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Catalytic Peptide of Human Glutaminyl-tRNA Synthetase Is Essential for Its Assembly to the Aminoacyl-tRNA Synthetase Complex*

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Human glutaminyl-tRNA synthetase (QRS) is one of several mammalian aminoacyl-tRNA synthetases (ARSs) that form a macromolecular protein complex. To understand the mechanism of QRS targeting to the multi-ARS complex, we analyzed both exogenous and endogenous QRSs by immunoprecipitation after overexpression of various Myc-tagged QRS mutants in human embryonic kidney 293 cells. Whereas a deletion mutant containing only the catalytic domain (QRS-C) was targeted to the multi-ARS complex, a mutant QRS containing only the N-terminal appended domain (QRS-N) was not. Deletion mapping showed that the ATP-binding Rossman fold was necessary for targeting of QRS to the multi-ARS complex. Furthermore, exogenous Myc-tagged QRS-C was co-immunoprecipitated with endogenous QRS. Since glutaminylation of tRNA was dramatically increased in cells transfected with the full-length QRS, but not with either QRS-C or QRS-N, both the QRS catalytic domain and the N-terminal appended domain were required for full aminoacylation activity. When QRS-C was overexpressed, arginyl-tRNA synthetase and p43 were released from the multi-ARS complex along with endogenous QRS, suggesting that the N-terminal appendix of QRS is required to keep arginyl-tRNA synthetase and p43 within the complex. Thus, the eukaryote-specific N-terminal appendix of QRS appears to stabilize the association of other components in the multi-ARS complex, whereas the C-terminal catalytic domain is necessary for QRS association with the multi-ARS complex.

Aminoacyl-tRNA synthetases (ARSs)[1] catalyze the ligation of a specific amino acid to its cognate tRNA, thereby ensuring the faithful translation of genetic code. Although catalytic domains from homologous synthetases are highly conserved from bacteria to mammals, mammalian ARSs have acquired unique peptide appendices that are not present in the corresponding prokaryotic enzymes (1–3). Eight of the mammalian ARSs are found in a high molecular weight multi-ARS complex with three noncatalytic proteins, p43, p38, and p18, whereas the corresponding bacterial ARSs do not form such a complex (1–4). This suggests that the peptide appendices might be involved in protein-protein interactions within the multi-ARS complex. Furthermore, intact rat aspartyl-tRNA synthetase (DRS) associates *in vivo* with the multi-ARS complex, whereas the N-terminal appendix-deleted form does not (5), indicating that the appended domain is indispensable for targeting DRS to this complex. Protein-protein interactions between the appendices of various ARSs have previously been shown using the yeast two-hybrid system (6). Similarly, p43 associates with the N-terminal appendix of human arginyl-tRNA synthetase (RRS), stimulating its aminoacylation activity (7).

In addition to a role in protein-protein interactions, ARS appended domains could also enhance aminoacylation activity by recruiting tRNAs. Previously it has been shown that the N-terminal appended domain of yeast QRS nonspecifically binds to both double- and single-stranded RNA (8). Fusion of this domain to *Escherichia coli* QRS confers the ability to aminoacylate yeast tRNA (9). Similarly, a tandemly repeated appendix of EPRS associates with various nucleic acids including RNA, tRNA, DNA, and minihelix (10, 11). Therefore, nonspecific RNA-protein interactions via the appended domain might increase aminoacylation efficiency.

Since chaotropic salts and detergents dissociate the components of the multi-ARS complex, it has been suggested that hydrophobic interactions between the protein components are responsible for its assembly (12–15). Despite extensive studies, the molecular architecture and physiological significance of the multi-ARS complex remain elusive. Although protein-protein interactions between the appended domains of the ARSs have been shown, it is not clear whether these interactions are essential for the assembly of the multi-ARS complex. Thus, we have investigated the role of QRS in the molecular mechanism of assembly of the multi-ARS complex. We demonstrate that the C-terminal catalytic domain of QRS plays a major role in promoting its association with the multi-ARS complex. Furthermore, the N-terminal appendix of QRS is necessary for the association of RRS and p43 to the multi-ARS complex and is indispensable for QRS aminoacylation activity.

EXPERIMENTAL PROCEDURES

Preparation of Antibodies—MRS, and IRS (denatured full-length), YRS, WRS, p43, and p38 (native full-length), EPRS (the native peptide of three repeats), LRS (denatured peptide of C-terminal 236 amino acids), QRS (native N-terminal 236 amino acids), and RRS (native N-terminal 72 amino acids) were overexpressed as His-tagged proteins using *E. coli* and then purified using nickel affinity chromatography following the manufacturer's protocol (Invitrogen). Rabbit polyclonal antibodies were then raised against these proteins as described previ-

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[1] The abbreviations used are: ARS, aminoacyl-tRNA synthetase; *X*RS, ARS for amino acid *X*; EV, empty vector.

ously (7). The IgG from each antiserum was purified by protein A affinity chromatography according to the manufacturer's protocol (Bio-Rad), and the antibody specificity was confirmed by immunoblotting (data not shown). Anti-Myc antibody was purchased from Roche Molecular Biochemicals.

Construction of QRS Deletion Plasmids—The cDNAs encoding the full-length, N-terminal 236 amino acids (QRS-N) and C-terminal 539 amino acids (QRS-C) of QRS were generated using polymerase chain reaction from pM191 (Dr. K. Shiba, Cancer Institute, Tokyo, Japan) and ligated into pcDNA3 (Invitrogen) containing Myc and FLAG tags using the *Eco*RI and *Not*I sites. The full-length QRS in pcDNA3 (pcDNA3-QRS-F) was cleaved with *Eco*RI and re-ligated to generate the DNA encoding the C-terminal 318 amino acids (QRS-(458–775)). pcDNA3-QRS-F was digested with *Xba*I (partial digestion) or *Apa*I and re-ligated to generate the DNAs encoding QRS-(1–552) and QRS-(1–653), respectively. QRS-(Δ380–568) and QRS-(Δ179–653) were generated by re-ligation after digestion of pcDNA3-QRS-F with *Nco*I and *Apa*I, respectively. Finally, QRS-(237–458) and QRS-(237–552) were constructed by intra-ligation of the larger fragments after digestion of pcDNA3-QRS-C with *Eco*RI and *Xba*I, respectively.

Cell Culture, DNA Transfection, and Immunoprecipitation—Human embryonic kidney 293 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 50 μg/ml penicillin and streptomycin in a 5% CO_2 incubator. 100-mm dishes of 293 cells were transfected with 4 μg of the indicated Myc-tagged pcDNA3-QRS plasmid using Geneporter (Gene Therapy Systems) according to the manufacturer's protocol. Twenty four hours after transfection, cells were washed twice with cold phosphate-buffered saline, lysed with lysis buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 5 mM EGTA, 1% Nonidet P-40, 10% glycerol, 2 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and 5 μg/ml aprotinin) for 30 min, and microcentrifuged (14,000 rpm, 4 °C, 10 min). The lysate (1 mg of protein) was incubated with anti-Myc antibody (5 μg) and protein A-agarose (50 μl) for 4 h. Samples were washed four times with lysis buffer and then analyzed by SDS-polyacrylamide gel electrophoresis.

Electrophoresis and Immunoblotting—Proteins were separated using SDS-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane. The membrane was blocked using 5% nonfat dry milk in Tris-buffered saline. The ARS complex and Myc-tagged QRS derivatives were visualized by sequential treatment with specific antibodies, horseradish peroxidase-conjugated secondary antibodies, and an enhanced chemiluminescence (ECL) substrate kit (Amersham Pharmacia Biotech).

Aminoacylation Assay—293 cells transfected with the indicated QRS expression vector or empty vector were harvested and washed twice with ice-cold phosphate-buffered saline. Cells were resuspended in a hypotonic buffer plus protease inhibitors (20 mM HEPES, pH 7.5, 10 mM KCl, 10 mM β-mercaptoethanol, 10% glycerol, 0.1 mM EDTA, 10 mM β-glycerophosphate 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin, 5 μg/ml chymostatin A, 5 μg/ml aprotinin, and 5 μg/ml L-1-tosylamido-2-phenylethyl chloromethyl ketone). Resuspended cells were lysed by gentle sonication and centrifuged at 33,000 × *g* for 20 min, at 4 °C. Protein concentration was determined by Bradford assay. Twenty micrograms of cell lysate was mixed with reaction buffer (50 mM HEPES, pH 7.5, 20 mM KCl, 25 mM $MgCl_2$, 20 mM β-mercaptoethanol, 0.2 mg/ml bovine serum albumin, 5 mM ATP, and 0.12 μCi/μl [^{3}H]glutamine), and the reaction was initiated by adding bovine liver total tRNA (0.4 μg/μl). Reaction samples were taken at 1.5-min intervals and spotted on filter discs presoaked with 5% trichloroacetic acid. After 1 min, the filter discs were added to ice-cold 5% trichloroacetic acid and washed three times with fresh 5% trichloroacetic acid at 4 °C. The filter discs were dehydrated by incubation with 95% ethanol at 4 °C, and the radioactivity adsorbed to the filters was quantitated by liquid scintillation counting.

Gel Filtration Chromatography—Cell lysate (5 mg of total protein) in hypotonic buffer was loaded into a Superdex 200 HR column (exclusion limit of 1300 kDa) using AKTA-fast protein liquid chromatography (Amersham Pharmacia Biotech) and eluted at the flow rate of 0.2 ml/min. 50 μl of each fraction (0.3 ml) was analyzed by immunoblotting using antibodies against Myc, EPRS, IRS, LRS, MRS, QRS, p43, and p38 as indicated.

RESULTS

QRS Catalytic Domain Alone Was Incorporated to the Multi-ARS Complex—We compared QRS sequences from *E. coli*, yeast, and human in an attempt to identify a domain of QRS that would target it to the multi-ARS complex. As shown in Fig. 1, human QRS contains a unique N-terminal appendix (amino acids from 1 to 236) that is not present in prokaryotes but conserved in eukaryotic organisms such as *Saccharomyces cerevisiae, Caenorhabditis elegans, and Drosophila melanogaster*. Although the N-terminal appendix of human QRS interacts with RRS, IRS, and MRS (6), it is not known whether this domain is a determinant for targeting QRS to the multi-ARS complex.

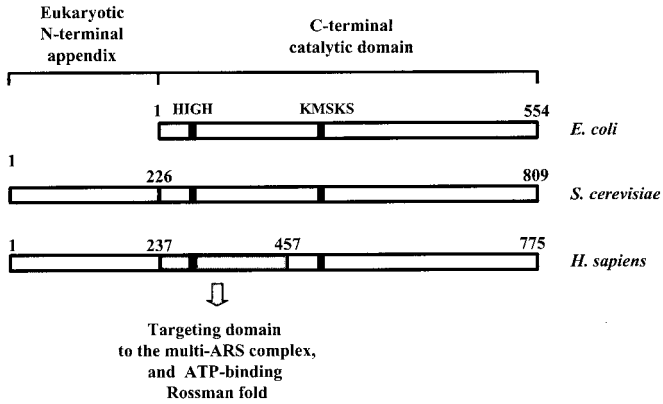

FIG. 1. **A schematic diagram of QRS from *E. coli*, *S. cerevisiae*, and *Homo sapiens*.** Although the active site-containing core of the eukaryotic enzymes is highly homologous to *E. coli* counterpart, each has an extra domain at its N terminus. The appended domain of human QRS is required for RRS and p43 to be incorporated into the multi-ARS complex, whereas its catalytic domain drives QRS targeting to the complex. A *gray box* represents the domain of QRS responsible for targeting it to the multi-ARS complex. *Thick lines* represent two sequence motifs, HIGH and KMSKS, that are specifically found in tRNA synthetase class I enzymes.

In order to map the domain of QRS targeting it to the multi-ARS complex, we constructed various Myc epitope-tagged deletion mutants of QRS. The mutant QRS constructs used in this study are shown schematically in Fig. 2*A*. QRS-N and -C represent the appended or catalytic domain, respectively. The constructs were transiently overexpressed in 293 cells, and cell lysates were immunoprecipitated with anti-Myc antibody. The precipitated complex was immunoblotted using a mixture of antibodies specific to EPRS, LRS, MRS, QRS, RRS, YRS, WRS, and p43. Fig. 2*B* shows that QRS-F and -C were co-immunoprecipitated with the EPRS, LRS, MRS, RRS, and p43 components of the multi-ARS complex, whereas QRS-N was not. Cells were then transiently transfected with expression vectors for Myc-GRS and -CRS, which are known not to be associated with the multi-ARS complex (1). As above, lysates were immunoprecipitated with anti-Myc antibody and then immunoblotted with the antibody mixture. No components of the multi-ARS complex were detected in association with either Myc-GRS or -CRS, thus confirming the specificity of multi-ARS complex interactions with QRS-F and -C. In addition, Amido Black staining of the membrane also confirmed that the immunoprecipitates of QRS-F and QRS-C contained bands corresponding to EPRS, IRS, LRS, MRS, RRS, and p43, whereas QRS-N did not (Fig. 2*C*). Interestingly, QRS could be present as an oligomer in the multi-ARS complex since QRS-C also co-immunoprecipitated endogenous QRS (endogenous QRS is indicated with an *arrowhead* in Fig. 2, *B* and *C*). These data suggest that QRS-C and endogenous QRS could co-exist in the multi-ARS complex.

In order to map the region of QRS-C interacting with the multi-ARS complex, different Myc-tagged QRS derivatives were transiently expressed and immunoprecipitated with anti-Myc. As shown in Fig. 3, QRS-(1–552) and QRS-(1–653) were co-immunoprecipitated with the multi-ARS complex, but QRS-(458–775) was not, suggesting that the region from amino acids

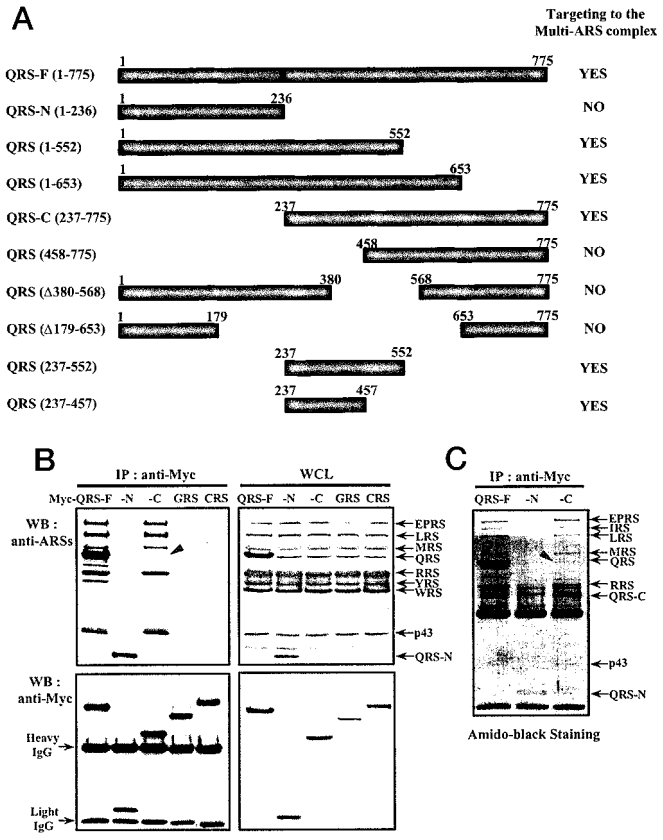

FIG. 2. **QRS-C binds to the multi-ARS complex.** *A,* schematic representation of Myc-tagged QRS mutant proteins. Interaction with the multi-ARS complex when transfected into 293 cells is indicated. *YES* indicates interaction with the multi-ARS complex, and *NO* indicates lack of interaction. *B,* interaction of Myc-tagged QRS-F and QRS-C with the multi-ARS complex. Plasmid pcDNA3-Myc-QRS-F, -N, or -C was transiently transfected into 293 cells. Lysates were immunoprecipitated (*IP*) with anti-Myc antibody and immunoblotted (*WB*) with mixture of antibodies raised against EPRS, LRS, MRS, QRS, YRS, WRS, and p43 (*left panel*). An *arrowhead* indicates endogenous QRS. *Right panel* shows immunoblotting of whole cell lysate (*WCL*). *C,* Amido Black staining of the membrane that was used in *B*. An *arrowhead* indicates endogenous QRS.

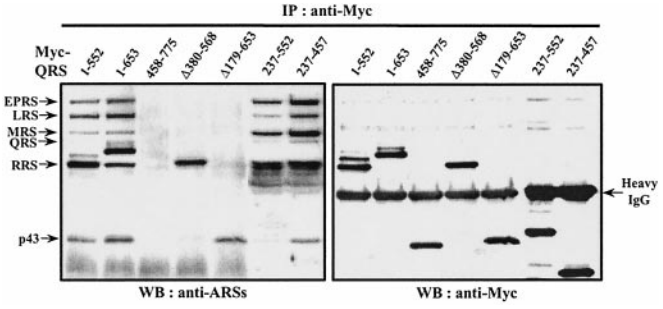

FIG. 3. **The Rossman fold of QRS is needed to target QRS to the multi-ARS complex.** Each indicated Myc-tagged QRS deletion mutant was transfected into 293 cells, the immunoprecipitated and immunoblotted as described in Fig. 2*B*. QRS-(1–552) and QRS-(1–653) were targeted to the multi-ARS complex, and QRS-(458–775), QRS-(Δ380–568), and QRS-(Δ179–653) were not. QRS-(237–552) and QRS-(237–457) were targeted to the multi-ARS complex, showing that the region from amino acids 237 to 457 is necessary for QRS targeting to the multi-ARS complex.

237–457 is necessary for QRS targeting to the multi-ARS complex. Furthermore, the QRS derivatives, QRS-(Δ380–568) and QRS-(Δ179–653), which also do not contain amino acids 380–568, were not targeted to the multi-ARS complex. Indeed, QRS deletion mutants containing the amino acid regions 237–457

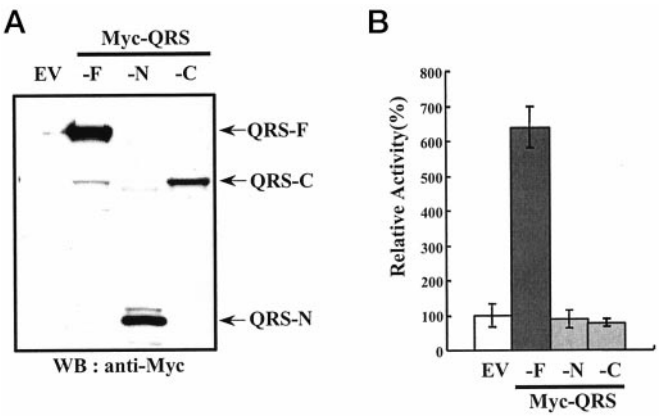

FIG. 4. **Aminoacylation activity of cell extracts overexpressing QRS-F, QRS-N, and QRS-C.** QRS-F, QRS-N, or QRS-C was transiently transfected into 293 cells as described in Fig. 2. *A,* immunoblotting (*WB*) with anti-Myc. Cells were immunoblotted with anti-Myc as in Fig. 2 to confirm protein expression. *B,* aminoacylation activity of QRS mutants. After lysis in hypotonic buffer, cell lysates were assayed for glutaminylation activity as described under "Experimental Procedures."

and 237–552 were all incorporated into the multi-ARS complex (Fig. 3). These mapping data demonstrate that the region of QRS from amino acids 237 to 457 is a domain needed to target QRS to the multi-ARS complex. This region is an ATP-binding domain that is called Rossman fold (16). Thus, our data indicate that the Rossman fold is necessary for protein-protein interaction within the multi-ARS complex.

QRS Appended Domain Is Essential for Aminoacylation Activity—Since QRS-C itself was sufficient for the association with the multi-ARS complex, we asked whether this domain alone could catalyze aminoacylation of $tRNA^{Gln}$. To address this issue, we transfected QRS-F, QRS-N, and QRS-C into 293 cells and measured the cytoplasmic aminoacylation activity. As shown in Fig. 4, the activity in the QRS-F transfected cells was about 6.5-fold that of cells transfected with empty vector (EV), whereas no increase was observed in cells transfected with QRS-N or QRS-C. This result indicates that QRS-C has very little, if any, aminoacylation activity, and the QRS N-terminal appendix is also required for the enzymatic activity of QRS.

QRS Appended Domain Is Required for Association of Other Components in the Multi-ARS Complex—The incorporation of QRS-C into the multi-ARS complex was also confirmed by gel filtration chromatography using fast protein liquid chromatography (Fig. 5). First, each fraction was analyzed by immunoblotting with anti-QRS and anti-Myc antibodies. Endogenous QRS from the cells transfected with EV was eluted near the exclusion limit in association with the multi-ARS complex (see fractions 24–26) (Fig. 5*A*). QRS in QRS-F-transfected cells showed an additional free, non-complexed form, well separated from the multi-ARS complex in fractions 38–48 (Fig. 5*A*). Because QRS-F was identified in fractions corresponding to both the multi-ARS complex and the free form when immunoblotted with anti-Myc antibody, the overexpressed QRS-F was thought to be largely incorporated into the multi-ARS complex. Like the profile of endogenous QRS protein, its enzymatic activity was also found in both the free form as well as in the multi-ARS complex (Fig. 5*B*), suggesting that association with the multi-ARS complex is not essential for the catalytic activity of QRS.

When cells transfected with QRS-C were analyzed for both QRS-C and endogenous QRS using immunoblotting with anti-Myc and anti-QRS antibodies after gel filtration, we found that QRS-C was targeted to the multi-ARS complex (Fig. 5*A*), consistent with the result of co-immunoprecipitation experiment in Fig. 2. Interestingly, a portion of endogenous QRS was also present in the free form, as well as the multi-ARS complex,

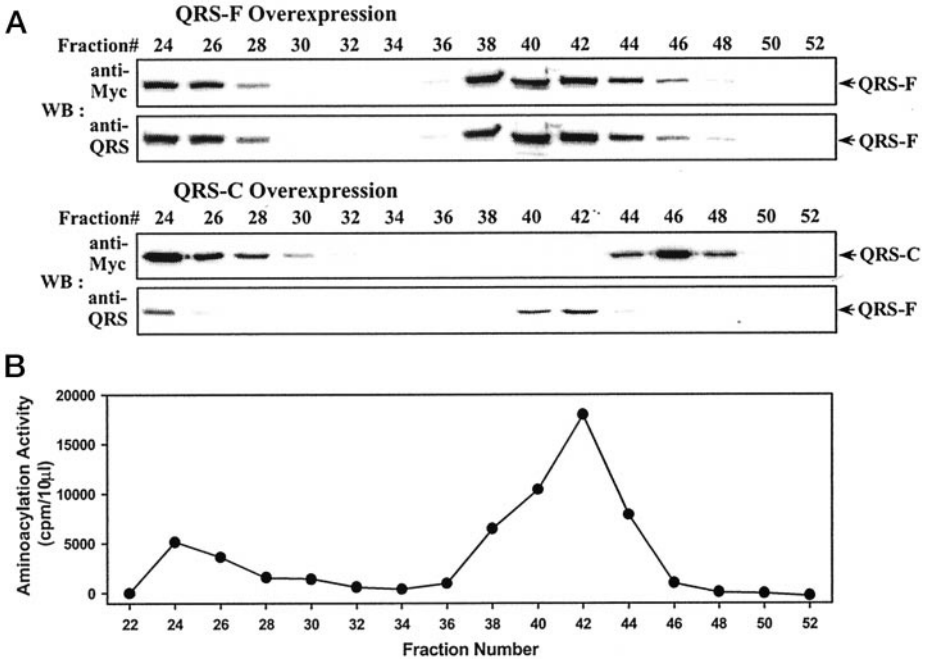

FIG. 5. **Myc-tagged QRS-F and QRS-C are incorporated to the multi-ARS complex.** *A,* the lysates (5 mg) from QRS-F- or QRS-C-transfected cells were separated on a Superset 200 HR column as described under "Experimental Procedures" and immunoblotted (*WB*) with anti-Myc and anti-QRS antibodies. Fractions *24–26* represent the void volume containing the multi-ARS complex, whereas fractions *38–48* contain free QRS. *B,* aminoacylation activity of QRS from each fraction after gel filtration of QRS-F-transfected cell extracts was measured as in Fig. 4.

suggesting that QRS-C replaces endogenous QRS in the multi-ARS complex. Since QRS-C did not change the aminoacylation activity of endogenous QRS (Fig. 4), the free endogenous QRS still appears to have enzymatic activity.

The absence of the QRS N-terminal appendix in the multi-ARS complex could disrupt the architecture of multi-ARS complex since the appended domain of QRS interacts with other ARSs such as IRS, RRS, and MRS (6). Thus, the removal of this domain may destabilize the association of the related components. To address this possibility, we analyzed each fraction in Fig. 6 with anti-EPRS, IRS, LRS, MRS, RRS, p43, and p38 after gel filtration. As shown in Fig. 6*A*, except for RRS, EV-transfected cells did not show any free form of other multi-ARS components including EPRS, IRS, LRS, MRS, p43, and p38, suggesting that DNA transfection itself does not seriously change the organization of the complex. It should be noted that RRS has been previously reported to exist as a truncated free form as well as in a multi-ARS complex form (17). Similarly, QRS-F-overexpressed cells also showed no free form of EPRS, IRS, LRS, MRS, p43, and p38 (data not shown). However, the incorporation of QRS-C into the complex caused the release of both RRS and p43 (Fig. 6*B*), suggesting that the absence of QRS N-terminal appendix in the multi-ARS complex disrupts the association of RRS and p43 within the complex. On the other hand, EPRS, IRS, LRS, MRS, and p38 were not released to the free forms from the multi-ARS complex, suggesting that these components were not affected by the absence of the QRS N-terminal appendix. Thus, we conclude that the catalytic domain of QRS is required for targeting QRS to the multi-ARS complex**,** although its appended domain is necessary for the stabilization of RRS and p43 within the multi-ARS complex.

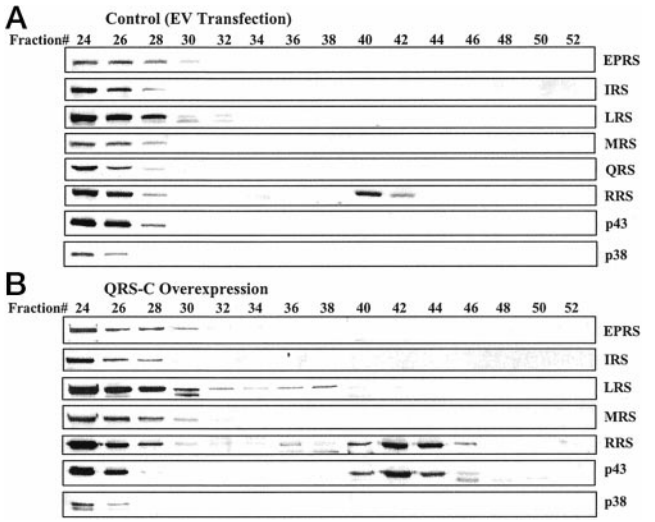

FIG. 6. **RRS and p43 are released from the multi-ARS complex and present in the free form in QRS-C-overexpressing cells.** Each fraction shown in Fig. 5 was further analyzed by immunoblotting with mixture of antibodies raised against the components of the multi-ARS complex. *A,* EV-transfected cells; *B,* QRS-C-transfected cells.

DISCUSSION

We have previously mapped the protein-protein interactions between various peptide domains of the ARSs that are components of the multi-ARS complex (6). However, it is not known whether these interactions are sufficient to determine the incorporation of each component into the multi-ARS complex. Here, we investigated the importance of various regions of QRS in the molecular organization of the multi-ARS complex by constructing different Myc-tagged derivatives of QRS, transfecting them into 293 cells, then immunoprecipitating these QRS derivatives with anti-Myc, and immunoblotting with antibodies against numerous components of the multi-ARS complex. In addition, we analyzed the multi-ARS complex using gel filtration of cell extracts overexpressing QRS-F or QRS-C. These *in vivo* experiments yielded several unexpected results that suggest important insights into the assembly of the multi-ARS complex.

The targeting domain of QRS to the multi-ARS complex is the Rossman fold that is an ATP-binding site (amino acids 380–457). It was previously demonstrated that the catalytic domain of human YRS binds to the interleukin-8 type A receptor and functions as an interleukin-8-like cytokine (18), indicating that the catalytic domains of ARSs could be involved in protein-protein interaction. Our results also indicate that there may be more than one QRS molecule in the multi-ARS complex. As shown in Fig. 2, endogenous QRS was also co-immunoprecipitated with Myc-tagged QRS-C, suggesting that at least two or more QRS molecules are present in a multi-ARS complex

contrary to the previous results in which QRS is found as a monomer within a multi-ARS complex (2, 3).

We also show that the QRS N-terminal appendix is required for enzymatic activity of QRS (Fig. 4). In many other ARSs, this domain is dispensable for cell survival and aminoacylation activity. For example, *S. cerevisiae* QRS does not require its extended domain for either cell survival or aminoacylation (19). In addition, human DRS and YRS express aminoacylation activity without their extended N-terminal domain (18, 20). However, the complete removal of N-terminal appendix in yeast MRS affects the aminoacylation activity of this enzyme (21). Similarly, the removal of the C-terminal appendix in SRS reduces both the stability of this enzyme and its affinity for substrate (22). Our data show that enzymatic activity of QRS requires the appended domain and thus implies that the appendices from some eukaryotic ARSs could play important roles in either enzyme stability or activity.

Gel filtration chromatography revealed that the free form of QRS in cells overexpressing QRS-F and QRS-C still possessed aminoacylation activity, suggesting that association with the multi-ARS complex is not essential for enzyme catalysis (Fig. 5). Similarly, human DRS overexpressed in COS cells had aminoacylation activity when not associated with the multi-ARS complex (23). Thus, inclusion in the multi-ARS complex does not appear to be essential for the catalytic activities of several ARSs.

The appended domain of QRS was responsible for targeting RRS and p43 into the multi-ARS complex (Fig. 6). When QRS-C was overexpressed, it was incorporated into the multi-ARS complex, whereas endogenous QRS, RRS, and p43 were removed from this complex, instead being found in free forms (Fig. 6). Thus, the removal of the N-terminal appendix disrupts molecular interactions among QRS, RRS, and p43 within a multi-ARS complex, releasing RRS and p43. It should be noted that previously yeast two-hybrid analysis has shown molecular association of QRS with RRS (6) and RRS with p43 via the appended domains (7). Since QRS-C overexpression does not affect other ARSs such as EPRS, IRS, LRS, MRS, and p38 (Fig. 6), the appended domain of QRS might have a stabilizing role in maintaining RRS and p43 in the multi-ARS complex.

Taken together, the data of this work suggest that the catalytic domains of the complex-forming ARSs play an important role in protein-protein interactions within the macromolecular ARS complex. This finding poses an interesting question on the architecture of the complex because it is intriguing how the component ARSs would execute their catalytic activities while their catalytic domains are involved in complex association.

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REFERENCES

1. Kisselev, L. L., and Wolfson, A. D. (1994) *Prog. Nucleic Acids Res. Mol. Biol.* **48,** 83–142
2. Mirande, M. (1991) *FEBS Lett.* **283,** 1–3
3. Yang, D. C. H. (1996) *Curr. Top. Cell. Regul.* **34,** 101–136
4. Dang, C. V., and Dang, C. V. (1986) *Biochem. J.* **239,** 249–255
5. Mirande, M., Lazard, M., Martinez, R., and Latreille, M. T. (1992) *Eur. J. Biochem.* **203,** 459–466
6. Rho, S. B., Kim, M. J., Lee, J. S., Seol. W., Montegi, H., Kim, S., and Shiba, K. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 4488–4493
7. Park, S. G., Jung, K. H., Lee, J. S., Jo, Y. J., Motegi, H., Kim, S., Shiba, K. (1999) *J. Biol. Chem.* **274,** 16673–16676
8. Wang C. C., Scimmel, P. (1999) *J. Biol. Chem.* **274,** 16508–16512
9. Whelihan, E. F., and Schimmel, P. (1997) *EMBO J.* **16,** 2968–2974
10. Rho, S. B., Lee, J. S., Jeong, E.-J., Kim, K.-S., Kim, Y. G., and Kim, S. (1998) *J. Biol. Chem.* **273,** 11267–11273
11. Cahuzac, B. B. E., Birlirakis, N., Guittet, E., and Mirande, M. (2000) *EMBO J.* **19,** 445–452
12. Norcum, M. T. (1991) *J. Biol. Chem.* **266,** 15398–15405
13. Sihag, R. K., and Deutscher, M. P. (1983) *J. Biol. Chem.* **258,** 11846–11850
14. Cirakoglu, B., and Waller, J. P. (1985) *Eur. J. Biochem.* **151,** 101–110
15. Deutscher, M. P. (1984) *J. Cell Biol.* **99,** 373–377
16. Freist, W., Gauss, D. H., Ibba, M., and Soll, D. (1997) *Biol. Chem.* **378,** 103–117
17. Sivaram, P., and Deutscher, M. P. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 3665–3669
18. Wakasugi, K., and Schimmel, P. (1999) *Science* **284,** 147–150
19. Ludmerer, S. W., Wright, D. J., and Schimmel, P. (1993) *J. Biol. Chem.* **268,** 5519–5523
20. Reed, V. S., and Yang, D. C. H. (1994) *J. Biol. Chem.* **269,** 32937–32941
21. Walter, P., Weygand-Durasevic, I., Sanni, A., Ebel, J. P., and Fasiolo, F. (1989) *J. Biol. Chem.* **264,** 17126–17130
22. Weygand-Durasevic, I., Lenhard, D., Filipic, S., and Soll, D. (1996) *J. Biol. Chem.* **271,** 2455–2461
23. Escalante, C., Qasba, P. K., and Yang, D. C. H. (1994) *Mol. Cell. Biochem.* **140,** 56–63