Cell. Mol. Life Sci. 63 (2006) 1813–1818
1420-682X/06/161813-6
DOI 10.1007/s00018-006-6092-9

Cellular and Molecular Life Sciences

Visions & Reflections

tRNA, new aspects in intracellular dynamics

T. Yoshihisa

Research Center for Materials Science, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8602 (Japan),
Fax: +81 52 789 2491, e-mail: tyoshihi@biochem.chem.nagoya-u.ac.jp

Received 2 March 2006; received after revision 15 April 2006; accepted 12 May 2006
Online First 23 June 2006

Abstract. The nuclear envelope divides a eukaryotic cell into two compartments, the nucleus and the cytoplasm. Transcription and maturation of RNAs encoded on nuclear chromosomes are carried out in the nucleus, while the proteins coded by these RNAs are translated and processed in the cytoplasm. Cytosolic tRNAs, essential factors for translation, are transcribed in the nucleus and undergo extensive processing before reaching functionality. It had previously been believed that tRNAs have only one-way tickets to pass through the nuclear envelope after maturation in the nucleus, and that the small amounts of mature tRNAs found in the nucleus are biosynthetic intermediates. However, two reports from our lab and Anita Hopper's group recently demonstrated that tRNAs have multi-round commuter tickets to shuttle between the nucleus and the cytoplasm in the yeast *Saccharomyces cerevisiae*. In fact, various tRNA species, including aminoacylated full-length tRNAs and 3′ end-shortened tRNAs, are actively imported into the nucleus under various conditions. These findings force a reconsideration of our view of intracellular dynamics of tRNAs, and re-evaluations of the physiological meanings of the nuclear mature tRNAs.

Keywords. tRNA, nuclear import, nuclear export, tRNA splicing, quality control, amino acid deprivation, aminoacylation.

Introduction

Eukaryotic cells have developed fine subcellular compartments to share the responsibility for various biological activities. Among such compartmentalization events, the separation of the nucleus and the cytoplasm by the nuclear envelope (NE) was the most crucial during the evolution of eukaryotic cells. This separation enabled eukaryotic cells to achieve more sophisticated function and regulation of replication, transcription, translation and signal transduction than achieved in prokaryotic cells. In turn, eukaryotic cells had to evolve nuclear-cytoplasmic transport systems to allow the regulated movement of macromolecules across the NE [1, 2]. For example, many RNAs have to move from the nucleus, their birthplace, to the cytoplasm, their workplace, while proteins functioning in the nucleus have to move in the opposite direction. The finding of leptomycin B, a potent inhibitor for the Crm1-mediated nuclear export pathway, revealed that some proteins dynamically shuttle between the nucleus and the cytoplasm [3–5]. On the other hand, most nuclear-encoded RNAs, including tRNAs, have been considered to experience nuclear transport only once after transcription and maturation in the nucleus [1, 6, 7]. The only known class of RNAs returning to the nucleus has been U snRNAs. In metazoan cells, U1, U2, U4 and U5 snRNAs are exported to the cytoplasm to receive hyper-methylation of their cap structures and to form complexes with proteins, and then are re-imported into the nucleus, their final destination [8, 9]. Most RNAs exported to the cytoplasm stay there to exert their functions, while a subset of tRNAs are further delivered to mitochondria and plastids, their functional sites, in some organisms [10, 11].

However, the fundamental notion that most RNAs have only 'one-way tickets' to cross the NE is called into question in the case of the tRNAs in *Saccharomyces cerevi-*

siae. We found that, during maturation of intron-containing pre-tRNAs, their splice sites are cleaved on the mitochondrial surface in *S. cerevisiae* [12]. tRNA splicing is governed by three enzymes, splicing endonuclease, ligase and 2′-phosphotransferase [13, 14]. Indeed, the splicing endonuclease activity and subunits were found to localize on the outer surface of yeast mitochondria. Unspliced pre-tRNAs accumulated in the cytoplasm of *sen2* mutants, which were defective in one of the two catalytic subunits of yeast tRNA splicing endonuclease [12, 13]. These results suggested that pre-tRNAs are exported to the cytoplasm prior to splicing. On the other hand, it is known that spliced forms of intron-containing tRNAs accumulate in the nucleus when nuclear transport is perturbed by mutations of RanGAP (Rna1p), nucleoporins (Nup116p, *etc.*), tRNA-specific exportin (Los1p), CCA transferase also acting on tRNA export (Cca1p), aminoacyl tRNA synthetases (ARS, for example Tys1p, *etc*.), nucleolar factor Utp8p and so on [15–21]. All the facts mentioned above led us to the unexpected notion that tRNAs must be re-imported from the cytoplasm into the nucleus after maturation. However, there has not been any direct evidence for nuclear import of mature tRNAs. Last year, our lab and Anita Hopper's group independently demonstrated that mature tRNAs are indeed re-imported from the cytoplasm to the nucleus [22, 23]. In this article, I summarize the findings from the two labs and discuss the physiological meanings of mature tRNAs in the nucleus.

Finding of tRNA import

To demonstrate the nuclear import of tRNAs, both groups first utilized heterokaryon assays. Two haploids of *S. cerevisiae* with opposite mating types undergo mating and transiently form a heterokaryon with two nuclei in a common cytoplasm. The duration of this situation is prolonged when nuclear fusion is inhibited by certain mutations [24, 25]. When an exogenous tRNA with a different sequence from the cognate endogenous tRNA is expressed from only one of the haploids, one can examine whether or not the exogenous tRNA transcribed from one nucleus can enter into the other nucleus in a heterokaryon. Although different exogenous tRNA genes (from *Schizosaccharomyces pombe* or *Dictyostelium discoideum*) were used, both groups detected mature tRNAs in the nucleus that did not express these tRNAs when nuclear export of tRNAs was compromised by the Δ*los1* mutation, which is deficient in one of exportin genes specific for tRNAs [22, 23].

To demonstrate tRNA import in the vegetative growth phase, we used a Δ*los1* Δ*msn5* double mutant, which displayed more prominent accumulation of mature tRNAs in the nucleus than the Δ*los1* single mutant. When Δ*los1* Δ*msn5* cells were treated with sodium azide and 2-deoxyglucose to deplete intracellular ATP, the tRNA gradient across the NE disappeared. The gradient was re-established by removing the energy poisons. The re-establishment of the tRNA gradient after removal of the energy poisons was also observed even when transcription of tRNAs was blocked by adding thiolutin, a potent inhibitor of all three classes of RNA polymerases [26]. The nuclear accumulation of mature tRNAs in the absence of transcription entails a supply of pre-existing mature tRNAs from the cytoplasm to the nucleus [22]. On the other hand, Hopper's group utilized different conditions to upregulate tRNA import. It is known that a mature tRNA is accumulated in the nucleus when its cognate amino acid is depleted from the medium [19]. They found that, under the amino acid-deprivation conditions, the reporter tRNA was accumulated in both nuclei of a heterokaryon in the absence of the Δ*los1* mutation background. They concluded that, also in the case of vegetatively growing cells, the nuclear accumulation of mature tRNAs observed under these conditions results from an enhancement of nuclear import from the cytoplasm [23]. Both of these results demonstrate that there exists retrograde transport of mature tRNAs to the nucleus and that tRNAs shuttle between the nucleus and the cytoplasm during their life.

Characteristics of tRNA import

What kinds of tRNAs are imported into the nucleus? Our group and Hopper's group found that tRNAs transcribed from both intron-containing and intron-less genes are imported into the nucleus [22, 23]. Furthermore, as mentioned above, tRNAs derived from both endogenous and exogenous genes are imported. Thus, nuclear import of tRNAs is not specific for intron-containing tRNAs, which must be exported to the cytoplasm before their final maturation, or certain tRNAs specific for *S. cerevisiae*. Rather, the nuclear import machinery seems to recognize the general features of tRNAs. At present, whether intron-containing pre-tRNAs can be re-imported into the nucleus remains to be analyzed.

Large portions of mature tRNAs were found to be aminoacylated when tRNA import was analyzed during recovery after energy depletion in Δ*los1* Δ*msn5* cells [22]. This suggests that aminoacylated tRNAs are substrates for the import machinery, although we still cannot rule out the possibility that free tRNAs are aminoacylated after their import into the nucleus. If aminoacylated tRNAs are imported into the nucleus, they may form complexes with eEF1A, which takes aminoacylated tRNAs to the ribosomal A site [27], during nuclear import. Certain eEF1A mutants cause nuclear accumulation of mature tRNAs, suggesting that eEF1A acts on tRNA export [19]. In fact, eEF1A is a shuttling protein. In mammalian cells,

exportin-5, one of the export carriers for tRNAs, exports tRNA-eEF1A complexes, and exportin-5 is a homologue of yeast Msn5p [28, 29]. It might be possible that nuclear eEF1A is also supplied from the cytoplasm in the form of complexes with tRNAs. On the other hand, under the amino acid-deprivation conditions that Hopper's group adopted, the cognate tRNAs should be mostly deacylated [23]. In fact, mature tRNA-Leu^{CAA} is accumulated in the nucleus when aminoacylation is blocked by adding a Leu-RS inhibitor or by deprivation of leucine from the medium [19]. It was originally proposed that the tRNA quality control machinery on the inner surface of the NE deselects deacylated tRNAs from nuclear export so as to accumulate mature tRNAs in the nucleus under amino acid-deprivation conditions [18, 30]. However, acceleration of nuclear import of cytoplasmic deacylated tRNAs would also contribute to such accumulation. Therefore, both aminoacylated and deacylated tRNAs can be imported into the nucleus.

We also found that tRNAs are imported into the *cca1-1* mutant nucleus. The *cca1-1* mutation causes CCA transfer defects both for newly transcribed tRNAs in the nucleus and for cytoplasmic mature tRNAs with a shortened 3′-end [31]. *cca1-1* cells also exhibit defects in tRNA export [17]. We found that mature tRNAs accumulate in the *cca1-1* nucleus under the condition that most of the tRNAs have the shortened 3′-end. Especially in the case of tRNA-Ile^{AAU}, more than 90% of the tRNA was in the short form [22]. Therefore, tRNAs with some structural defects can be imported. These results suggest that various tRNA species are imported into the nucleus.

How are mature tRNAs imported into the nucleus?

As described above, nuclear import of mature tRNAs apparently requires energy. Most of the nuclear transport pathways are driven by the Ran GTPase gradient across the NE [1, 2]. Ran GTPase is a Ras-like small GTPase mainly localized in the nucleus. The GTP-binding form of Ran is restricted to the nucleus, while its GDP form is exclusively cytoplasmic. This gradient is achieved by two essential regulators, guanine nucleotide exchange factor RCC1 and GTPase activating protein RanGAP. When nuclear import of tRNAs is measured during recovery after energy depletion, the Ran GTPase gradient is not required for the import. A temperature sensitive mutant of yeast RanGAP, *rna1-1*, accumulates mature tRNAs in the nucleus [16]. This tRNA gradient across the NE in *rna1-1* cells was disrupted by the energy poisons, and was re-established when intracellular ATP was replenished in the absence of transcription at a restrictive temperature [22]. These results indicate that tRNA import continues even when the Ran GTPase cycle is perturbed. On the other hand, Hopper's group reported different results. They observed that when tRNA import was measured by the heterokaryon assay with *rna1-1* mutant cells under amino acid-deprivation conditions, the mutant heterokaryons did not import *D. discoideum* tRNA-Glu^{D} into the nucleus [23]. They also analyzed accumulation of mature tRNAs in the nucleus under amino acid-deprivation conditions in 9 out of 14 importin/exportin mutants of *S. cerevisiae*. They found that one of the importin mutants, Δ*mtr10*, did not accumulate mature tRNAs in the nucleus under these conditions [23]. Mtr10p was originally reported as a nuclear import carrier of the mRNA-binding protein Npl3p [32]. Mtr10p also acts on nuclear import of Tlc1 RNA, an RNA component of telomerase [33]. There are several ways to explain this discrepancy. One possibility is that Ran- and Mtr10p-dependent nuclear transport is required not for the import of mature tRNAs *per se* but for the requisite signal transduction to up-regulate nuclear import of tRNAs. As discussed later, tRNA dynamics are somehow regulated according to their respective cellular environments. Such regulation may require some nuclear-cytoplasmic transport of a regulatory factor(s), and this transport may be governed by Ran and Mtr10p. A more likely explanation is that nuclear import of tRNAs is driven by two or more different mechanisms. In the case of tRNA export, it has been shown that there are several parallel pathways. At least in yeast, two exportins, Los1p and Msn5p, and Cca1p are responsible for tRNA export in parallel [15–20, 22]. This may be true as well for nuclear import: one of the tRNA import machineries active under non-starvation conditions is Ran independent, and another machinery activated by amino acid depletion is Ran dependent.

Physiological roles of mature tRNAs in the nucleus

Both our group and Hopper's group have revealed that yeast cells actively maintain nuclear-cytoplasmic shuttling of mature tRNAs. One essential but unsolved question is the purpose for which yeast cells require such tRNA shuttling activity, in other words, what the physiological roles of the mature tRNAs in the nucleus specifically are. As mentioned above, yeast cells import various kinds of tRNA species from the cytoplasm into the nucleus under various conditions. There are several possibilities that are not mutually exclusive, and four of these are considered here (Fig. 1).

One interesting but highly controversial possibility is that nuclear tRNAs are utilized by translation in the nucleus. Iborra *et al.* [34] reported that some level of translation is detected in the mammalian nucleus, and suggested that this level of translation is used for non-sense mediated mRNA decay (NMD) to search premature termination codons on mRNAs. In fact, some reports have claimed that NMD is coupled with alternative splicing and that certain aberrant mRNAs with premature termination codons accumulate in the nucleus [35, 36]. There are translation-competent large and small ribosomal subunits (or

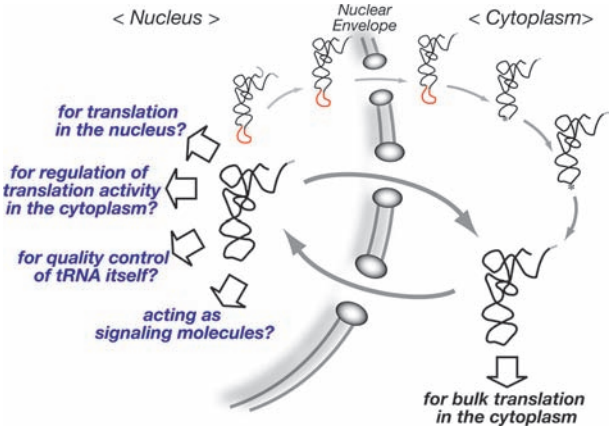

Figure 1. Possible functions of mature tRNAs in the nucleus. A new view of intracellular dynamics of tRNAs is schematically represented. Primary transcripts of tRNAs (upper left) become end-matured in the nucleus, and are exported to the cytoplasm. After removal of an intron (red) and the 2′-phosphate group (asterisk) at the splice junction, the resulting mature tRNAs function in cytoplasmic bulk translation. Mature tRNAs shuttle between the cytoplasm and the nucleus. Four possible roles of nuclear tRNAs are summarized on the left.

their late intermediates) in the nucleus, since the ribosomal subunits are assembled from RNA and protein components in the nucleolus, supporting the possibility of nuclear translation [37, 38]. On the other hand, many translation factors, *e.g.* eIF4AI, eEF1A, eRF1, *etc.*, are excluded from the nucleus, and skepticism has been expressed about NMD driven by intranuclear translation [28, 39]. Furthermore, in the case of *S. cerevisiae*, most of the mRNA decay is carried out in the cytoplasm [40]. Nevertheless, very limited translation may occur in the nucleus for quality control of certain mRNAs, or the ribosome itself [37]. If so, nuclear tRNAs might be used for such activity.

The second possibility is that the nucleus is used as a reservoir of tRNAs to control cytoplasmic bulk translation. This has been proposed by Hopper's group [23]. Since amino acid deprivation, which activates nuclear import of tRNAs, results in a shortage of aminoacyl-tRNAs, substrates for translation, it is reasonable for cells to sequester uncharged tRNAs from the cytoplasm to maintain the appropriate ratio of aminoacylated/deacylated tRNAs in the cytoplasm. The supply of tRNAs is also regulated at the transcriptional level by the TOR signaling pathway according to nutrient conditions [41]. Cells may strictly control the cytoplasmic amounts of aminoacylated tRNAs through both transcription and nuclear-cytoplasmic transport.

The third possibility is that tRNAs shuttle between the nucleus and cytoplasm for the purpose of quality control. Recently, degradation pathways for unstable or aberrant tRNAs have been discovered. In particular, the poly(A) polymerase-dependent nuclear exosomal pathway has become well characterized [42–46]. Accumulating evidence supports the notion that unstable tRNAs are recognized by Trf4p/Trf5p-containing poly(A) polymerase complexes specialized for RNA degradation, received a poly(A) tail and then degraded by the nuclear exosome. Another report has described a more rapid degradation pathway of hypo-modified tRNAs independent of polyadenylation [47]. Of course, these surveillance systems act during the biogenesis of tRNAs to eliminate mal-processed intermediates [43]. However, some of aberrant tRNAs may escape from the initial screening to reach the cytoplasm, and may be degraded after reimport into the nucleus. Alternatively, since tRNAs are rather long-lived RNA species, they may be susceptible to undesired modification and/or partial degradation in the cytoplasm. As mentioned above, at least certain kinds of 'wounded' tRNAs, such as CCA-less tRNAs, are substrates for nuclear import. From this point of view, the rather non-selective import of tRNAs into the nucleus is a sharp contrast to the selective export from the nucleus. Just before tRNA export, ARSs in the nucleus hand over only amino acylatable tRNAs to the export machineries [18, 19, 30]. The nucleus may import various tRNAs species in bulk, survey non-functional tRNAs to be repaired or degraded, and then export only healthy tRNAs back to the cytoplasm for translation.

The fourth possibility is that tRNAs act as messengers of cytoplasmic information. It is known that there is some relationship between general amino acid control and tRNA transport. Yeast cells sense the amounts of amino acids available for translation. If there are sufficient amino acids in the surrounding environment, they repress the production of enzymes required for amino acid biosynthesis [48]. The main regulator is Gcn2p, which has a protein-kinase activity specific for translation initiation factor 2α and a deacylated tRNA-binding activity that activates the kinase activity [49]. This phosphorylation activates translation of Gcn4p, a general transcription factor for amino acid biosynthetic pathways. Even in the absence of Gcn2p, yeast cells partially respond to amino acid starvation, and this response is activated by perturbation of tRNA export [50]. Another example is that Asp-RS expression is down-regulated in a post-transcriptional manner by an interaction between Asp-RS and its mRNA [51]. The binding of Asp-RS to its mRNA results in degradation of the mRNA in the nucleus. The cognate tRNA, tRNA-Asp, competes with the mRNA for binding to Asp-RS [52]. Since the level of nuclear tRNA-Asp is a key factor for this regulation, this level needs to be correlated to that in the cytoplasm. Nuclear import may contribute to a monitoring of the tRNA status in the cytoplasm by the nucleus. Or we may still miss the true explanation of the physiological roles of nuclear tRNAs.

Perspectives

The finding of tRNA import into the nucleus has revealed a more precise view of the life of tRNAs. However, we are far from a complete understanding, but are just in front of many questions. The specific carrier(s) of tRNAs from the cytoplasm to the nucleus remains unknown. How many pathways are operating for this import in yeast? If there is a Ran-independent but energy-dependent pathway, how is this transport system energized? Is tRNA import common to other organisms? And what is the real role(s) of mature tRNAs in the nucleus? Identification of the factors required for this novel nuclear transport is an urgent task, and should provide important clues for unraveling the physiological roles of nuclear tRNAs. Powerful yeast genetics, including the isolation and analysis of nuclear import mutants, will contribute toward opening the door to the complete view of the life of tRNAs.

1 Görlich, D. and Kutay, U. (1999) Transport between the cell nucleus and the cytoplasm. Annu. Rev. Cell Dev. Biol. 15, 607–660.
2 Weis, K. (2002) Nucleocytoplasmic transport, cargo trafficking across the border. Curr. Opin. Cell Biol. 14, 328–335.
3 Fukuda, M., Asano, S., Nakamura, T., Adachi, M., Yoshida, M., Yanagida, M. and Nishida, E. (1997) CRM1 is responsible for intracellular transport mediated by the nuclear export signal. Nature 390, 308–311.
4 Fornerod, M., Ohno, M., Yoshida, M. and Mattaj, I. W. (1997) CRM1 is an export receptor for leucine-rich nuclear export signals. Cell 90, 1051–1060.
5 Ossareh-Nazari, B., Bachelerie, F. and Dargemont, C. (1997) Evidence for a role of CRM1 in signal-mediated nuclear protein export. Science 278, 141–144.
6 Hopper, A. K. and Phizicky, E. M. (2003) tRNA transfers to the limelight. Genes Dev. 17, 162–180.
7 Wolin, S. L. and Matera, A. G. (1999) The trials and travels of tRNA. Genes Dev. 13, 1–10.
8 Mattaj, I.W. (1986) Cap trimethylation of U snRNA is cytoplasmic and dependent on U snRNP protein binding. Cell 46, 905–911.
9 Huber, J., Cronshagen, U., Kadokura, M., Marshallsay, C., Wada, T., Sekine, M. and Lührmann, R. (1998) Snurportin1, an m_3G-cap-specific nuclear import receptor with a novel domain structure. EMBO J. 17, 4114–4126.
10 Schneider, A. and Maréchal-Drouard, L. (2000) Mitochondrial tRNA import: are there distinct mechanisms? Trends Cell Biol. 10, 509–513.
11 Lohan, A. J. and Wolfe, K. H. (1998) A subset of conserved tRNA genes in plastid DNA on nongreen plants. Genetics 150, 425–433.
12 Yoshihisa, T., Yunoki-Esaki K,. Ohshima, C., Tanaka, N. and Endo, T. (2003) Possibility of cytoplasmic pre-tRNA splicing: the yeast tRNA splicing endonuclease mainly localizes on the mitochondria. Mol. Biol. Cell 14, 3266–3279.
13 Trotta, C. R., Miao, F., Arn, E. A., Stevens, S. W., Ho, C. K., Rauhut, R. and Abelson, J. N. (1997) The yeast tRNA splicing endonuclease: a tetrameric enzyme with two active site subunits homologous to the archaeal tRNA endonuclease. Cell 89, 849–858.
14 Abelson, J., Trotta, C. R. and Li, H. (1998) tRNA splicing. J. Biol. Chem. 273, 12685–12688.
15 Hellmuth, K., Lau, D. M., Bischoff, F. R., Künzler, M., Hurt, E. and Simos, G. (1998) Yeast Los1p has properties of an exportin-like nucleocytoplasmic transport factor for tRNA. Mol. Cell. Biol. 18, 6374–6386.
16 Sarkar, S. and Hopper, A. K. (1998) tRNA nuclear export in *Saccharomyces cerevisiae*: *in situ* hybridization analysis. Mol. Biol. Cell 9, 3041–3055.
17 Feng, W. and Hopper, A. K. (2002). A Los1p-independent pathway for nuclear export of intronless tRNAs in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 99, 5412–5417.
18 Sarkar, S., Azad, A. K. and Hopper, A. K. (1999) Nuclear tRNA aminoacylation and its role in nuclear export of endogenous tRNAs in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 96, 14366–14371.
19 Grosshans, H., Hurt, E. and Simos, G. (2000) An aminoacylation-dependent nuclear tRNA export pathway in yeast. Genes Dev. 14, 830–840.
20 Azad, A. K., Stanford, D. R., Sarkar, S. and Hopper, A. K. (2001) Role of nuclear pools of aminoacyl-tRNA synthetases in tRNA nuclear export. Mol. Biol. Cell 12, 1381–1392.
21 Steiner-Mosonyi, M., Leslie, D. M., Dehghani, H., Aitchison, J. D. and Mangroo, D. (2003) Utp8p is an essential intranuclear component of the nuclear tRNA export machinery of *Saccharomyces cerevisiae*. J. Biol. Chem. 278, 32236–32245.
22 Takano, A., Endo, T. and Yoshihisa, T. (2005) tRNA actively shuttles between the nucleus and cytosol in yeast. Science 309, 140–142.
23 Shaheen, H. H. and Hopper, A. K. (2005) Retrograde movement of tRNAs from the cytoplasm to the nucleus in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 102, 11290–11295.
24 Azpiroz, R. and Butow, R. A. (1993) Patterns of mitochondrial sorting in yeast zygotes. Mol. Biol. Cell 4, 21–36..
25 Bucci, M. and Wente, S. R. (1997) *In vivo* dynamics of nuclear pore complexes in yeast. J. Cell Biol. 136, 1185–1199.
26 Kadowaki, T., Chen, S., Hitomi, M., Jacobs, E., Kumagai, C., Liang, S., Schneiter, R., Singleton, D., Wisniewska, J. and Tartakoff, A. M. (1994) Isolation and characterization of *Saccharomyces cerevisiae* mRNA transport-defective (*mtr*) mutants. J. Cell Biol. 126, 649–659.
27 Andersen, G. R. and Nyborg, J. (2001) Structural studies of eukaryotic elongation factors. Cold Spring Harb. Symp. Quant. Biol. 66, 425–437.
28 Bohnsack, M. T., Regener, K., Schwappach, B., Saffrich, R., Paraskeva, E., Hartmann, E. and Görlich, D. (2002) Exp5 exports eEF1A via tRNA from nuclei and synergizes with other transport pathways to confine translation to the cytoplasm. EMBO J. 21, 6205–6215.
29 Calado, A., Treichel, N., Müller, E.-C., Otto, A. and Kutay, U. (2002) Exportin-5-mediated nuclear export of eukaryotic elongation factor 1A and tRNA. EMBO J. 21, 6216–6224.
30 Lund, E. and Dahlberg, J. E. (1998) Proofreading and aminoacylation of tRNAs before export from the nucleus. Science 282, 2082–2085.
31 Wolfe, C. L., Hopper, A. K. and Martin, N. C. (1996) Mechanisms leading to and the consequences of altering the normal distribution of ATP(CTP):tRNA nucleotidyltransferase in yeast. J. Biol. Chem. 271, 4679–4686.
32 Pemberton, L. F., Rosenblum, J. S. and Blobel, G. (1997) A distinct and parallel pathway for the nuclear import of an mRNA-binding protein. J. Cell Biol. 139, 1645–1653.
33 Ferrezuelo, F., Steiner, B., Aldea, M. and Futcher, B. (2002) Biogenesis of yeast telomerase depends on the importin Mtr10. Mol. Cell. Biol. 22, 6046–6055.
34 Iborra, F. J., Jackson, D. A. and Cook, P. R. (2001) Coupled transcription and translation within nuclei of mammalian cells. Science 293, 1139–1142.
35 Cheng, J. and Maquat, L. E. (1993) Nonsense codons can reduce the abundance of nuclear mRNA without affecting the abundance of pre-mRNA or the half-life of cytoplasmic mRNA. Mol. Cell. Biol. 13, 1892–1902.

36 Mühlemann, O., Mock-Casagrande, C. S., Wang, J., Li, S., Custódio, N., Carmo-Fonseca, M., Wilkinson, M. F. and Moore, M. J. (2001) Precursor RNAs harboring nonsense codons accumulate near the site of transcription. Mol. Cell 8, 33–44.
37 Pederson, T. and Politz, J. C. (2000) The nucleolus and the four ribonucleoproteins of translation. J. Cell Biol. 148, 1091–1095.
38 Mangiarotti, G., Chiaberge, S. and Bulfone, S. (1997) rRNA maturation as a 'quality' control step in ribosomal subunit assembly in *Dictyostelium discoideum*. J. Biol. Chem. 272, 27818–27822.
39 Dahlberg, J. E. and Lund, E. (2004) Does protein synthesis occur in the nucleus? Curr. Opin. Cell Biol. 16, 335–338.
40 Kuperwasser, N., Brogna, S., Dower, K. and Rosbash, M. (2004) Nonsense-mediated decay does not occur within the yeast nucleus. RNA 10, 1907–1915.
41 Upadhya, R., Lee, J.-H. and Willis, I. M. (2002) Maf1 is an essential mediator of diverse signals that repress RNA polymerase III transcription. Mol. Cell 10, 1489–1494.
42 Kadaba, S., Krueger, A., Trice, T., Krecic, A. M., Hinnebusch, A. G. and Anderson, J. (2004) Nuclear surveillance and degradation of hypomodified initiator $tRNA^{Met}$ in *S. cerevisiae*. Genes Dev. 18, 1227–1240.
43 Kadaba, S., Wang, X. and Anderson, J. T. (2006) Nuclear RNA surveillance in *Saccharomyces cerevisiae*: Trf4p-dependent polyadenylation of nascent hypomethylated tRNA and an aberrant form of 5S rRNA. RNA 12, 508–521.
44 LaCava, J., Houseley, J., Saveanu, C., Petfalski, E., Thompson, E., Jacquier, A. and Tollervey, D. (2005) RNA degradation by the exosome is promoted by a nuclear polyadenylation complex. Cell 121, 713–724.
45 Wyers, F., Rougemaille, M., Badis, G., Rousselle, J.-C., Dufour, M.-E., Boulay, J., Régnault, B., Devaux, F., Namane, A., Séraphin, B., Libri, D. and Jacquier, A. (2005) Cryptic Pol II transcripts are degraded by a nuclear quality control pathway involving a new poly(A) polymerase. Cell 121, 725–737.
46 Vaňáčová, S., Wolf, J., Martin, G., Blank, D., Dettwiler, S., Friedlein, A., Langen, H., Keith, G. and Keller, W. (2005) A new yeast poly(A) polymerase complex involved in RNA quality control. PLOS Biol. 3, e189.
47 Alexandrov, A., Chernyakov I, Gu, W., Hiley, S. L., Hughes, T. R., Grayhack, E. J. and Phizicky, E. M. (2006) Rapid tRNA decay can result from lack of nonessential modifications. Mol. Cell 21, 87–96.
48 Hinnebusch, A. G. (1997) Translational regulation of yeast *GCN4*, a window on factors that control initiator-tRNA binding to the ribosome. J. Biol. Chem. 272, 21661–21664.
49 Wek, S. A., Zhu, S. and Wek, R. C. (1995) The histidyl-tRNA synthetase-related sequence in the eIF-2 α protein kinase GCN2 interacts with tRNA and is required for activation in response to starvation for different amino acids. Mol. Cell. Biol. 15, 4497–4506.
50 Qiu, H., Hu, C., Anderson, J., Björk, G. R., Sarkar, S., Hopper, A. K. and Hinnebusch, A. G. (2000) Defects in tRNA processing and nuclear export induce *GCN4* translation independently of phosphorylation of the α subunit of eukaryotic translation initiation factor 2. Mol. Cell. Biol. 20, 2505–2516.
51 Frugier, M., Ryckelynck, M. and Giegé, R. (2005) tRNA-balanced expression of a eukaryal aminoacyl-tRNA synthetase by an mRNA-mediated pathway. EMBO Rep. 6, 860–865.
52 Frugier, M. and Giegé, R. (2003) Yeast aspartyl-tRNA synthetase binds specifically its own mRNA. J. Mol. Biol. 331, 375–383.