Mutational Switching of a Yeast tRNA Synthetase into a Mammalian-like Synthetase Cytokine†

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ABSTRACT: Aminoacyl-tRNA synthetases catalyze the attachment of amino acids to their cognate tRNAs. A link was recently established between protein biosynthesis and cytokine signal transduction. Human tyrosyl-tRNA synthetase can be split into two fragments, each of which has a distinct cytokine function. This activity is specific to the human enzyme. It is absent in the enzymes from lower organisms such as bacteria and yeast. Here, yeast tyrosyl-tRNA synthetase (TyrRS), which lacks cytokine activity, was used as a model to explore how a human tyrosyl-tRNA synthetase during evolution acquired novel functions beyond aminoacylation. We found that a rationally designed mutant yeast TyrRS(ELR) gained cytokine function. The mutant yeast enzyme gained this function without sacrifice of aminoacylation activity. Therefore, relatively simple alteration of a basic structural motif imparts cytokine activity to a tRNA synthetase while preserving its canonical function. Further work established that mutational switching of a yeast protein to a mammalian-like cytokine was specific to this synthetase and not to just any yeast ortholog of a mammalian cytokine.

Aminoacyl-tRNA synthetases catalyze the attachment of amino acids to their cognate tRNAs (*1*−*4*). They are essential for the viability of all organisms in the three kingdoms of life. On the basis of their sequences and the architectures of their catalytic domains, they are divided into two classes, each of which contains 10 members (*5*−*8*). Class I tRNA synthetases have an 11 amino acid signature sequence that ends in HXGH (*5*, *6*) and a KMSKS pentapeptide (*9*). Each are part of a Rossmann fold that binds ATP and the 3′-end of tRNA. The catalytic domain of class II tRNA synthetases contains three highly degenerate sequence motifs, known as motifs 1, 2, and 3 (*7*, *8*), which are part of a structure of alternating β-strands and α-helices. During their long evolution, tRNA synthetases incorporated new domains by insertions into or fusions to the class-defining catalytic core (*10*). For example, certain eukaryotic tRNA synthetases incorporated extra N- or C-terminal appended domains that were not essential for aminoacylation but that acted as nonspecific tRNA-binding platforms (*11*, *12*) or as elements required for assembly of multisynthetase complexes (*13*, *14*).

As a result of the long evolution, the activities of individual tRNA synthetases expanded to include new functions in addition to aminoacylation (*15*, *16*). For example, tRNA synthetases participate in nuclear tRNA export (*17*, *18*), mitochondrial RNA splicing (*19*, *20*), transcriptional and translational regulation control (*21*, *22*), ribosomal RNA maturation (*23*), apoptosis inhibition (*24*), and possibly retrovirus packaging (*25*). Recently, human tyrosyl-tRNA synthetase (TyrRS)[1] and tryptophanyl-tRNA synthetase (TrpRS) were demonstrated to have novel functions in signal transduction pathways (*26*−*30*), thus establishing a link between protein synthesis and cytokine signal transduction in mammalian systems.

Tyrosyl-tRNA synthetase (TyrRS) is a dimeric class I tRNA synthetase (*10*). Compared to its counterparts in bacteria and lower eukaryotes such as yeast, human TyrRS has an appended C-terminal domain similar to the pro-inflammatory cytokine endothelial-monocyte-activating polypeptide II (EMAP II) (*31*). A similar domain also exists as a distinct protein (Trbp111 (*32*)) or as a fused domain in other proteins such as Arc1p and methionyl-tRNA synthetase (MetRS) (*33*, *34*). In human TyrRS, two distinct cytokines, each with distinct activities, are produced by proteolysis (*26*). The N-terminal catalytic fragment (human mini-TyrRS) is active in aminoacylation and functions as a cytokine—similar to interleukin 8 (IL-8)—that induces migration of polymorphonuclear leukocytes (PMNs). The isolated C-domain of human TyrRS is a homologue of the cytokine EMAP II. It has potent chemotaxis activity for PMNs and mononuclear phagocytes (MPs), and stimulates production of myeloperoxidase, tumor necrosis factor-α, and tissue factor (*26*).

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[1] Abbreviations: TyrRS, tyrosyl-tRNA synthetase; PMN, polymorphonuclear leukocyte; MP, mononuclear phagocyte; PCR, polymerase chain reaction; CXC chemokine, chemokine with the first two conserved cysteine residues separated by an intervening amino acid; IL-8, interleukin 8; EMAP II, endothelial-monocyte-activating polypeptide II; FBS, fetal bovine serum; HPF, high power field; IPTG, isopropyl β-D-thiogalactopyranoside; HUVEC, human umbilical vein endothelial cell.

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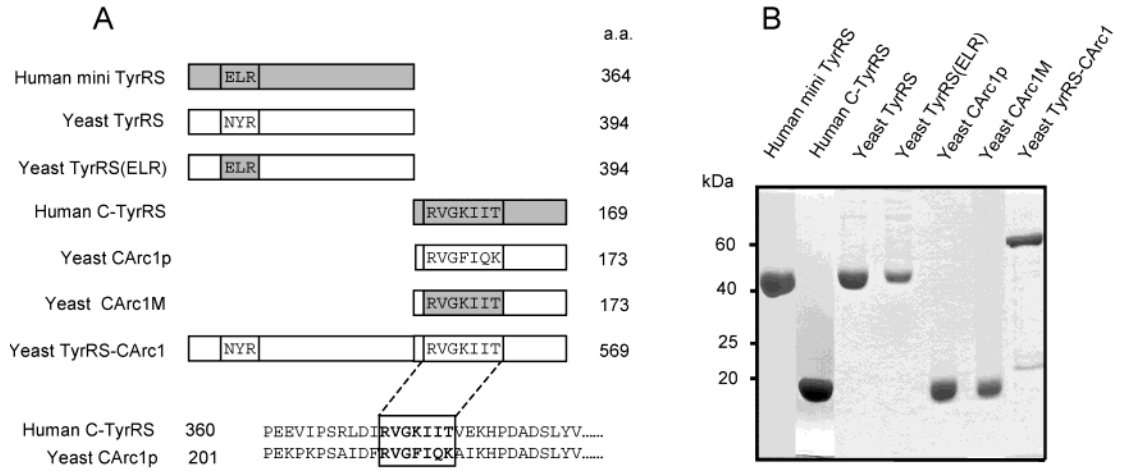

FIGURE 1: Expression and purification of recombinant proteins. **(**A**)** Schematic presentation of constructs used in this study. Numbers at the right indicate the total number of amino acids. Tripeptide motif ELR or NYR and heptapeptide motif RVGFIQK or RVGKIIT are indicated. All constructs were cloned into an *E. coli* expression vector, pET-20b, to produce recombinant proteins with a COOH-terminal His_6-tag upon IPTG induction. **(**B**)** Analysis of purified recombinant proteins on 12% SDS−PAGE. The gel was stained with Coomassie brilliant blue.

All CXC chemokines—such as IL-8—that act as PMN chemoattractants have an ELR motif, which is essential for PMN receptor binding and the activity of PMNs (*35*, *36*). In the case of mini-TyrRS, an ELR motif is found within the Rossmann fold of the catalytic site. The PMN chemoattractant activity of mini-TyrRS is dependent on this ELR motif. Mutation of ELR to ELQ abolished activity for PMN migration (*26*). The ELR motif is conserved among higher eukaryote TyrRSs but is absent from lower organisms. For example, the homologous sequence in yeast *Saccharomyces cerevisiae* is NYR. Although *S. cerevisiae* TyrRS was shown to bind to PMNs, it nonetheless lacked cell signaling activity (*27*). Thus, to further explore the relationship among cell signaling activity, the ELR motif, and the capacity of a tRNA synthetase to incorporate new activities, we manipulated *S. cerevisiae* TyrRS with the aim of making it active as a PMN attractant. At the same time, we recognize that a significant constraint was the maintenance of aminoacylation activity. The idea was to see whether the evolution of cytokine activity could be a straightforward adaptation requiring simple mutations, or whether the broader context of the protein sequence and architecture also needed to change to preserve the canonical function.

EXPERIMENTAL PROCEDURES

Protein Expression and Purification. Plasmids carrying wild-type yeast TyrRS, human mini-TyrRS, or the C-terminal domain of human TyrRS were previously described (*26*). For other constructs used in this study, coding regions of different genes flanked by *Nde*I and *Xho*I restriction sites were amplified by the polymerase chain reaction (PCR) and ligated into the same sites in the bacterial expression vector pET-20b encoding a C-terminal His_6-tag (Novagen, Madison, WI). Mutant yeast TyrRS (TyrRS(ELR)) containing NYR → ELR mutations (N95E/Y96L) and the C-terminal domain of Arc1p (CArc1p) containing RVGFIQK → RVGKIIT exchanges (F215K/Q217I/K218) were generated with a QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). The chimeric protein TyrRS-CArc1p was constructed by inserting the DNA segment encoding CArc1p flanked by the *Xho*I restriction site into the *Xho*I site of pET-TyrRS to give pET-TyrRS-CArc1p.

The recombinant proteins used in this study are schematically illustrated in Figure 1A. Recombinant proteins were expressed in *E. coli* host strain BL21(DE3) grown in LB medium with 100 μg/mL ampicillin. Expression was induced at $A_{600} = 0.6$ by addition of 1 mM isopropyl β-D-thiogalactopyranoside (IPTG) at room temperature or 37 °C for 3 h. The cells were harvested by centrifugation and lysed by French press in a binding buffer (20 mM Tris−HCl (pH 7.9), 500 mM NaCl, 30 mM imidazole) supplemented with 5 mM β-mercaptalethanol and 1 mM PMSF. After centrifugation at 35000*g* for 30 min, the proteins were purified from the clear lysate by Ni−NTA affinity chromatography according to the manufacturer's instructions (Qiagen, Valencia, CA). Some proteins were further purified by ion exchange chromatography (Mono-Q) (Pharmacia Biotech, Piscataway, NJ). All purified proteins were more than 95% pure as judged by 12% SDS−polyacrylamide gel electrophoresis (Figure 1B). Proteins produced in *E. coli* may be contaminated with endotoxin, which derives from cell wall lipopolysaccharide and is highly toxic in vivo (*37*). Endotoxin was removed by phase separation using the detergent Triton X-114 (*38*, *39*) and was determined to be less than 0.01 EU/mg by Limulus Amebocyte Lysate gel-clot assay (Sigma, St. Louis, MO). The protein concentration was determined by Bradford assay (Bio-Rad, Hercules, CA).

RNA Substrates. Yeast total tRNA was purchased from Sigma. Human $tRNA^{Tyr}$ was transcribed in vitro from a linearized plasmid DNA template and processed using a 5′ *cis*-acting hammerhead ribozyme (*40*). This method of transcription was used because the $tRNA^{Tyr}$ gene has an inefficient 5′ end for T7 RNA polymerase (*40*). The plasmid is a derivative of pUC18 (*41*) with an internal promoter of T7 RNA polymerase followed by the hammerhead ribozyme and $tRNA^{Tyr}$ gene cloned into the polylinker site. A Bst N1 site at the 3′ end of the gene allowed generation of a linear template that provided the correct 3′-CCA end in the tRNA by in vitro runoff transcription. The transcription reaction mixture contained 0.1 μg/μL Bst N1 linearized plasmid, 40

mM Tris−HCl (pH 7.9), 24 mM $MgCl_2$, 25 mM NaCl, 10 mM DTT, 3.7 mM ATP, 5.4 mM GTP, 3.7 mM CTP, 3.2 mM UTP (the ratio of nucleoside triphosphates in the reaction was adjusted to correspond to the ratio of corresponding nucleotides in the RNA), 80 units/mL ribonuclease inhibitor, 2 μg/mL yeast pyrophosphatase, and 60 μg/mL T7 RNA polymerase. The reaction was carried out at 37 °C for 4 h. Afterward, the reaction mixture was incubated at 60 °C for 2 h to cleave the $tRNA^{Tyr}$ transcript in *cis* orientation with the hammerhead ribozyme. The $tRNA^{Tyr}$ transcript was then purified by phenol/chloroform extraction, denaturing polyacrylamide gel electrophoresis (12%), electroelution, and ethanol precipitation. The concentration of tRNA was determined by the plateau level of aminoacylation (*42*).

Aminoacylation Assays. The enzyme concentration was determined by Bradford assay and active site titration (*43*). Aminoacylation assays were performed in a 100 μL reaction mixture as described (*44*).

Preparation of Polymorphonuclear Leukocytes (PMNs). Human polymorphonuclear leukocytes were prepared from the whole blood of normal healthy volunteers. Human PMNs were isolated from the blood by centrifugation (700*g*) over Histopaque 1077 and 1119 as described by the manufacturer (Sigma), washed twice with phosphate-buffered saline (PBS), and resuspended in RPMI 1640 (Sigma) containing heat-inactivated fetal bovine serum (0.5%, Sigma) at a concentration of 4×10^5 cells/mL.

Chemotaxis Assays. Migration of PMNs was performed in an AP48 chemotaxis chamber (Neuro Probe, Gaithersburg, MD) containing polycarbonate filters (5.0 μm pore size) without poly(vinylpyrrolidone) (Neuro Probe) as described (*27*). The chemotactic stimulus was placed in the lower chamber, and 50 μL of cell suspension was added to the upper chamber. Cells were allowed to migrate for 45 min at 37 °C in a 5% CO_2 incubator. After incubation, nonmigrating cells were removed, and migrating cells were stained with the Diff-Quik stain set (Dade Behring, Newark, DE) and then counted under a light microscope in high power fields (HPFs).

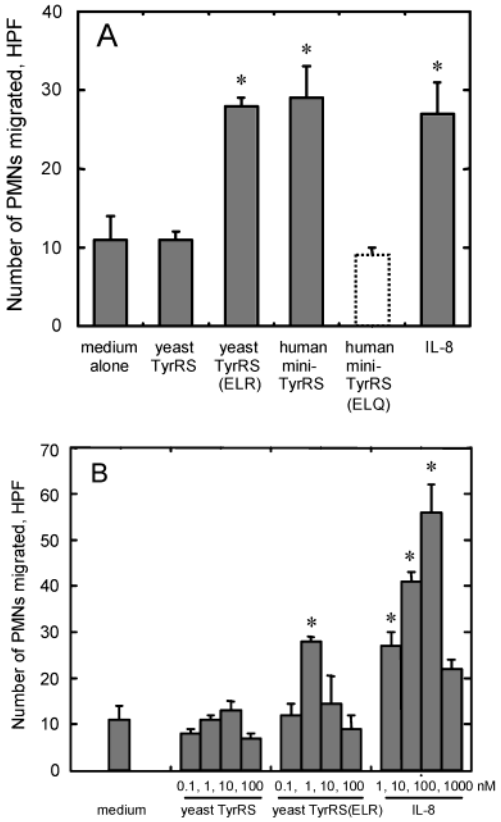

FIGURE 2: Effect of TyrRSs on human PMN cell migration. PMN chemotaxis assay was performed using an AP48 chemotaxis chamber (Neuro Probe, Gaithersburg, MD) with polycarbonate filters (PVP free, 5.0 μm). Each protein or medium alone was placed in the lower wells in triplicate, and freshly isolated human PMNs (2×10^4 cells) were placed in the upper wells. After 45 min in a 37 °C incubator containing 5% CO_2, migrating cells were stained and counted under a light microscope in HPFs. Values represent the mean ± SE (error bars) from at least three experiments. The asterisk denotes a measurement that has a *p*-value < 0.01. **(A)** PMN chemotaxis stimulated by 1 nM yeast TyrRS and 1 nM yeast TyrRS(ELR). Human mini-TyrRS (1 nM) and interleukin 8 (1 nM) serve as positive controls, and the medium serves as a negative control. The open column represents previously reported data (*26*) on the migration of human mini-TyrRS(ELQ) for comparison. **(B)** Dose dependence of PMN chemotaxis for yeast TyrRS, yeast TyrRS(ELR), and IL-8. Medium alone serves as a negative control.

RESULTS

Mutational Creation of Cytokine Activity for Yeast TyrRS. *S. cerevisiae* TyrRS shares 50% amino acid identity with human TyrRS (excluding the C-terminal domain). Both enzymes are active on the tRNA substrates from either species (*31*, *44*). We considered that the absence of the ELR motif may be responsible, in part, for the lack of cytokine function for the yeast enzyme. To evaluate whether directed mutation of yeast TyrRS would impart cytokine signaling activity to the enzyme, two point mutations were made to introduce an ELR motif in yeast TyrRS in place of the natural sequence NYR (N95E/Y96L) (Figure 1A). As measured by PMN migration, wild-type yeast TyrRS at a concentration of 1 nM did not induce PMN migration compared to the medium alone control. In contrast, mutant yeast TyrRS (TyrRS(ELR)) induced cell migration (Figure 2A). Yeast TyrRS(ELR) enzyme was active at the same concentrations as those of the positive controls IL-8 and human mini-TyrRS.

The hallmark of cytokines that induce PMN migration is the bell-shaped response of migration to the concentration of cytokine (*45*). A bell-shaped response of PMN migration to yeast TyrRS(ELR) with maximal migration occurring at 1 nM was seen (Figure 2B). This response was similar to that of human mini-TyrRS, which gives a PMN migration curve with maximum migration between 1 and 10 nM (*26*). In contrast, wild-type yeast TyrRS did not show significant induction of PMN migration over the same concentration range (0.1−100 nM). Stimulation of HUVEC migration is also seen with cytokines such as human mini-TyrRS and IL-8 (*28*, *46*). Significantly, yeast TyrRS(ELR) at a concentration of 1 nM induced HUVEC migration, while wild-type yeast TyrRS did not (data not shown). Thus, introduction of an ELR motif was associated with a gain of cytokine activity in yeast TyrRS.

Aminoacylation Activity of Mutant Yeast TyrRS. To determine whether the ELR motif introduced into yeast TyrRS affected catalytic activity, we tested the mutant and wild-type enzymes in an aminoacylation assay. Mutant TyrRS(ELR) protein had 2-fold-enhanced aminoacylation

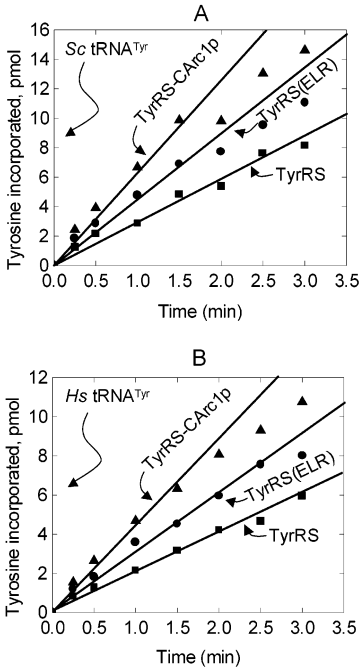

FIGURE 3: Aminoacylation activity of yeast TyrRSs. Aminoacylation activity was measured as the incorporation of [^{3}H]tyrosine onto **(**A**)** yeast tRNA^{Tyr} or **(**B**)** human tRNA^{Tyr} transcripts over time.

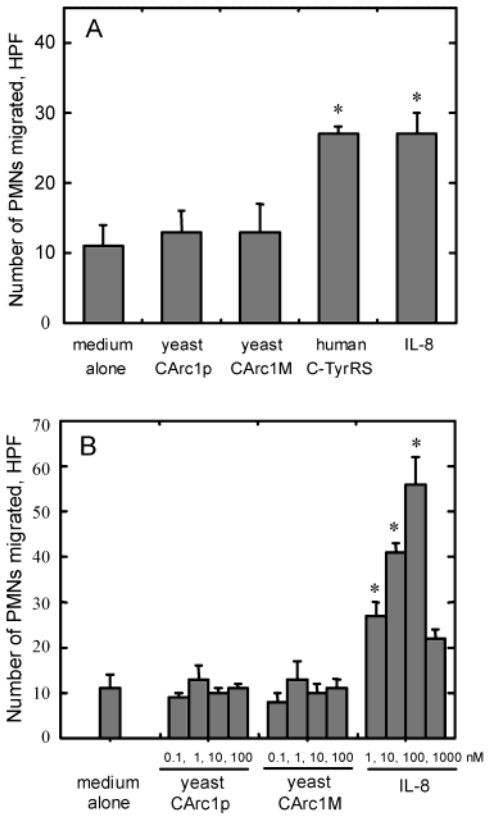

FIGURE 4: Effect of CArc1p on human PMN cell migration. Values represent the mean ± SE (error bars) from at least three experiments. The asterisk denotes a measurement that has a *p*-value < 0.01. (A**)** PMN chemotaxis stimulated by medium, CArc1p (1 nM), CArc1M (1 nM), human mini-TyrRS (1 nM), and IL-8 (1 nM). **(**B**)** PMN chemotaxis assay for CArc1p and CArc1M over a range of 0.1−100 nM. IL-8 serves as a positive control and the medium as a negative control.

efficiency (K_{cat}/K_m) compared to the wild-type yeast enzyme (Figure 3A). The K_m of TyrRS(ELR) for yeast tRNA^{Tyr} substrate was 2.8 μM, the same as for wild-type TyrRS. Thus, the incorporated ELR motif modestly increased the catalytic efficiency of yeast TyrRS. Human tRNA^{Tyr} transcript was also tested as a substrate, and yeast TyrRS(ELR) also had enhanced activity on this substrate (Figure 3B). Thus, when the ELR motif was introduced into the yeast TyrRS, the enzyme gained both catalytic enhancement and cytokine activity.

Attempt To Convert Yeast Arc1p into an EMAP II-like Cytokine. The C-terminal domain of human TyrRS shares 49% sequence identity with the human cytokine EMAP II and has EMAP II-like cytokine activities (*26*). In *S. cerevisiae* there is a "stand alone" homologue of the human TyrRS C-domain. The C-terminal portion of the RNA binding protein Arc1p (*33*) has 43% sequence identity to the C-terminal portion of human TyrRS and 54% identity to EMAP II. On the basis of their high similarity, we investigated whether the C-terminal domain of yeast Arc1p (CArc1p) had cytokine activities similar to those of the C-domain of human TyrRS or EMAP II.

While human C-TyrRS stimulated migration of PMN cells, yeast CArc1p did not (Figure 4A). Previous studies showed that a peptide corresponding to a seven amino acid motif in human C-TyrRS and EMAP II stimulated PMN migration. Peptides with the sequence RVGKIIT (human C-TyrRS) or RIGCIIT (human EMAP II) induced PMN and MP migration, while the corresponding motif from yeast Arc1p (RVGFIQK) did not (*27*, *47*). To determine whether this yeast ortholog of a mammalian cytokine could be switched by mutation into a cytokine, we replaced the RVGFIQK motif in yeast CArc1p with the sequence RVGKIIT from human C-TyrRS, creating the recombinant mutant CArc1M (Figure 1A). Despite the mutation, CArc1M did not stimulate measurable PMN migration over a concentration range of 0.1−100 nM (Figure 4). These data show that, although a specific motif is important for the cytokine function, the context is critical. Despite the overall similarity between yeast CArc1M and human C-TyrRS, additional amino acid substitutions are required to acquire the cytokine activity present in the human homologue.

DISCUSSION

This work demonstrates that a lower eukaryote TyrRS can be transformed into a mammalian-like cytokine with a few simple amino acid substitutions. In addition to creating cytokine activity, the placement of an ELR motif in a lower eukaryote TyrRS improved the enzyme efficiency. According to the crystal structure of the bacterial TyrRS (*48*), comparison of the TyrRS sequences from human, yeast, and the bacterium predicts that the ELR motif is close to the active site for aminoacylation. (Recently, the crystal structure of human mini-TyrRS was solved, revealing that the ELR motif is partially exposed on the surface of the Rossmann fold near the active site (*49*).) In principle, this improvement could be a driving force to fix the ELR motif in TyrRS. Organisms could then utilize the ELR motif in TyrRS for signal transduction in angiogenesis or immune system modulation without compromising the canonical function (*26*, *28*). Likewise, selective pressure that strengthened or maintained productive aminoacylation may have supported a fusion

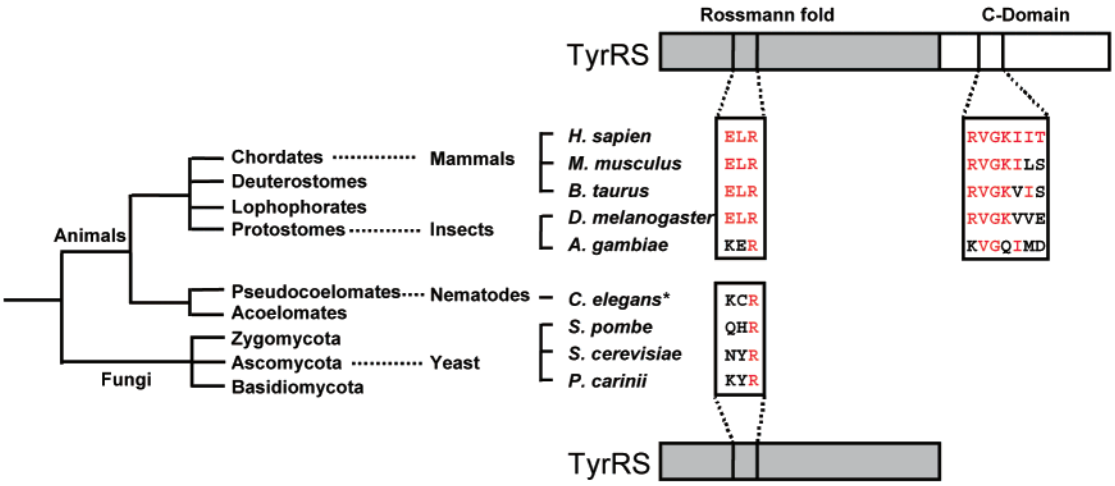

FIGURE 5: Comparison of ELR and RVGKIIT motifs in eukaryote TyrRSs. The C-domain and ELR motif are found in segmented animals. Red letters denote amino acids similar to those conferring cytokine activity in human TyrRS. **C. elegans* TyrRS contains a C-terminal domain which does not have sequence similarity to human TyrRS C-terminal domain.

between the EMAP II-like C-domain and TyrRS. This fusion is unique to TyrRSs in higher eukaryotes.

Examining all available eukaryote TyrRS sequences, we found that the C-domain and ELR motif appear in segmented animals, such as mammals and insects, whereas both features were absent from yeast and the lower animal *Caenorhabditis elegans* (Figure 5). The enlargement of the enzyme by the C-domain and the expansion of activity by the addition of the ELR motif occurred relatively late during evolution, and their addition to TyrRS coincides with the existence of vessels in these organisms. As the two modifications occur together for the most part, both were apparently fixed into the enzyme at a similar stage. Thus, the order in which the ELR motif and C-domain were initially fixed into the enzyme is difficult to determine. Interestingly, the ELR motif and C-domain are already present in insects, such as *Drosophila*, which have a primitive circulatory system and cells with an immune-system-like function, suggesting that TyrRS may have had a role early in the development of vascular and immune systems. In any case, the modern day mammalian TyrRS is active in both translation and cell signaling.

In human TyrRS, the isolated C-domain was demonstrated to have cell signaling activity in vitro (*26*, *27*). The N-terminal peptide RVGKIIT from the C-domain was also shown to have cell signaling activity on its own (*27*). This sequence was not sufficient to create cytokine activity in a yeast ortholog (Arc1p) of the C-domain of TyrRS (Figure 4). Thus, the activity found in the C-domain of TyrRS required the context of the synthetase. Sequence comparisons of the C-domains in higher eukaryote TyrRSs show that the RVGKIIT sequence is not completely conserved, even among mammals. It remains to be determined whether these alternative sequences in mammals are active as peptide elements. If so, then the cytokine activity of the C-domain would appear to be activated by a broader array of sequences than the IL-8 activity found in mini-TyrRS.

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REFERENCES

1. Schimmel, P. (1987) *Annu. Rev. Biochem. 56*, 125−158.
2. Moras, D. (1992) *Trends Biochem. Sci. 17*, 159−164.
3. Cusack, S. (1999) *Curr. Opin. Struct. Biol. 9*, 66−73.
4. Ribas de Pouplana, L., and Schimmel, P. (2001) *J. Biol. Chem. 276*, 6881−6884.
5. Webster, T., Tsai, H., Kula, M., Mackie, G. A., and Schimmel, P. (1984) *Science 226*, 1315−1317.
6. Ludmerer, S. W., and Schimmel, P. (1987) *J. Biol. Chem. 262*, 10801−10806.
7. Eriani, G., Delarue, M., Poch, O., Gangloff, J., and Moras, D. (1990) *Nature 347*, 203−206.
8. Cusack, S., Berthet-Colominas, C., Hartlein, M., Nassar, N., and Leberman, R. (1990) *Nature 347*, 249−255.
9. Hountondji, C., Dessen, P., and Blanquet, S. (1986) *Biochimie 68*, 1071−1078.
10. Mirande, M. (1991) *Prog. Nucleic Acid Res. Mol. Biol. 40*, 95−142.
11. Wang, C. C., and Schimmel, P. (1999) *J. Biol. Chem. 274*, 16508−16512.
12. Cahuzac, B., Berthonneau, E., Birlirakis, N., Guittet, E., and Mirande, M. (2000) *EMBO J. 19*, 445−452.
13. Simos, G., Segref, A., Fasiolo, F., Hellmuth, K., Shevchenko, A., Mann, M., and Hurt, E. C. (1996) *EMBO J. 15*, 5437−5448.
14. Rho, S. B., Kim, M. J., Lee, J. S., Seol, W., Motegi, H., Kim, S., and Shiba, K. (1999) *Proc. Natl. Acad. Sci. U.S.A. 96*, 4488−4493.
15. Francklyn, C., Musier-Forsyth, K., and Martinis, S. A. (1997) *RNA 3*, 954−960.
16. Martinis, S. A., Plateau, P., Cavarelli, J., and Florentz, C. (1999) *EMBO J. 18*, 4591−4596.
17. Lund, E., and Dahlberg, J. E. (1998) *Science 282*, 2082−2085.
18. Sarkar, S., Azad, A. K., and Hopper, A. K. (1999) *Proc. Natl. Acad. Sci. U.S.A. 96*, 14366−14371.
19. Dujardin, G., and Herbert, C. J. (1997) in *Ribosomal RNA and group I introns* (Green, R., and Schroeder, R., Eds.) pp 179−198, Lands Bioscience, Austin, TX.
20. Rho, S. B., and Martinis, S. A. (2000) *RNA 6*, 1882−1894.
21. Putney, S. D., and Schimmel, P. (1981) *Nature 291*, 632−635.
22. Putzer, H., Grunbery-Manago, M., and Springer, M. (1995) in *tRNA: Structure, Biosynthesis and Function* (Söll, D., and RajBhandary, U., Eds.) pp 293−333, ASM Press, Washington, DC.
23. Ko, Y. G., Kang, Y. S., Kim, E. K., Park, S. G., and Kim, S. (2000) *J. Cell Biol. 149*, 567−574.
24. Ko, Y. G., Kim, E. Y., Kim, T., Park, H., Park, H. S., Choi, E. J., and Kim, S. (2001) *J. Biol. Chem. 276*, 6030−6036.
25. Cen, S., Khorchid, A., Javanbakht, H., Gabor, J., Stello, T., Shiba, K., Musier-Forsyth, K., and Kleiman, L. (2001) *J. Virol. 75*, 5043−5048.
26. Wakasugi, K., and Schimmel, P. (1999) *Science 284*, 147−151.

27. Wakasugi, K., and Schimmel, P. (1999) *J. Biol. Chem. 274*, 23155−23159.
28. Wakasugi, K., Slike, B. M., Hood, J., Ewalt, K. L., Cheresh, D. A., and Schimmel, P. (2002) *J. Biol. Chem. 277*, 20124−20126.
29. Wakasugi, K., Slike, B. M., Hood, J., Otani, A., Ewalt, K. L., Friedlander, M., Cheresh, D. A., and Schimmel, P. (2002) *Proc. Natl. Acad. Sci. U.S.A. 99*, 173−177.
30. Otani, A., Slike, B. M., Dorrell, M. I., Hood, J., Kinder, K., Ewalt, K. L., Cheresh, D., Schimmel, P., and Friedlander, M. (2002) *Proc. Natl. Acad. Sci. U.S.A. 99*, 178−183.
31. Kleeman, T. A., Wei, D., Simpson, K. L., and First, E. A. (1997) *J. Biol. Chem. 272*, 14420−14425.
32. Swairjo, M. A., Morales, A. J., Wang, C. C., Ortiz, A. R., and Schimmel, P. (2000) *EMBO J. 19*, 6287−6298.
33. Simos, G., Tekotte, H., Grosjean, H., Segref, A., Sharma, K., Tollervey, D., and Hurt, E. C. (1996) *EMBO J. 15*, 2270−2284.
34. Kaminska, M., Deniziak, M., Kerjan, P., Barciszewski, J., and Mirande, M. (2000) *EMBO J. 19*, 6908−6917.
35. Clark-Lewis, I., Dewald, B., Geiser, T., Moser, B., and Baggiolini, M. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 3574−3577.
36. Daly, T. J., LaRosa, G. J., Dolich, S., Maione, T. E., Cooper, S., and Broxmeyer, H. E. (1995) *J. Biol. Chem. 270*, 23282−23292.
37. Sharma, S. K. (1986) *Biotechnol. Appl. Biochem. 8*, 5−22.
38. Aida, Y., and Pabst, M. J. (1990) *J. Immunol. Methods 132*, 191−195.
39. Liu, S., Tobias, R., McClure, S., Styba, G., Shi, Q., and Jackowski, G. (1997) *Clin. Biochem. 30*, 455−463.
40. Fechter, P., Rudinger, J., Giegé, R., and Théobald-Dietrich, A. (1998) *FEBS Lett. 436*, 99−103.
41. Yanisch-Perron, C., Vieira, J., and Messing, J. (1985) *Gene 33*, 103−119.
42. Shepard, A., Shiba, K., and Schimmel, P. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 9964−9968.
43. Fersht, A. R., Ashford, J. S., Bruton, C. J., Jakes, R., Koch, G. L., and Hartley, B. S. (1975) *Biochemistry 14*, 1−4.
44. Wakasugi, K., Quinn, C. L., Tao, N., and Schimmel, P. (1998) *EMBO J. 17*, 297−305.
45. Bertini, R., Howard, O. M., Dong, H. F., Oppenheim, J. J., Bizzarri, C., Sergi, R., Caselli, G., Pagliei, S., Romines, B., Wilshire, J. A., Mengozzi, M., Nakamura, H., Yodoi, J., Pekkari, K., Gurunath, R., Holmgren, A., Herzenberg, L. A., and Ghezzi, P. (1999) *J. Exp. Med. 189*, 1783−1789.
46. Strieter, R. M., Polverini, P. J., Kunkel, S. L., Arenberg, D. A., Burdick, M. D., Kasper, J., Dzuiba, J., Van Damme, J., Walz, A., Marriott, D., and et al. (1995) *J. Biol. Chem. 270*, 27348−27357.
47. Kao, J., Fan, Y. G., Haehnel, I., Brett, J., Greenberg, S., Clauss, M., Kayton, M., Houck, K., Kisiel, W., Seljelid, R., and et al. (1994) *J. Biol. Chem. 269*, 9774−9782.
48. Brick, P., Bhat, T. N., and Blow, D. M. (1989) *J. Mol. Biol. 208*, 83−98.
49. Yang, X.-L., Skene, R. J., McRee, D. E., and Schimmel, P. (2002) *Proc. Natl. Acad. Sci. U.S.A.* (in press).

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