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Hypothesis

Occurrence of the aminoacyl-tRNA synthetases in high-molecular weight complexes correlates with the size of substrate amino acids

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Abstract One of the distinctive and mysterious features of mammalian aminoacyl-tRNA synthetases (AARSs) is the existence of stable high-molecular weight complexes containing 10 out of 20 AARSs. The composition and structure of these complexes are conserved among multicellular animals. No specific function associated with these structures has been found, and there is no evident rationale for a particular separation of AARSs in "complex-bound" and "free" forms. We have demonstrated a strong association between the occurrence of AARSs in the complexes and the volume of their substrate amino acids. The significance of this association is discussed in terms of the structural organization of translation in the living cell.

Keywords: Aminoacyl-tRNA synthetase; tRNA; Elongation factor; Translation; Amino acid; Channeling

The structural organization of aminoacyl-tRNA synthesis is a unique feature of animal cells. Ten out of twenty aminoacyl-tRNA synthetases (AARSs) participate in high-molecular weight (Mr) complexes. Of these, nine AARSs form a single multienzyme complex, whose composition is strictly conserved in animals ranging from nematodes to mammals [1–3]. This multi-AARS complex contains nine AARSs specific for Asp, Arg, Glu, Gln, Met, Ile, Leu, Pro and Lys. The complex also contains three non-AARS proteins (p43, p38 and p18) with largely unclear function, although p43 is known to have non-specific tRNA-binding activity [4]. A tenth AARS, valyl-tRNA synthetase (ValRS) does not participate in this complex, but forms a separate complex together with four subunits (eEF1A, eEF-1Bα, β, and γ) of translation elongation factor 1 (EF-1) [5,6]. Multienzyme complexes typically unite polypeptides that are either involved in the same catalytic function or that catalyze consecutive reactions. While the ValRS complex might catalyze consecutive tRNA aminoacylation and binding to EF, the multi-AARS complex is known to catalyze parallel and independent tRNA aminoacylation reactions. The reasons for the formation of the stable multi-AARS complex, and for the association of ValRS with the EF, remain mysterious.

Numerous biochemical studies have demonstrated that the association of AARSs into complexes is not essential for their catalytic function ([1,7,8] for review). However, the conservation of the structure of the complexes throughout the multicellular animals strongly suggests the existence of some essential function(s) associated with this AARS organization. The reason for the highly conserved pattern of grouping is unclear: no obvious association between the occurrence of AARSs in the complexes and the physical parameters of AARSs or their corresponding substrates has been identified. For example, complexes contain both class I and class II AARSs, AARSs specific for both polar and hydrophobic amino acids, and so on. However, the association of AARSs into complexes does not appear to be random. For example, the multi-AARS complex contains the AARSs for all of the hydrophobic non-aromatic amino acids, all of which contain U at the second position of their cognate codons. The complex also contains AARSs for all charged amino acids, and some of the amino acids in the complex are metabolically related (Glu, Gln, Pro and Arg, for example). However, none of these observed associations can fully explain the existing grouping of enzymes.

When looking at the particular AARSs present in the multi-AARS complex, we observed that the AARSs for the biggest and the smallest amino acids were absent. The association between relative Mr of amino acids and occurrence of corresponding AARSs in the complex is presented in Fig. 1A. Since Mr most likely reflects other parameters more important for molecular interaction, we tested for an association between amino acid volume and occurrence of the corresponding AARS in the complexes. Although, the association between the volumes of amino acids occurring in the complex is not stronger than the Mr association (Fig. 1B), a much better association is observed between the occurrence in the complexes and the buried volume of amino acids in the protein (Fig. 1C). Amino acids with extremely large or small buried volumes have AARSs that are excluded from the complex.

The statistical significance of the observed association was confirmed by Monte Carlo simulations, in which we asked how surprising it would be if the complex included a set of amino acids as similar as those observed. Since the complexes contain AARSs for 10 amino acids, we chose sets of 10 amino acids at random and measured the mean and S.D. of each random set for a range of physical and chemical parameters. We then tested whether the actual set of 10 amino acids whose AARSs are found in the complex was higher (or lower) on the scale, and whether it was more (or less) variable, than each

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Abbreviations: AARS, aminoacyl-tRNA synthetase; ValRS, valyl-tRNA synthetase; EF, elongation factor; Mr, molecular weight

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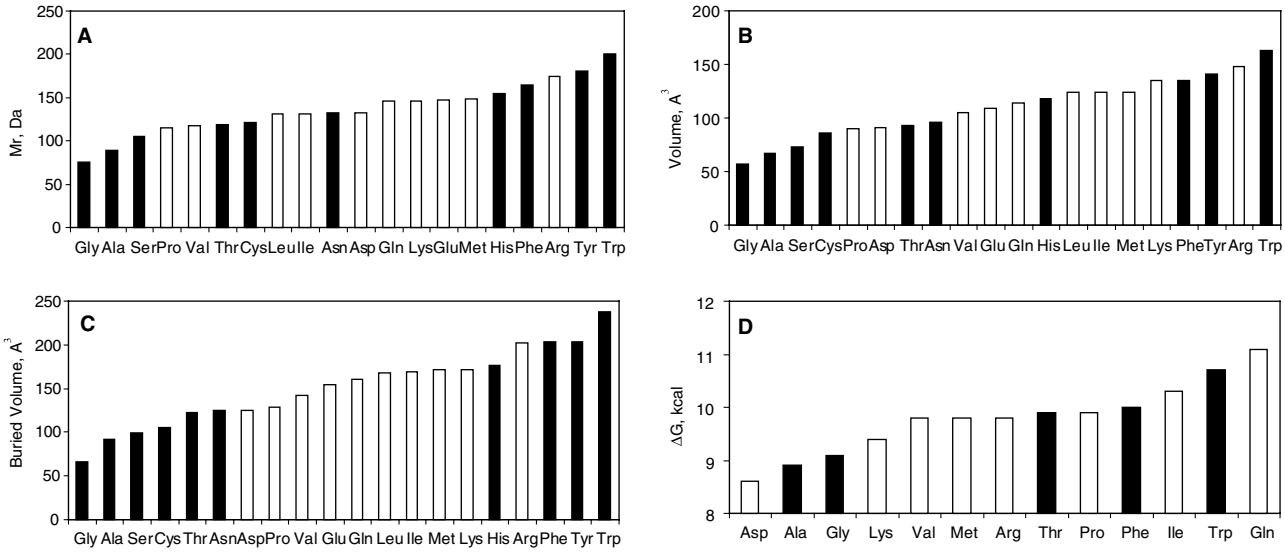

Fig. 1. Association of AARS occurrence in the complexes with different measurements of amino acid properties. Amino acids with AARSs in the complex are indicated by white bars, while those not in the complex are indicated by black bars. A measure with significantly low variance would be expected to group the white bars together more than chance expectations. Note that the order of amino acids changes on the different graphs. (A) Mr, bars sorted by Mr. (B) Amino acid volume, bars sorted by volume. (C) Amino acid buried volume, bars sorted by buried volume. Note the almost perfect separation between black and white bars, the latter occurring in the center of the list. (D) Effect of amino acid on the binding energy of aminoacylated tRNA to bacterial elongation factor EF-Tu (for the 12 amino acids where it has been measured), bars sorted by binding energy. The buried volume data were obtained from Creighton [24] and EF-Tu affinity data from Dale et al. [14].

of the random sets. This procedure allowed us to derive a *P*-value for the mean and S.D. of the physical properties of a set of amino acids: the *P*-value is calculated as the fraction of random amino acid sets that exceeded the actual amino acid set on each of these scales. The corresponding *P*-values are presented in Table 1.

The average values of these parameters for the AARSs contained in the complexes sometimes differ significantly from the "small" and "big" groups, but not from the set of all 20 amino acids. This lack of an overall difference stems from the fact that this group was initially selected as average. In contrast, the S.D. observed within this group is significantly smaller than would be expected from a randomly chosen set of amino acids, with corresponding *P*-values below 0.1, demonstrating the non-random nature of amino acid selection into this group. Specifically, the amino acids in the complex are less variable than we would expect by chance, consistent with the original observation that extreme amino acids (on either end of the scale) are excluded.

To further confirm the relevance of these associations, we analyzed the AAIndex database [9–11] to find the properties of amino acids that are most strongly associated with the occurrence in the complexes by repeating the Monte Carlo procedure described above for each of the 494 measures of amino acid properties in AAIndex. As presented in Table 2, 11 out of the 12 properties most significantly associated with AARS occurrence in the complex are parameters related to the volume of amino acids (refractivity and polarizability are highly correlated with measures of volume, with correlation coefficients ranging in the range of 0.8–0.9; the appearance of mutability in the list is somewhat surprising, since mutability is not highly correlated with volume). The amino acid properties that are least associated with occurrence in complexes are also listed. They include such properties related to hydro-

Table 1
Statistical analysis of association of occurrence of AARSs in the complexes with Mr, volume and buried volume of amino acid residues

Amino acid group	Mr		Amino acid volume		Amino acid buried volume	
	Average (Da)	S.D.	Average (A^3)	S.D.	Average (A^3)	S.D.
All 20	118.3	31.8	109.7	28.1	151.2	43.5
Complex DEIKLMPQRV	120.9	16.6	116.4	18.4	159.3	22.8
P-value	0.42	0.013	0.87	0.073	0.21	0.0005
Small GASTCN	88.8	24.3	77.2	18.2	100.8	24.1
P-value	0.001	0.21	0.0001	0.13	0.00008	0.0237
Big WYFH	158.3	21.4	139.3	18.5	205.3	25.4
P-value	0.0011	0.18	0.012	0.046	0.0003	0.15

The values of amino acids volume and buried volume were obtained from Creighton [24]. *P*-values were calculated by randomly choosing sets of amino acids with the same number as each of the designated sets ('complex', 'small', and 'big'), and measuring the fraction of random sets with values of the mean or S.D. smaller (or greater, depending on the direction of the prediction) than the actual sets.

Table 2
Association of the AARSs occurrence in the complexes with the properties of the substrate amino acids

Measure	*P*-value
(A) Best associations	
MCMT640101:Refractivity [25], cited by Jones [26]	0.0009
LEVM760107:van der Waals parameter epsilon [27]	0.0011
CHOC750101:Average volume of buried residue [28]	0.0023
BIGC670101:Residue volume [29]	0.0025
GOLD730102:Residue volume [30]	0.0034
TSAJ990101:Volumes including the crystallographic waters using the ProtOr [31]	0.0039
TSAJ990102:Volumes not including the crystallographic waters using the ProtOr [31]	0.0039
JOND920102:Relative mutability [32]	0.004
CHOC760101:Residue accessible surface area in tripeptide [33]	0.0044
FAUJ880103:Normalized van der Waals volume [34]	0.0046
CHAM820101:Polarizability parameter [35]	0.0084
KRIW790103:Side chain volume [36]	0.089
(B) Worst associations	
YUTK870101:Unfolding Gibbs energy in water, pH 7.0, [37]	0.9948
CHOP780204:Normalized frequency of N-terminal helix [38]	0.9954
PONP930101:Hydrophobicity scales [39]	0.996
RADA880101:Transfer free energy from chx to wat [40]	0.9965
MANP780101:Average surrounding hydrophobicity [41]	0.9967
NAKH900104:Normalized composition of mt-proteins [42]	0.9968
NAKH900106:Normalized composition from animal [42]	0.9968
NAKH920108:AA composition of MEM of multi-spanning proteins [43]	0.9968
KYTJ820101:Hydropathy index [44]; JURD980101:Modified Kyte-Doolittle hydrophobicity scale [45]	0.9981
NAKH920105:AA composition of MEM of single-spanning proteins [43]	0.9995
KLEP840101:Net charge [46]	1
FAUJ880112:Negative charge [34]	1

P-values showing physical and/or chemical properties with significantly less (or more) variability for the sample of amino acids in the multi-AARS complex than would be expected by chance. We generated 10000 random sets of 10 amino acids and measured the S.D. of the property (a measure of spread) for each of the 493 measured properties in the AAIndex database. This Monte Carlo simulation showed directly which properties were highly conserved in the 10 amino acids participating in the multi-AARS complex via a one-tailed test: a property in which few random samples had lower variability than the actual set of AARSs had a low *P*-value, and a property in which almost all random samples had higher variability had a high *P*-value. The corresponding references are reproduced from AAIindex database.

phobicity and charge, which is intuitively clear since complexes contain AASRs specific for all charged and most of hydrophobic amino acids. Thus, all the amino acid properties most strongly associated with occurrence of the corresponding AARS in the complexes are related to volume. This observation suggests that the AARS complexes serve as a molecular sieve, separating the AARS specific for medium-volume amino acids from AARSs specific for large and small amino acids.

What might the functional significance of the observed correlation be? One possibility is that it may reflect molecular events occurring after tRNA aminoacylation. It has long been proposed and experimentally confirmed that the process of tRNA transfer from AARS to the ribosome in eukaryotic cell requires a special structural organization that facilitates tRNA movement between the components of the translation machinery [12,13]. Thus, it is possible that different aminoacylated tRNAs may behave differently in interactions with components of the translation machinery, which occur after tRNA aminoacylation and involve recognition of the amino acid side chain of aminoacyl-tRNA. The most drastic example of the amino acid affecting the recognition properties of aminoacyl-tRNA is the binding of aminoacyl-tRNA translation EF-1 [14–16]. Evidence demonstrating the effect of the nature of the amino acid moiety on aminoacyl-tRNA binding to A-site of the ribosome has recently been presented [17].

After aminoacylation, all elongator tRNAs bind to EF-1A, which delivers them to the A-site of the ribosome. Interaction of EF-1A with aminoacylated tRNA requires the presence of amino acid residue attached to tRNA. The amino acid residue resides in a deep pocket on the EF-Tu (bacterial EF-1A) surface [18]. Although EF-Tu binds all cognate aminoacylated tRNAs with approximately the same efficiency despite differences in the nature of the amino acid or the tRNA sequence, the nature of the amino acid strongly affects the strength of binding. Bacterial EF-Tu binds amino acids and tRNA moieties of aminoacyl-tRNAs independently, with significant variation in binding energy between different amino acids and tRNAs [14–16,19]. In cognate aminoacylated tRNAs, the binding energy of the amino acid and the tRNA body are balanced to achieve the uniform binding of elongator tRNAs [15,16].

No correlation is evident between AARSs occurrence in the complex and amino acid binding energy to bacterial EF-Tu (Fig. 1D), but the mechanism of aminoacyl-tRNA recognition by mammalian eEF-1A may be significantly different from that of bacterial Tu. Differences in tRNA binding between EF-Tu and eEF-1A are indicated, for example, by a different method of discriminating against initiator tRNA. The structure of the first base pair of the tRNA acceptor stem is a crucial determinant for EF-Tu affinity, but plays a lesser role in tRNA recognition by eukaryotic EF-1A [20]. Some of the residues involved in tRNA recognition by EF-Tu are not conserved in mammalian eEF-1A. The significant changes in the composition of the amino acid binding pocket also indicate differences in the mechanism of amino acid recognition between bacterial and eukaryotic EFs. The amino acid binding pocket of EF-Tu

surrounds the amino acid in a deep cleft within the protein (Fig. 2). Several residues forming the amino acid binding pocket of bacterial EF-Tu are substituted with more hydrophobic ones in mammalian eEF-1A (Fig. 2). Substitutions in the amino acid binding pocket of bacterial EF-Tu selectively alter its affinity for the amino acid [14]. Notably, the substitution of Glu226 (conserved in bacterial EF-Tu) to Gln (conserved in eukaryotic eEF-1A) specifically decreased EF-Tu affinity to strongly binding amino acids, decreasing the range of Tu affinity in response to variation in the amino acid nature [14]. The structural differences between amino acid binding pocket of bacterial and mammalian eEF-1A suggest significant differences in the specificity of aminoacyl-tRNA binding.

What mechanism might explain why the amino acid volume would affect aminoacyl-tRNA binding? The amino acid substitutions in the amino acid binding pocket, which are conserved in eukaryotic EF-1A, make it more hydrophobic than in bacterial EF-Tu. Introduction of an amino acid residue into the binding pocket requires the displacement of water molecules. The interplay of enthalpic and entropic contributions toward amino acid binding in the pocket may specifically disfavor binding of medium-size amino acids.

What, then, might the structural organization of AARS have to do with the strength of aminoacyl-tRNA binding by eEF-1A? In bacteria, the difference in the energy of amino acid binding is counterbalanced by corresponding adjustments in the binding strength of cognate tRNAs by changes in the sequence [16]. It is possible that animal cells, where tRNA diversity is much higher than in bacteria, use a different compensation mechanism. The difference in aminoacyl-tRNA binding may be compensated for by the structural organization of aminoacyl-tRNA delivery to eEF-1A. If we assume that aminoacyl-tRNAs made by AARSs in the complexes are weaker binders, the reduced binding strength may be balanced by the specific mechanism(s) of aminoacyl-tRNA transfer to the EF. It is obvious in the case of the ValRS complex with the EF, where aminoacylated $tRNA^{Val}$ can be directly transferred to the eEF-1A subunit of the complex [21]. As for the multi-AARS complex, tRNA transfer might be mediated by the non-specific tRNA-binding protein p43, the exact function of which is unknown. The possibility that class 1 AARS and *Escherichia coli* analog of p43 bind tRNA simultaneously has been demonstrated [22]. There is also evidence for interactions between some of the complex components and subunits of EF-1 [23], which might allow eEF-1A to dock on the multi-AARS complex and to transfer the aminoacyl-tRNA to eEF-1A. The idea that different classes of aminoacyl-tRNAs may enter the ribosome through different pathways is indirectly confirmed by the existence of multiple forms of EF-1 in mammalian cells. The activity of the factor is distributed between "free" EF-1A, heavy form of EF-1 (EF-1H) that contains eEF-1A associated with three other subunits (eEF1Bα, β, and γ), and the Val-RS*EF-1H complex. All these forms of EF-1 are functional in vitro [5], but their specificity toward aminoacyl-tRNAs has never been addressed.

The hypothesis presented here suggests that association of AARSs into the complexes correlates with the ability of the corresponding aminoacyl-tRNAs to bind eEF-1A. It predicts the significant differences in aminoacyl-tRNA binding specificity between bacterial EF-Tu and mammalian eEF-1A. It also predicts the existence of classes of elongator tRNAs with visible differences in binding to eEF-1A, and differences in aminoacyl-tRNA binding specificity between different forms of EF-1. The function of the association of particular AARSs into the complexes may be to provide a pathway that channels distinct classes of aminoacyl-tRNAs to the ribosome. Disruption of

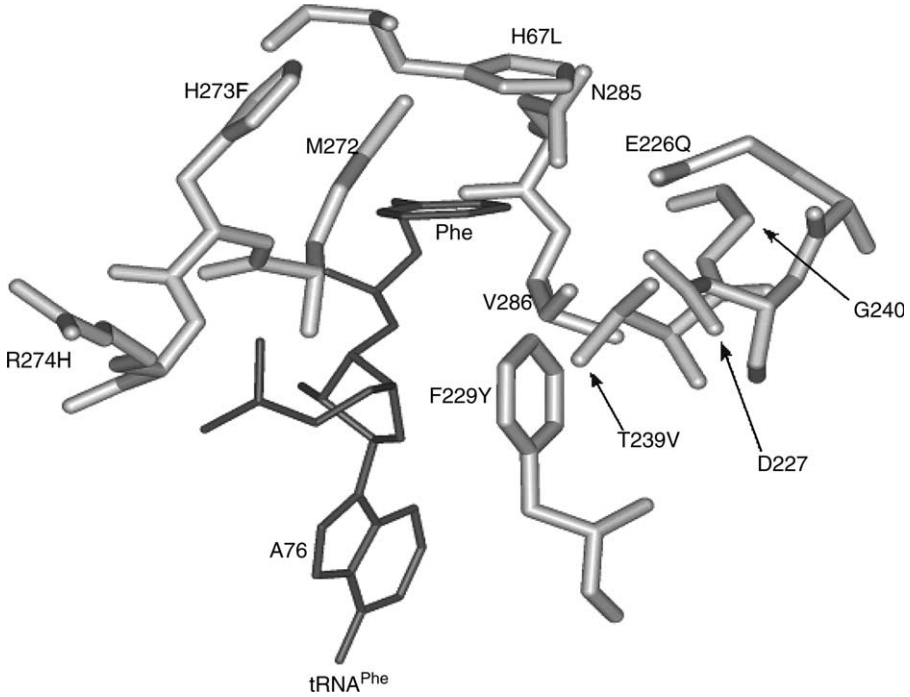

Fig. 2. Amino acid binding pocket of *Thermus thermophilus* EF-Tu [18]. Amino acid replacements that occurred in human eEF-1A are indicated.

the complex structure would then result in deregulation of aminoacyl-tRNA delivery to the ribosomes, and, consequently, in decreased translational efficiency.

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References

[1] Kisselev, L.L. and Wolfson, A.D. (1994) Aminoacyl-tRNA synthetases from higher eukaryotes. Prog. Nucleic Acid Res. Mol. Biol. 48, 83–142.
[2] Kerjan, P., Cerini, C., Semeriva, M. and Mirande, M. (1994) The multienzyme complex containing nine aminoacyl-tRNA synthetases is ubiquitous from *Drosophila* to mammals. Biochim. Biophys. Acta 1199, 293–297.
[3] Han, J.M., Kim, J.Y. and Kim, S. (2003) Molecular network and functional implications of macromolecular tRNA synthetase complex. Biochem. Biophys. Res. Commun. 303, 985–993.
[4] Renault, L., Kerjan, P., Pasqualato, S., Menetrey, J., Robinson, J.C., Kawaguchi, S., Vassylyev, D.G., Yokoyama, S., Mirande, M. and Cherfils, J. (2001) Structure of the EMAPII domain of human aminoacyl-tRNA synthetase complex reveals evolutionary dimer mimicry. Embo J. 20, 570–578.
[5] Motorin, Y.A., Wolfson, A.D., Lohr, D., Orlovsky, A.F. and Gladilin, K.L. (1991) Purification and properties of a high-molecular-mass complex between Val-tRNA synthetase and the heavy form of elongation factor 1 from mammalian cells. Eur. J. Biochem. 201, 325–331.
[6] Bec, G., Kerjan, P., Zha, X.D. and Waller, J.P. (1989) Valyl-tRNA synthetase from rabbit liver. I. Purification as a heterotypic complex in association with elongation factor 1. J. Biol. Chem. 264, 21131–21137.
[7] Mirande, M., Cirakoglu, B. and Waller, J.P. (1983) Seven mammalian aminoacyl-tRNA synthetases associated within the same complex are functionally independent. Eur. J. Biochem. 131, 163–170.
[8] Mirande, M. (1991) Aminoacyl-tRNA synthetase family from prokaryotes and eukaryotes: structural domains and their implications. Prog. Nucleic Acid Res. Mol. Biol. 40, 95–142.
[9] Kawashima, S., Ogata, H. and Kanehisa, M. (1999) AAindex: amino acid index database. Nucleic Acids Res. 27, 368–369.
[10] Nakai, K., Kidera, A. and Kanehisa, M. (1988) Cluster analysis of amino acid indices for prediction of protein structure and function. Protein Eng. 2, 93–100.
[11] Tomii, K. and Kanehisa, M. (1996) Analysis of amino acid indices and mutation matrices for sequence comparison and structure prediction of proteins. Protein Eng. 9, 27–36.
[12] Stapulionis, R. and Deutscher, M.P. (1995) A channeled tRNA cycle during mammalian protein synthesis. Proc. Natl. Acad. Sci. USA 92, 7158–7161.
[13] Negrutskii, B.S., Stapulionis, R. and Deutscher, M.P. (1994) Supramolecular organization of the mammalian translation system. Proc. Natl. Acad. Sci. USA 91, 964–968.
[14] Dale, T., Sanderson, L.E. and Uhlenbeck, O.C. (2004) The affinity of elongation factor Tu for an aminoacyl-tRNA is modulated by the esterified amino acid. Biochemistry 43, 6159–6166.
[15] LaRiviere, F.J., Wolfson, A.D. and Uhlenbeck, O.C. (2001) Uniform binding of aminoacyl-tRNAs to elongation factor Tu by thermodynamic compensation. Science 294, 165–168.
[16] Wolfson, A.D., LaRiviere, F.J., Pleiss, J.A., Dale, T., Asahara, H. and Uhlenbeck, O.C. (2001) tRNA conformity. Cold Spring Harb. Symp. Quant. Biol. 66, 185–193.
[17] Fahlman, R.P., Dale, T. and Uhlenbeck, O.C. (2004) Uniform binding of aminoacylated transfer RNAs to the ribosomal A and P sites. Mol. Cell 16, 799–805.
[18] Nissen, P., Kjeldgaard, M., Thirup, S., Polekhina, G., Reshetnikova, L., Clark, B.F. and Nyborg, J. (1995) Crystal structure of the ternary complex of Phe-tRNAPhe, EF-Tu, and a GTP analog. Science 270, 1464–1472.
[19] Asahara, H. and Uhlenbeck, O.C. (2002) The tRNA specificity of *Thermus thermophilus* EF-Tu. Proc. Natl. Acad. Sci. USA 99, 3499–3504.
[20] Mayer, C., Stortchevoi, A., Kohrer, C., Varshney, U. and RajBhandary, U.L. (2001) Initiator tRNA and its role in initiation of protein synthesis. Cold Spring Harb. Symp. Quant. Biol. 66, 195–206.
[21] Negrutskii, B.S., Shalak, V.F., Kerjan, P., El'skaya, A.V. and Mirande, M. (1999) Functional interaction of mammalian valyl-tRNA synthetase with elongation factor EF-1alpha in the complex with EF-1H. J. Biol. Chem. 274, 4545–4550.
[22] Nomanbhoy, T., Morales, A.J., Abraham, A.T., Vortler, C.S., Giege, R. and Schimmel, P. (2001) Simultaneous binding of two proteins to opposite sides of a single transfer RNA. Nat. Struct. Biol. 8, 344–348.
[23] Sang Lee, J., Gyu Park, S., Park, H., Seol, W., Lee, S. and Kim, S. (2002) Interaction network of human aminoacyl-tRNA synthetases and subunits of elongation factor 1 complex. Biochem. Biophys. Res. Commun. 291, 158–164.
[24] Creighton, T.E. (1993) Proteins: Structure and Molecular Properties, 2nd edn, W.H. Freeman & Co., New York.
[25] McMeekin, T.L., Groves, M.L. and Hipp, N.J. (1964) in: Amino Acids and Serum Proteins (stekol, J.A., Ed.), p. 54, American Chemical Society, Washington, DC.
[26] Jones, D.D. (1975) Amino acid properties and side-chain orientation in proteins: a cross correlation approach. J. Theor. Biol. 50, 167–183.
[27] Levitt, M. and Chothia, C. (1976) Structural patterns in globular proteins. Nature 261, 552–558.
[28] Chothia, C. and Janin, J. (1975) Principles of protein–protein recognition. Nature 256, 705–708.
[29] Bigelow, C.C. (1967) On the average hydrophobicity of proteins and the relation between it and protein structure. J. Theor. Biol. 16, 187–211.
[30] Goldsack, D.E. and Chalifoux, R.C. (1973) Contribution of the free energy of mixing of hydrophobic side chains to the stability of the tertiary structure of proteins. J. Theor. Biol. 39, 645–651.
[31] Tsai, J., Taylor, R., Chothia, C. and Gerstein, M. (1999) The packing density in proteins: standard radii and volumes. J. Mol. Biol. 290, 253–266.
[32] Jones, D.T., Taylor, W.R. and Thornton, J.M. (1992) The rapid generation of mutation data matrices from protein sequences. Comput. Appl. Biosci. 8, 275–282.
[33] Chothia, C. (1976) The nature of the accessible and buried surfaces in proteins. J. Mol. Biol. 105, 1–12.
[34] Fauchere, J.L., Charton, M., Kier, L.B., Verloop, A. and Pliska, V. (1988) Amino acid side chain parameters for correlation studies in biology and pharmacology. Int. J. Pept. Protein Res. 32, 269–278.
[35] Charton, M. and Charton, B.I. (1982) The structural dependence of amino acid hydrophobicity parameters. J. Theor. Biol. 99, 629–644.
[36] Krigbaum, W.R. and Komoriya, A. (1979) Local interactions as a structure determinant for protein molecules: II. Biochim. Biophys. Acta 576, 204–248.
[37] Yutani, K., Ogasahara, K., Tsujita, T. and Sugino, Y. (1987) Dependence of conformational stability on hydrophobicity of the amino acid residue in a series of variant proteins substituted at a unique position of tryptophan synthase alpha subunit. Proc. Natl. Acad. Sci. USA 84, 4441–4444.
[38] Chou, P.Y. and Fasman, G.D. (1978) Empirical predictions of protein conformation. Annu. Rev. Biochem. 47, 251–276.
[39] Ponnuswamy, P.K. (1993) Hydrophobic characteristics of folded proteins. Prog. Biophys. Mol. Biol. 59, 57–103.
[40] Radzicka, A., Pedersen, L. and Wolfenden, R. (1988) Influences of solvent water on protein folding: free energies of solvation of *cis* and *trans* peptides are nearly identical. Biochemistry 27, 4538–4541.

[41] Manavalan, P. and Ponnuswamy, P.K. (1978) Hydrophobic character of amino acid residues in globular proteins. Nature 275, 673–674.
[42] Nakashima, H., Nishikawa, K. and Ooi, T. (1990) Distinct character in hydrophobicity of amino acid compositions of mitochondrial proteins. Proteins 8, 173–178.
[43] Nakashima, H. and Nishikawa, K. (1992) The amino acid composition is different between the cytoplasmic and extracellular sides in membrane proteins. FEBS Lett. 303, 141–146.
[44] Kyte, J. and Doolittle, R.F. (1982) A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. 157, 105–132.
[45] Juretic, D., Zucic, D., Lucic, B. and Trinajstic, N. (1998) Preference functions for prediction of membrane-buried helices in integral membrane proteins. Comput. Chem. 22, 279–294.
[46] Klein, P., Kanehisa, M. and DeLisi, C. (1984) Prediction of protein function from sequence properties. Discriminant analysis of a data base. Biochim. Biophys. Acta 787, 221–226.