (48). Therefore, it is not by chance that Arc1p was originally identified by its strong genetic interaction with Los1p, a yeast nuclear tRNA export factor (49, 50). Even in the cytoplasm, the concentration of free tRNA is thought to be very low, as tRNAs are “channeled” between the components of the translation machinery and do not simply dissociate and diffuse between ribosomes, aminoacyl-tRNA synthetases, and translation factors (51, 52). Therefore, the importance of the inter- or even intramolecular association of aminoacyl-tRNA synthetases with protein domains that increase tRNA affinity may be understood by in vitro experiments and only become vital for efficient aminoacylation as well as transport of tRNA in vivo.
Arc1p Organizes the Yeast Aminoacyl-tRNA Synthetase Complex and Stabilizes Its Interaction with the Cognate tRNAs
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