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Review

Aminoacyl-tRNA synthetases and aminoacylation of tRNA in the nucleus✪

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This review is focused on findings concerning the presence of translation apparatus components (aminoacyl-tRNA synthetases, aminoacyl-tRNA, elongation factors) as well as translation itself in the nucleus. A nuclear role of these molecules is unknown. New findings suggest that well-accepted model of spatial segregation of transcription and translation in eukaryotic cell may be oversimplifcation. Nuclear coupling of both these processes show us how exciting and surprising may be the world of the living cell.

Aminoacyl-tRNA synthetases (aaRSs, EC 6.1.1) play the central role in the translation of the language of nucleotides into amino-acid sequence of proteins. *In vitro,* several examples of non-proteinogenic aminoacylation of tRNA by ribozymes or high pressure are known, however, *in vivo* tRNA aminoacylation is exclusively catalyzed by aaRSs [1–3]. The role of aaRS in the preribosomal phase of translation in the cytosol is well known [2, 4, 5]. Recently the presence of both aaRS and aminoacyl-tRNA in the nucleus has been discovered [6–8]. Their nuclear function is unknown. Aminoacylation enhances the efficiency of nuclear export of tRNA and may be the last step of a proofreading mechanism of its maturation, however, it is not critical for cell viability. Recent experiments emphasized

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Abbreviations: aa, amino acid; aaRS, aminoacyl-tRNA synthetase; ATA, aurintricarboxylic acid; HTH, helix-turn-helix motif; NLS, nuclear localization signal; NMD, nonsense mediated decay; NPC, nuclear pore complex; RNAP, RNA polymerase.

the presence of translation in the nucleus. These discoveries were a big surprise in the "well-established" knowledge of the aminoacylation and translation processes. Our knowledge about the compartmentalization of discrete steps of gene expression in the eukaryotic cell has been changed.

In higher *eukaryota* most cytoplasmatic aaRSs exist as high molecular mass (1.1–1.4 MDa) supramolecular multienzymatic complexes [2, 7, 9–15]. Number of synthetases in such fragile complexes varies, but a stable core is composed of nine aaRSs* (Arg, Lys, Asp, Gln, Glu, Ile, Leu, Met, Pro) and three non-enzymatic proteins (of 18, 38 and 43 kDa) [2, 9, 11, 13, 16]. These accessory, non-enzymatic proteins are responsible for a transient interaction of the complex with eEF1A (p18), assembly of the complex (p38) and enhancing the affinity for tRNA (p43) [9]. The detailed structure of the multienzymatic complexes is unknown. Taking into account the specific shape and size of the tRNA molecule, the multienzymatic complex of aaRSs should have a rather open architecture. Electron microscopy studies suggest a cap-like or elongated U-shape of the complexes [17–19]. Homologues bacterial and lower *eukaryota* aaRSs do not form such multienzymatic complexes [7]. This suggests that the structure of the aaRS complex is highly associated with its cellular function. Until recently it was believed that aaRS and aminoacyl-tRNA are confined to the cytoplasm. However, recent experiments have provided a clear evidence for their presence in the nucleus [22–31].

To enter the nucleus from the cytoplasm, aaRS must cross over the nuclear envelope (Fig. 1). Nuclear import of aaRS occurs through nuclear-pore complexes (NPC) [12, 32–36]. The multienzymatic complexes of aaRS are too large to be imported into the nucleus by a passive mechanism, like diffusion [7, 13, 22]. They seem to be actively transported into the nucleus by special translocating proteins called importins, however, no specific receptor has been identified so far. Also, no experiments have yet explicitly demonstrated the nuclear import of aaRS.

The nuclear import of such "unusual" for nuclear environment proteins like aaRSs suggests that there is a huge amount of traffic going in and out of the nucleus. It has been established that about one million molecules per minute are transferred between the nucleus and the cytoplasm. Importins recognize specific sequence signature(s) located on the surface of a transported protein, the nuclear localization signal (NLS) [37–39]. NLS sequences were found in many aaRSs (Table 1) [22].

Table 1. NLSs of selected yeast aaRSs and Arc1p.

A sequence analysis of the whole set of aaRSs can be found in [22]. Basic residues are bolded.

Protein	NLS
GlnRS	**K**^{667}P**KK**^{680}
	K^{679}**K**P**K**^{682}
	P^{678}**KK**P**K**TY^{684}
LysRS	P^{56}AS**KKK**T^{62}
	K^{33}**KR**I**K**Q**R**QVEA**KK**AA**KK**^{49}
ValRS	**K**^{65}**K**P**K**^{68}
	K^{66}P**KK**^{69}
	P^{67}**KKK**^{70}
	K^{68}**KKK**^{71}
	P^{67}**KKKK**EV^{73}
	K^{38}**KK**AE**K**LL**K**FAA**K**QA**KK**^{54}
	K^{53}**K**NAAATTGASQ**KK**P**KK**^{69}
	K^{132}**KKK**^{135}
Arc1p	P^{321}**KKK**^{324}

NUCLEAR LOCALIZATION SIGNAL OF aaRS

Two classic types of NLSs are known, the SV40 large antigen T type and the bipartite type [40–42]. NLS of both types or sequences of high homology are present in aaRSs (Ta-

*GluRS and ProRS are carried by a single polypeptide chain, which displays two distinct catalytic domains [20, 21].

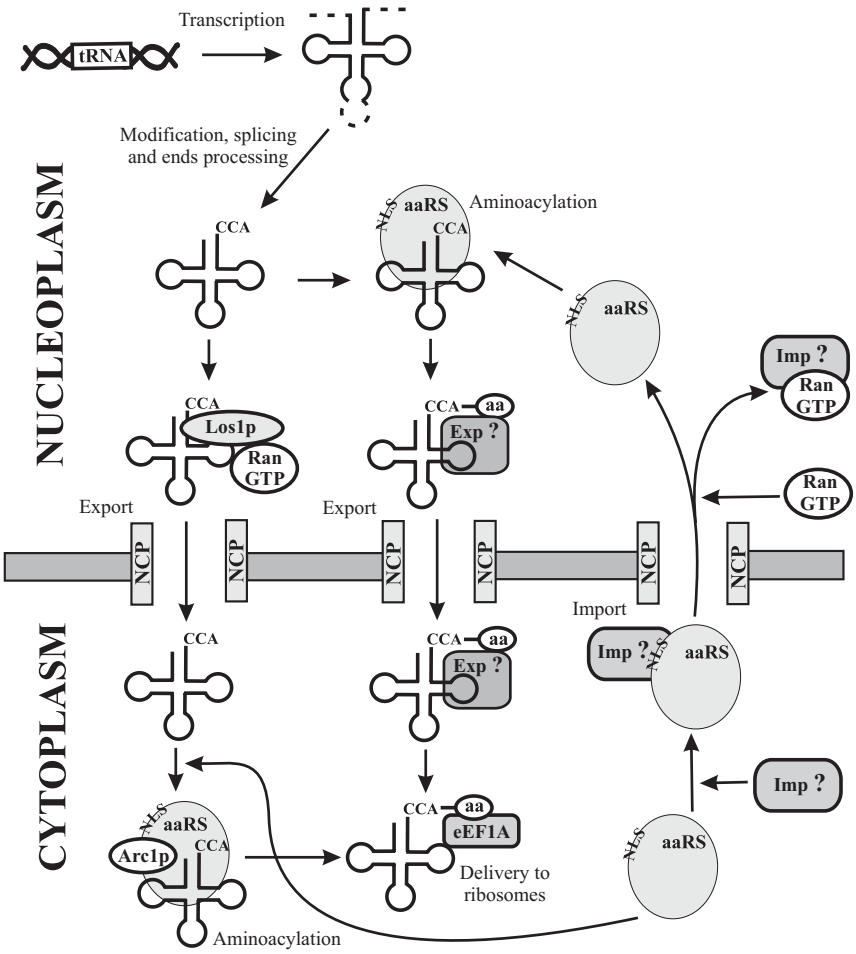

Figure 1. A model of nuclear import/export of aaRS and aminoacyl-tRNA.

Hypothetical receptors are labeled as "Exp?" and "Imp?". The nuclear export of uncharged and aminoacylated tRNA is presented as two independent pathways. It is unknown whether any correlation between them exists. The figure has been modified from [34].

ble 1) [22]. The first type is composed of a cluster of four arginine (Arg) or lysine (Lys) residues, or three basic residues (Arg or Lys) and a histidine (His) or a proline (Pro) residue, or a Pro residue followed by three basic residues. The bipartite NLS consists of two clusters of basic residues separated by a 10-aa long unspecific sequence [22]. NLS mutations of TyrRS reduce its concentration in the nucleus [43]. The mutations do not significantly affect the aminoacylation activity of TyrRS, but do cause retardation in nuclear $tRNA^{Tyr}$ export [43]. Some aaRSs, like yeast MetRS lack the typical NLSs [22]. To get into the nucleus they have to bind other NLS-containing proteins, like Arc1p (three NLSs of the SV40 large antigen T type) [22].

It has been found that NLS sequences bind to DNA and change its conformation [44]. The role of this phenomenon is unknown but it may be involved in DNA-conformation dependent regulation of gene expression. The 8-aa long fragment of *Lupinus luteus* GlnRS, $K^{197}PKKKKEK^{204}$ (NLS of the SV40 large antigen T type) specifically binds linear duplex of poly d(Gm^5C) and, in contrast to poly d(GC), changes its conformation from B to Z form [44, 45]. The conformational transition of the right-handed helix of B form into the left-handed Z helix is one of the most radical observed for DNA [45]. The Z form is somehow correlated with DNA transcription. Z DNA is transiently generated *in vitro* adjacent to a moving RNA polymerase (RNAP)

[46–48]. Such conformation of DNA is not transcribed by RNAP but may recruit specific proteins responsible for post-transcriptional processing of RNA [45–47]. It has been found that ADAR1, a protein responsible for the guanine to inosine (G→I) modification, specifically recognizes Z DNA regions [47, 48]. Recently it has been shown that PheRS is able to bind specifically certain B DNA sequences [49, 50]. The winged helix-turn-helix (HTH) motif of the B5 domain of *Thermus thermophilus* PheRS binds B DNA causing DNA bending and looping [50].

AaRS IN THE NUCLEUS

AaRSs have been found in the cytoplasm and as well as in the nucleus. However, their physicochemical properties were different [2, 7]. The nuclear activity represents only a small percentage (2–3%) of the cytoplasmatic activity of aaRSs [7]. Nuclear aaRSs form more stable and larger multienzymatic complexes than their cytoplasmatic homologues (2.5 MDa *versus* 1.3 MDa) [7]. Mammalian (rat, Chinese hamster, rabbit) ArgRS which exists simultaneously in a multienzymatic complex and as a "free" form in the cytoplasm, forms exclusively the high molecular mass multienzymatic complex in the nucleus [7, 51, 52]. It was postulated that the non-complexed, "free" ArgRS provides Arg-tRNA^{Arg} used for the N-terminal arginylation of proteins targeted for ubiquitin-dependent degradation [25]. This process is carried out exclusively in the cytoplasm. The complexation is not directly linked with the basal enzymatic activity of aaRSs but may modulate their kinetics [2].

The nuclear aminoacylation of tRNA is highly specific. After injection of ^{35}S-Met into the nucleus only tRNA^{Met} is aminoacylated [6]. Leucine starvation causes nuclear accumulation only of its cognate tRNA [34]. Mutated MetRS or inhibited TyrRSs (or LeuRS or IleRS) block nuclear export mostly of their cognate tRNAs [6, 8, 22, 34, 53]. However, in some cases nuclear accumulation of non-cognate as well cognate tRNA has been observed [53]. So far the question whether there is a small immobile nuclear pool of aaRSs or they are able to shuttle between the nucleus and the cytoplasm is unanswered.

THE "CLASSICAL" PATHWAY OF NUCLEAR EXPORT OF tRNA

The separation of the nucleus from the cytoplasm by the nuclear envelope necessitates effective bi-directional transport of biomolecules across the envelope [37–39]. This transport is carried out through NPC channels [32–35]. Before leaving the nucleus tRNA undergoes complex, not yet fully understood processes collectively called maturation [12, 54–56]. Maturation is a proofreading mechanism (base modifications, processing of the ends and in some cases splicing) that insures that incompletely processed or mutated tRNA will be retained in the nucleus. Mature tRNA is exported from the nucleus by a member of the importin/karyopherin superfamily, Los1p (in yeast) or its mammalian homologue Xpo-t (Fig. 1) [34, 55–59]. The export is an energy-dependent process that requires the presence of the GTPase Ran [34, 58, 60–62]. Los1p and Xpo-t are unique among karyopherin proteins because they can directly interact with RNA [34, 63]. Yeast cells lacking Los1p are viable which suggests alternative, Los1p-independent mechanisms of nuclear export of tRNA [6, 8, 34, 55, 56]. Xpo-t almost exclusively interacts with the TΨC loop and the acceptor stem of tRNA [12, 34]. A minihelix composed of these two elements is not recognized by Xpo-t, suggesting that structural features are important for tRNA/Xpo-t interaction [12, 34, 64]. Beyond the functional tertiary structure, Xpo-t requires correctly maturated ends with the CCA sequence at the 3′-end of tRNA [6, 12, 34]. Introns are tolerated only in regions which do not interact with

Xpo-t, like the anticodon domain [34]. However, such not fully maturated tRNA may be exported from the nucleus only in the presence of Xpo-t excess [65–67]. Under physiological conditions, splicing occurs before processing of the ends thus kinetic control of maturation restricts the access of Xpo-t to intron-containing tRNA [6, 65]. Xpo-t displays high affinity (nanomolar range) for processed but unmodified tRNA. Native, modified nucleosides-containing tRNA is bound with even higher affinity [58, 59, 64, 65]. It is possible that the modified nucleosides play some role in quality control of tRNA, mediating of tRNA interaction with Xpo-t.

THE "ALTERNATIVE" PATHWAY OF NUCLEAR EXPORT OF tRNA

Los1p and Xpo-t are major nuclear proteins responsible for export of maturated tRNA. However, while mammalian Xpo-t is the main tRNA export receptor, in yeast the Los1p-dependent tRNA export pathway is much less important. Los1p is essential only for nuclear export of tRNA in Arc1p and eIF-2γ defective cells [56, 57]. These proteins are involved in tRNA aminoacylation and in translation. The first evidence of alternative, aminoacylation-dependent nuclear export of tRNA was the discovery of aminoacyl-tRNA in the nucleus [6]. How aminoacylation of tRNA acts on its nuclear export is not clear but it has been shown that inhibition of the nuclear aminoacylation led to a retardation of tRNA export [6, 34, 53]. The mechanism of nuclear export of aminoacylated tRNA and the influence of tRNA aminoacylation on its affinity for the main nuclear tRNA carrier, Xpo-t/Los1p, is unknown [12, 58, 65]. It is possible that nuclear aminoacylation of tRNA is not connected with Xpo-t/Los1p and a fully independent pathway of nuclear export of aminoacyl-tRNA exists (Fig. 1). Although the nuclear aminoacylation of tRNA is not necessary for cell viability, it is known that aminoacylated tRNA is exported from the nucleus much more efficiently than uncharged tRNA [6]. The preferential nuclear export of aminoacylated tRNA may act as a proofreading step of the maturation.

TRANSLATION IN THE NUCLEUS

The presence of translation apparatus components (aaRS, aminoacyl-tRNA, elongation factors) in the nucleus rises the question about the possibility of nuclear translation. *Prokaryota* do not separate transcription from translation and their ribosomes bind at the 5′-end of mRNA while the transcript is still being made. It is widely assumed that eukaryotic transcription and translation are separated by the nuclear envelope. Only mature, intron-free mRNA transported to the cytoplasm is translated. New results show that this "standard model" of eukaryotic translation may be an oversimplification. Although nuclear translation was claimed back in the 1950s, a direct proof of this was obtained only in 2001 [67]. Biotin-Lys-tRNA^{Lys} was used to trace peptide chain elongation [67]. Suboptimal conditions of nuclear export/import to prevent transport of nascent biotinylated peptides were used. Although most of the biotinylated peptides (synthesis of peptides on average 15-aa long was allowed) were found in the cytoplasm, some were detected in the nucleus, at discrete nuclear sites known as transcription "factories" [67]. The nuclear translation was estimated to be about 15% of total cytoplasmatic protein biosynthesis. Similar results were obtained with isolated nuclei. The translation inhibitor cycloheximide strongly blocked the nuclear translation [67]. Low concentration of a translation initiation inhibitor, aurintricarboxylic acid (ATA), did not reduce the nuclear translation, whereas higher concentration of ATA that blocked the elongation step strongly inhibited (90%) nuclear biotinylation of the peptides [67]. These results led to the conclusion that the transla-

tion inhibitors block the incorporation of biotin-Lys-tRNA^{Lys} into already working structures of the nuclear translation machinery. The role of the nuclear translation is unknown. Experimental data showed that after elongation of the label time much of the biotinylated products of the nuclear translation are high molecular mass proteins [67]. So far there is no clue where these proteins go or what they do. It was proposed that the nuclear translation is connected with nonsense-mediated decay (NMD), a quality-control mechanism in which mRNA is surveyed for the presence of nonsense codons [67].

Some experiments showed that nuclear transcription and translation might be carried out not only in the same place, but also might be correlated with each other [67]. Nuclear concentration of the biotinylated peptides increased with increasing nuclear concentration of ribonucleoside triphosphates. This effect was not observed when RNAP II was specifically inhibited with α-amanitin [67]. These results strongly resemble the correlation between prokaryotic transcription and translation.

CONCLUSIONS

The results obtained so far on nuclear export and aminoacylation of tRNA suggest the existence of at least two parallel (independent?) pathways of tRNA export. Cells lacking the major nuclear tRNA receptor, Los1p (or Xpo-t) are viable and may use an alternative, aminoacyl-tRNA-dependent pathway of tRNA export. It seems that there are no preferences for any tRNA species for the nuclear aminoacylation. If so, that implies the presence of all twenty aaRSs in the nucleus. At least thirteen species of aaRSs have been identified in the nucleus so far [7]. No details about translocation of aminoacyl-tRNA and aaRS across the nuclear envelope are known. It is unknown whether aminoacylated tRNA alone or its complex with aaRS crosses the nuclear envelope. There is a possibility that aaRSs are able to shuttle between the nucleus and the cytoplasm, serving as a nuclear export receptor of aminoacylated tRNA. Recent experiments have shown that both aaRS and proteins involved in translation were necessary for efficient nuclear export of tRNA [6, 34]. The elongation factor eEF1A has been localized in the nucleus [6, 8, 30, 31, 68]. eEF1A is involved in nuclear aminoacylation and export of tRNA [8]. Aminoacylated tRNA has a higher affinity for eEF1A than uncharged tRNA [22, 68]. Aminoacylation and following binding eEF1A were found in both the cytoplasm and the nucleus. Thus, aminoacylation of tRNA may be a prerequisite for eEF1A-dependent nuclear tRNA export. The involvement of eEF1A in the nuclear export of tRNA and translation suggests a correlation of these processes.

Until recently, it was widely assumed that eukaryotic transcription and translation are spatially separated by the nuclear envelope. Recent experiments have shown that translation also occurs in the nucleus. Moreover, the nuclear translation is somehow correlated with RNAP II-dependent transcription. The role of the nuclear translation and the fate of nucleus-born proteins are unknown. The finding of the nuclear translation necessitates a reappraisal of the widely accepted ideas on the compartmentalization of discrete steps of the eukaryotic cell metabolism. The discovery in the nucleus of aaRS-dependent aminoacylation of tRNA and translation shows us how exciting and surprising is the world of the living cell.

REFERENCES

1. Lee, N., Bessho, Y., Wei, K., Szostak, J.W. & Suga, H. (2000) Ribozyme-catalyzed tRNA aminoacylation. *Nat. Struct. Biol.* **7**, 28–33.

2. Kisselev, L.L. & Wolfson, A.D. (1994) Aminoacyl-tRNA synthetases from higher

eukaryotes. *Prog. Nucleic Acid Res. Mol. Biol.* **48**, 83–142.

3. Krzyzaniak, A., Salanski, P., Twardowski, T., Jurczak, J. & Barciszewski, J. (1998) tRNA aminoacylated at high pressure is a correct substrate for protein biosynthesis. *Biochem. Mol. Biol. Int.* **45**, 489–500.

4. Beuning, P.J. & Musier-Forsyth, K. (1999) Transfer RNA recognition by aminoacyl-tRNA synthetases. *Biopolymers* **52**, 1–28.

5. Martinis, S.A., Plateau, P., Cavarelli, J. & Florentz, C. (1999) Aminoacyl-tRNA synthetases: A new image for a classical family. *Biochimie* **81**, 683–700.

6. Lund, E. & Dahlberg, J.E. (1998) Proofreading and aminoacylation of tRNAs before export from the nucleus. *Science* **282**, 2082–2085.

7. Nathanson, L. & Deutscher, M.P. (2000) Active aminoacyl-tRNA synthetases are present in nuclei as a high molecular weight multienzyme complex. *J. Biol. Chem.* **275**, 31559–31562.

8. Grosshans, H., Hurt, E. & Simos, G. (2000) An aminoacylation-dependent nuclear tRNA export pathway in yeast. *Genes Dev.* **14**, 830–840.

9. Ibba, M. & Soll, D. (2000) Aminoacyl-tRNA synthesis. *Annu. Rev. Biochem.* **69**, 617–650.

10. Bandyopadhyay, A.K. & Deutscher, M.P. (1971) Complex of aminoacyl-transfer RNA synthetases. *J. Mol. Biol.* **60**, 113–122.

11. Yang, D.C. (1996) Mammalian aminoacyl-tRNA synthetases. *Curr. Top. Cell. Regul.* **34**, 101–136.

12. Simos, G. & Hurt, E. (1999) Transfer RNA biogenesis: A visa to leave the nucleus. *Curr. Biol.* **9**, R238–R241.

13. Robinson, J.C., Kerjan, P. & Mirande, M. (2000) Macromolecular assemblage of aminoacyl-tRNA synthetases: Quantitative analysis of protein–protein interactions and mechanism of complex assembly. *J. Mol. Biol.* **304**, 983–994.

14. Norcum, M.T. & Warrington, J.A. (1998) Structural analysis of the multienzyme aminoacyl-tRNA synthetase complex: A three-domain model based on reversible chemical crosslinking. *Protein Sci.* **7**, 79–87.

15. Deinert, K., Fasiolo, F., Hurt, E.C. & Simos, G. (2001) Arc1p organizes the yeast aminoacyl-tRNA synthetase complex and stabilizes its interaction with the cognate tRNAs. *J. Biol. Chem.* **276**, 6000–6008.

16. Mirande, M. (1991) Aminoacyl-tRNA synthetase family from prokaryotes and eukaryotes: Structural domains and their implications. *Progr. Nucleic Acid Res. Mol. Biol.* **40**, 95–142.

17. Norcum, M.T. (1999) Ultrastructure of the eukaryotic aminoacyl-tRNA synthetase complex derived from two dimensional averaging and classification of negatively stained electron microscopic images. *FEBS Lett.* **447**, 217–222.

18. Norcum, M.T. (1989) Isolation and electron microscopic characterization of the high molecular mass aminoacyl-tRNA synthetase complex from murine erythroleukemia cells. *J. Biol. Chem.* **264**, 15043–15051.

19. Norcum, M.T. & Dignam, J.D. (1999) Immunoelectron microscopic localization of glutamyl-prolyl-tRNA synthetase within the eukaryotic multisynthetase complex. *J. Biol. Chem.* **274**, 12205–12208.

20. Cerini, C., Kerjan, P., Astier, M., Gratecos, D., Mirande, M. & Semeriva, M. (1991) A component of the multisynthetase complex is a multifunctional aminoacyl-tRNA synthetase. *EMBO J.* **10**, 4267–4277.

21. Kaiser, E., Hu, B., Becher, S., Eberhard, D., Schray, B., Baack, M., Hameister, H. & Knippers, R. (1994) The human EPRS locus (formerly the QARS locus): A gene encoding a class I and a class II aminoacyl-tRNA synthetase. *Genomics* **19**, 280–290.

22. Schimmel, P. & Wang, C.C. (1999) Getting tRNA synthetases into the nucleus. *Trends Biochem. Sci.* **24**, 127–128.

23. Ibba, M., Curnow, A.W. & Soll, D. (1997) Aminoacyl-tRNA synthesis: Divergent routes to a common goal. *Trends Biochem. Sci.* **22**, 39–42.

24. Negrutskii, B.S., Stapulionis, R. & Deutscher, M.P. (1994) Supramolecular organization of the mammalian translation system. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 964–968.

25. Sivaram, P. & Deutscher, M.P. (1990) Existence of two forms of rat liver arginyl-tRNA synthetase suggests channeling of aminoacyl-tRNA for protein synthesis. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 3665–3669.

26. Amar-Costesec, A. & Turu, C. (1989) Subcellular topology of rat liver methionyl-, leucyl-, and arginyl-tRNA synthetases. *Biol. Cell* **65**, 21–28.

27. Hampel, A. & Enger, M.D. (1973) Subcellular distribution of aminoacyl-transfer RNA synthetases in Chinese hamster ovary cell culture. *J. Mol. Biol.* **79**, 285–293.

28. Mirande, M., Le Corre, D. & Waller, J.P. (1985) A complex from cultured Chinese hamster ovary cells containing nine aminoacyl-tRNA synthetases. Thermolabile leucyl-tRNA synthetase from the tsH1 mutant cell line is an integral component of this complex. *Eur. J. Biochem.* **147**, 281–289.

29. Popenko, V.I., Ivanova, J.L., Cherny, N.E., Filonenko, V.V., Beresten, S.F., Wolfson, A.D. & Kisselev, L.L. (1994) Compartmentalization of certain components of the protein synthesis apparatus in mammalian cells. *Eur. J. Cell. Biol.* **65**, 60–69.

30. Barbarese, E., Koppel, D.E., Deutscher, M.P., Smith, C.L., Ainger, K., Morgan, F. & Carson, J.H. (1995) Protein translation components are colocalized in granules in oligodendrocytes. *J. Cell Sci.* **108**, 2781–2790.

31. Sanders, J., Brandsma, M., Janssen, G.M., Dijk, J. & Moller, W. (1996) Immunofluorescence studies of human fibroblasts demonstrate the presence of the complex of elongation factor-1 beta gamma delta in the endoplasmic reticulum. *J. Cell Sci.* **109**, 1113–1117.

32. Gamblin, S.J. & Smerdon, S.J. (1999) Nuclear transport: What a kary-on! *Structure Fold. Des.* **7**, R199–R204.

33. Cullen, B.R. (2000) Nuclear RNA export pathways. *Mol. Cell. Biol.* **20**, 4181–4187.

34. Grosshans, H., Simos, G. & Hurt, E. (2000) Review: Transport of tRNA out of the nucleus – direct channeling to the ribosome? *J. Struct. Biol.* **129**, 288–294.

35. Adam, S.A. (1999) Transport pathways of macromolecules between the nucleus and the cytoplasm. *Curr. Opin. Cell Biol.* **11**, 402–406.

36. Nigg, E.A. (1997) Nucleoplasmatic transport: Signals, mechanisms and regulation. *Nature* **386**, 779–786.

37. Strom, A.C. & Weis, K. (2001) Importin-beta-like nuclear transport receptors. *Genome Biol.* **2**, 1–9.

38. Weis, K. (1998) Importins and exportins: How to get in and out of the nucleus. *Trends Biochem. Sci.* **23**, 185–189.

39. Kutay, U., Hartmann, E., Treichel, N., Calado, A., Carmo-Fonseca, M., Prehn, S., Kraft, R., Gorlich, D. & Bischoff, F.R. (2000) Identification of two novel RanGTP-binding proteins belonging to the importin beta superfamily. *J. Biol. Chem.* **275**, 40163–40168.

40. Corbett, A.H. & Silver, P.A. (1997) Nucleocytoplasmic transport of macromolecules. *Microbiol. Mol. Biol. Rev.* **61**, 193–211.

41. Kalderon, D., Roberts, B.L., Richardson, W.D. & Smith, A.E. (1984) A short amino acid sequence able to specify nuclear location. *Cell* **39**, 499–509.

42. Makkerh, J.P., Dingwall, C. & Laskey, R.A. (1996) Comparative mutagenesis of nuclear localization signals reveals the importance of neutral and acidic amino acids. *Curr. Biol.* **6**, 1025–1027.

44. Krzyzaniak, A., Siatecka, M., Szyk, A., Mucha, P., Rekowski, P., Kupryszewski, G. & Barciszewski, J. (2000) Specific induction of Z-DNA conformation by a nuclear localization signal peptide of lupin glutaminyl tRNA synthetase. *Mol. Biol. Rep.* **27**, 51–54.

45. Schade, M., Behlke, J., Lowenhaupt, K., Herbert, A., Rich, A. & Oschkinat, H. (1999) A 6 bp Z-DNA hairpin binds two Z alpha domains from the human RNA editing enzyme ADAR1. *FEBS Lett.* **458**, 27–31.

46. Herbert, A. & Rich, A. (1996) The biology of left-handed Z-DNA. *J. Biol. Chem.* **271**, 11595–11598.

47. Peck, L.J. & Wang, J.C. (1985) Transcriptional block caused by a negative supercoiling induced structural change in an alternating CG sequence. *Cell* **40**, 129–137.

48. Kim, Y.G., Lowenhaupt, K., Maas, S., Herbert, A., Schwartz, T. & Rich, A. (2000) The zab domain of the human RNA editing enzyme ADAR1 recognizes Z-DNA when surrounded by B-DNA. *J. Biol. Chem.* **275**, 26828–26833.

49. Dou, X., Limmer, S. & Kreutzer, R. (2001) DNA-binding of phenylalanyl-tRNA synthetase is accompanied by loop formation of the double-stranded DNA. *J. Mol. Biol.* **305**, 451–458.

50. Lechler, A. & Kreutzer, R. (1998) The phenylalanyl-tRNA synthetase specifically binds DNA. *J. Mol. Biol.* **278**, 897–901.

51. Deutscher, M.P. & Ni, R.C. (1982) Purification of a low molecular weight form of rat liver arginyl-tRNA synthetase. *J. Biol. Chem.* **257**, 6003–6006.

52. Vellekamp, G., Sihag, R.K. & Deutscher, M.P. (1985) Comparison of the complexed and free forms of rat liver arginyl-tRNA synthetase and origin of the free form. *J. Biol. Chem.* **260**, 9843–9847.

53. Sarkar, S., Azad, A.K. & Hopper, A.K. (1999) Nuclear tRNA aminoacylation and its role in nuclear export of endogenous tRNAs in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 14366–14371.

54. Wolin, S.L. & Matera, A.G. (1999) The trials and travels of tRNA. *Genes Dev.* **13**, 1–10.

55. Hopper, A.K., Schultz, L.D. & Shapiro, R.A. (1980) Processing of intervening sequences: A new yeast mutant which fails to excise intervening sequences from precursor tRNAs. *Cell* **19**, 741–751.

56. Hellmuth, K., Lau, D.M., Bischoff, F.R., Kunzler, M., Hurt, E. & Simos, G. (1998) Yeast Los1p has properties of an exportin-like nucleocytoplasmic transport factor for tRNA. *Mol. Cell. Biol.* **18**, 6374–6386.

57. Gorlich, D., Dabrowski, M., Bischoff, F.R., Kutay, U., Bork, P., Hartmann, E., Prehn, S. & Izaurralde, E. (1997) A novel class of RanGTP binding proteins. *J. Cell Biol.* **138**, 65–80.

58. Kutay, U., Lipowsky, G., Izaurralde, E., Bischoff, F.R., Schwarzmaier, P., Hartmann, E. & Gorlich, D. (1998) Identification of a tRNA-specific nuclear export receptor. *Mol. Cell* **1**, 359–369.

59. Arts, G.J., Fornerod, M. & Mattaj, I.W. (1998) Identification of a nuclear export receptor for tRNA. *Curr. Biol.* **8**, 305–314.

60. Dahlberg, J.E. & Lund, E. (1998) Functions of the GTPase Ran in RNA export from the nucleus. *Curr. Opin. Cell. Biol.* **10**, 400–408.

61. Izaurralde, E. & Adam, S. (1998) Transport of macromolecules between the nucleus and the cytoplasm. *RNA* **4**, 351–364.

62. Sarkar, S. & Hopper, A.K. (1998) tRNA nuclear export in *Saccharomyces cerevisiae*: *In situ* hybridization analysis. *Mol. Biol. Cell* **9**, 3041–3055.

63. Hurt, D.J., Wang, S.S., Lin, Y.H. & Hopper, A.K. (1987) Cloning and characterization of Los1, a *Saccharomyces cerevisiae* gene that affects tRNA splicing. *Mol. Cell. Biol.* **7**, 1208–1216.

64. Arts, G.J., Kuersten, S., Romby, P., Ehresmann, B. & Mattaj, I.W. (1998) The role of exportin-t in selective nuclear export of mature tRNAs. *EMBO J.* **17**, 7430–7441.

65. Lipowsky, G., Bischoff, F.R., Izaurralde, E., Kutay, U., Schafer, S., Gross, H.J., Beier, H. & Gorlich, D. (1999) Coordination of tRNA nuclear export with processing of tRNA. *RNA* **5**, 539–549.

66. Simos, G., Sauer, A., Fasiolo, F. & Hurt, E.C. (1998) A conserved domain within Arc1p delivers tRNA to aminoacyl-tRNA synthetases. *Mol. Cell.* **1**, 235–242.

67. Iborra, F.J., Jackson, D.A. & Cook, P.R. (2001) Coupled transcription and translation within nuclei and mammalian cells. *Science* **293,** 1139–1142.

68. Dreher, T.W., Uhlenbeck, O.C. & Browning, K.S. (1999) Quantitative assessment of EF-1alpha. GTP binding to aminoacyl-tRNAs, aminoacyl-viral RNA, and tRNA shows close correspondence to the RNA binding properties of EF-Tu. *J. Biol. Chem.* **274**, 666–672.