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tRNA dynamics between the nucleus, cytoplasm and mitochondrial surface: Location, location, location*

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Abstract

Although tRNAs participate in the essential function of protein translation in the cytoplasm, tRNA transcription and numerous processing steps occur in the nucleus. This subcellular separation between tRNA biogenesis and function requires that tRNAs be efficiently delivered to the cytoplasm in a step termed “primary tRNA nuclear export”. Surprisingly, tRNA nuclear-cytoplasmic traffic is not unidirectional, but, rather, movement is bidirectional. Cytoplasmic tRNAs are imported back to the nucleus by the “tRNA retrograde nuclear import” step which is conserved from budding yeast to vertebrate cells and has been hijacked by viruses, such as HIV, for nuclear import of the viral reverse transcription complex in human cells. Under appropriate environmental conditions cytoplasmic tRNAs that have been imported into the nucleus return to the cytoplasm via the 3rd nuclear-cytoplasmic shuttling step termed “tRNA nuclear re-export”, that again is conserved from budding yeast to vertebrate cells. We describe the 3 steps of tRNA nuclear-cytoplasmic movements and their regulation. There are multiple tRNA nuclear export and import pathways. The different tRNA nuclear exporters appear to possess substrate specificity leading to the tantalizing possibility that the cellular proteome may be regulated at the level of tRNA nuclear export. Moreover, in some organisms, such as budding yeast, the pre-tRNA splicing heterotetrameric endonuclease (SEN), which removes introns from pre-tRNAs, resides on the cytoplasmic surface of the mitochondria. Therefore, we also describe the localization of the SEN complex to mitochondria and splicing of pre-tRNA on mitochondria, which occurs prior to the participation of tRNAs in protein translation. This article is part of a Special Issue entitled: SI: Regulation of tRNA synthesis and modification in physiological conditions and disease edited by Dr. Boguta Magdalena.

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Transparency document

The Transparency document associated with this article can be found, in online version.

1. Introduction

tRNA biosynthesis has been studied for decades and there are sophisticated and detailed understandings of the transcription, processing, and modification steps that are required to generate mature, functional tRNAs that bring amino acids to the ribosomes as specified by mRNA codons during protein synthesis. Less well understood is how this biosynthesis process occurs within the 3-dimensional landscape of eukaryotic cells. A full understanding of the spatial-temporal regulation of tRNAs is important as tRNA biology impacts the human condition. Literally scores of human diseases, from metabolic disorders, to neuromuscular diseases, to cancer, are caused by defects in various steps of tRNA biology, with discovery of new tRNA-related diseases occurring regularly [[1,2]; Review [3]]. Many of the processing steps between transcription of pre-tRNAs in the nucleus and their function in the cytoplasm occur prior to nuclear export, whereas other steps, such as addition of a subset of nucleotide modifications, occur post nuclear export, in the cytoplasm; and, in some, but not all organisms, intron-containing pre-tRNAs travel to the mitochondrial surface where splicing occurs. Surprisingly, tRNAs travel bi-directionally between the nucleus and cytoplasm. This bidirectional travel serves numerous functions ranging from tRNA modification, to tRNA quality control, to regulation of translation, and to HIV replication. Moreover, tRNA subcellular traffic is responsive to environmental conditions. We discuss the bidirectional movement of tRNA between the nucleus to the cytoplasm and also the movement of the tRNAs in the cytoplasm to the outer mitochondrial membrane, where pre-tRNA splicing occurs in budding yeast. In most organisms tRNAs are also imported into mitochondria - a topic which has been recently reviewed by others [Review [4]]. We describe a theme that is arising that each of the dynamic steps of tRNA movement within cells is achieved by the activities of multiple proteins that function in parallel. If such parallel pathways regulate species-specific tRNA subcellular trafficking under particular environmental conditions, it is possible that tRNA cell biology could serve as a novel mechanism to regulate gene expression in response to changes in the environment. Here, we address how tRNAs locate to the correct subcellular place, at the correct time, and under the correct environmental conditions: location, location, location.

2. Primary export of tRNAs to the cytoplasm

2.1. Multiple primary tRNA nuclear export pathways

Precursor tRNAs (pre-tRNAs) are transcribed with extra 5' leader (~12 nucleotides (nt) in budding yeast) and 3' trailer (~12 nt in yeast) sequences [5,6]. The 5' leader and 3' trailer sequences are removed by various post-transcriptional endo- and exonucleolytic cleavages [Review [7]]. Transcription of eukaryotic tRNA genes also generates two populations of pre-tRNAs in the nucleus, as a subset of tRNA encoding genes contains introns that must be spliced from pre-tRNAs to generate functional, mature tRNAs. The percentage of intron-containing tRNA genes varies greatly in eukaryotes as does the subcellular locations where pre-tRNA splicing takes place. In vertebrate cells, the SEN complex is located in the nucleoplasm and pre-tRNA splicing occurs in the nucleus, whereas in budding yeast, the SEN complex is peripherally associated with the mitochondrial outer membrane and pre-tRNA splicing occurs in the cytoplasm post nuclear export [8–11].

Although tRNAs are smaller than the 40 kD cut-off range for passive diffusion across the nuclear pore complexes, exit of tRNAs from the nucleus is an active process [12]. The best described nuclear exporter for tRNA primary nuclear export is the conserved β -importin family member Los1/Exportin-t/PAUSED. β -Importin family members utilize the Ran pathway. Ran, a small GTPase, is primarily in the GTP bound form in the nucleus and the GDP bound form in the cytoplasm [Reviews [13,14]]. The nuclear-cytoplasmic gradient of RanGTP dictates the directionality of movement across the nuclear membrane. However, as detailed below, recently described parallel pathways for tRNA primary, as well as tRNA re-export and retrograde nuclear import, are Ran-independent (Fig. 1).

The well-studied β -importin, yeast Los1, and its orthologues -Exportin-t or Exp-t in vertebrates, Xpot in *S. pombe*, and PAUSED or PSD in *Arabidopsis*, function in the Ran-dependent primary tRNA nuclear export step [15–20]. Los1 and its orthologues bind both intron-containing as well as spliced tRNAs in a RanGTP-dependent mechanism [21–26]. As pre-tRNA export from the nucleus is an essential process, the observations that *LOS1*, *XPOT* and *PSD* are unessential genes, led to the conclusion that Los1 cannot be the only protein participating in the primary tRNA nuclear export [18,20,27]. Also, no orthologues of Los1 have been identified in *Drosophila melanogaster* [28]. In vertebrate ~80% reduction in the levels of cytoplasmic tRNAs was observed upon inhibition of tRNA export by introduction of antibodies against Exp-t. This led to the hypothesis that Exp-t is the major exportin required for tRNA export in vertebrate cells [21,26]. However, Exp-t has been scored as unessential in multiple human haploid cancer cell lines by 3 independent genome-wide screens, suggesting that there are also alternate primary tRNA nuclear export pathways in human cells [29–31]. Yeast Msn5 and its orthologues, Exportin-5 in vertebrates and HASTY in plants, likely provided an alternate pathway for primary tRNA nuclear export [32–34]. However, in budding yeast, Msn5 does not export, intron-containing pre-tRNAs to the cytoplasm and hence does not participate in the primary nuclear export step for tRNAs encoded by intron-containing tRNA genes [25].

A recent genome-wide screen to uncover novel genes potentially involved in tRNA processing and subcellular dynamics, revealed two potential additional exporters for primary tRNA nuclear export in budding yeast [35]. One is Crm1 (Xpo1 in vertebrates) - a protein essential for cell survival with established roles in exporting proteins with Leucine-rich motifs and multiple ribonuclear protein complex cargoes from the nucleus to the cytoplasm [Review [36]]. The temperature sensitive (ts) mutant of yeast *CRM1*, *crm1-1*, accumulates unspliced pre-tRNA and exhibits tRNA accumulation at the nuclear periphery when cells are incubated at the non-permissive temperature [35]. Moreover, *crm1-1* has negative synthetic genetic interaction with *los1* at semi-permissive temperatures for *crm1-1* [35]. Although the data support a role for Crm1 in tRNA nuclear export, additional investigations to assess whether tRNA binds with Crm1 are necessary to validate its direct role in tRNA nuclear export.

The second potential tRNA nuclear exporter uncovered [35] is the Mex67-Mtr2 heterodimer (NXF-NXT or TAP-p15 in metazoans) [37]. This essential heterodimer has a well-studied role in mRNA nuclear export [Review [38]]. Cells possessing either ts *mex67-5* or *mtr2-ts* alleles accumulate unspliced tRNA in the nucleus at the non-permissive temperature.

Moreover, mere 5-fold over-expression of Mex67-Mtr2 substitutes for Los1 in *los1* cells, thereby confirming its role in tRNA nuclear export. Finally, tRNAs co-purify with Mex67, documenting a direct role for Mex67 in tRNA nuclear export [37]. The essential functions of Mex67-Mtr2 are evolutionarily conserved, as human TAP-p15 suppresses lethality of a yeast *mex67 mtr2* double mutant strain [39]. The Mex67-Mtr2-mediated mRNA export pathway is also conserved from yeast to humans [39]. Although, it is unknown whether the role of Mex67-Mtr2 in tRNA export is also evolutionarily conserved, p15 has been reported to stimulate nuclear tRNA export in permeabilized HeLa cells [40]. Since, Mex67 and Mtr2 are not β -importins, Mex67-Mtr2-dependent tRNA nuclear export is independent of the Ran gradient.

Ohira and Suzuki recently detected 5'-methylguanosine capped, intron-containing pre-tRNAs in human and yeast cells as well as capped spliced tRNAs in budding yeast [6]. Since capped tRNAs can be spliced in yeast, they must be exported to the cytoplasm for splicing as pre-tRNAs with capped 5' leaders [6]. However, capped pre-tRNAs cannot be recognized by Los1 for nuclear export since numerous biochemical and structural studies have documented that Los1 binds only end-processed tRNAs [20–25]. Hence, nuclear export of capped pre-tRNAs requires a Los1-independent nuclear export mechanism. Since Mex67-Mtr2 exports capped mRNAs from the nucleus, the heterodimer may mediate primary tRNA nuclear export of capped tRNAs. However, accumulation of capped pre-tRNAs was not observed in *mex67-5* or *mtr2*ts cells at the non-permissive temperature. Rather, northern hybridization data document that *mex67-5* or *mtr2*ts cells accumulate pre-tRNAs with processed ends [37]. Perhaps, the lack of accumulation of 5' extended, capped pre-tRNAs in *mex67-5* or *mtr2*ts cells is due to increased retention in the nucleus, allowing sufficient time for end-processing. Collectively, the data document Ran-dependent and Ran-independent parallel pathways for primary tRNA nuclear export.

2.2. Regulation of primary tRNA nuclear export

Transport of cargo through nuclear pores is closely associated with cell physiology and changes in the cellular environment alter nuclear-cytoplasmic traffic [Reviews [41,42]]. For example, in budding yeast, tRNA nuclear accumulation is observed under growth conditions with non-fermentable carbon sources like glycerol or upon certain stresses [Review [43]]. Sequestration of tRNA in the nucleus, away from the cytoplasmic protein synthesis machinery may be a mechanism to reduce global protein synthesis [Review [44]]. However, accumulation of significant amounts of cytoplasmic tRNAs in the nucleus did not cause global inhibition of translation [45,46]. Nuclear accumulation of tRNAs may thus play an as yet unidentified role for specific gene regulation. The reduction in cytoplasmic tRNA levels during stress due to regulation of nuclear-cytoplasmic shuttling of tRNAs, could result from altering the subcellular distribution of the various tRNA nuclear exporters. Cytosolic tRNA levels can also be altered at the level of tRNA transcription. With the emergence of new proteins participating in tRNA subcellular trafficking, the mechanisms by which tRNA subcellular distribution are regulated need further exploration.

Although tRNAs accumulate in the nucleus of budding yeast under conditions of glucose and amino acid deprivation, the accumulated tRNAs have been spliced and therefore have

been imported into the nucleus from the cytoplasm via the tRNA retrograde step [47,48]. Thus, under conditions of nutrient deprivation, pre-tRNAs are able to access the cytoplasm via the primary nuclear export, but not the re-export step. The data support the notion that primary nuclear tRNA export is constitutive and regulation of tRNA trafficking is likely regulated at the re-export step. As detailed below, Los1 participates in both the primary and the tRNA nuclear re-export steps. Interestingly, the steady state of Los1 levels decrease in response to amino acid deprivation, but not in response to glucose deprivation. The data support the model that there are distinct mechanisms by which cells respond to amino acid versus glucose availability [48]. In yeast cells grown under glucose deprivation conditions or with non-fermentable carbon sources like glycerol or ethanol, a portion of the Los1 pool, which normally resides in the nucleoplasm under fed conditions, re-distributes to the nuclear periphery and the cytoplasm [48,49]. Redistribution of Los1 is mediated by the metabolic sensor kinase Snf1 [49], likely as a result of the collapse of the Ran gradient in glucose-deprived cells [48]. It is possible that export pathways that work in parallel to Los1 and do not rely on the Ran gradient for tRNA nuclear export, like Mex67-Mtr2, play predominant roles under conditions of glucose deprivation. Future studies are required to understand the cross-talk between various tRNA export pathways under stress conditions.

Though Mex67-Mtr2 has been recently been characterized as a bona fide tRNA exporter [37], the mechanism of Mex67-Mtr2-mediated tRNA export is not clear. Various biochemical as well as structural studies of Mex67-Mtr2 and their homologs have identified two mechanisms by which the heterodimer exports specific RNA cargoes [Reviews [38,50–52]]. Mex67-Mtr2 is recruited on most mRNAs through the concerted action of various adapter proteins like yeast Yra1 or human Aly/REF. However, Mex67-Mtr2 also can directly bind to RNA cargoes, for example, viral RNAs that contain Cis-acting Transport Element (CTE) motifs and 5S rRNA [53–56]. Moreover, under heat shock stress, Mex67 has been shown to directly bind mRNAs transcribed from various heat shock protein (*HSP*) genes that contain heat shock responsive elements (HSE) in their promoters [57]. Nuclear export of most mRNA are inhibited following heat shock and other forms of stresses in yeast [Review [58]]. Nuclear retention of the mRNAs is due to alterations of various adapter proteins that associate with Mex67-Mtr2. For example, Npl3, one of the shuttling proteins required for Mex67-Mtr2 mediated nuclear mRNA export, is rapidly exported from the nucleus under stress, thus negatively regulating mRNA nuclear export [59]. Since it is not known whether tRNA binding by Mex67-Mtr2 is adapter protein-mediated, it is unknown whether Mex67-Mtr2-mediated primary tRNA nuclear export is regulated.

2.3. Interplay between transcription and primary nuclear export

Yeast cells treated with DNA damaging agents rapidly accumulate pre-tRNAs in the nucleus in yeast as a result of Los1 mislocalization to the cytoplasm via an unknown cell signal-dependent mechanism [60]. Nuclear accumulation of pre-tRNAs upon DNA damage triggers activation of the Gcn4 transcription factor, which in turn leads to cell cycle arrest at the G1 stage. Deletion of *LOS1*, which results in accumulation of intron-containing pre-tRNAs in the nucleus, also leads to constitutive activation of Gcn4 [61]. Gcn4 activation, in turn, results in a reduced rate of general protein synthesis, while concomitantly inducing expression of proteins required for adaptation of starvation and stress [61,62]. A recent study

has also reported that similar to *los1* cells, several tRNAs accumulate in the nuclei of *nup100* cells, activating the Gcn4 pathway [63]. This Gcn4 activation is related to replicative lifespan and occurs by an unknown mechanism. Future studies are required to fully understand this functional coupling between tRNA subcellular dynamics and regulation of cellular proliferation rates in cells.

Los1 has been reported to coordinate tRNA transcription with tRNA nuclear export by facilitating the docking of tRNA genes to nuclear-pore complexes (NPC) [64]. This Los1-mediated tethering of tRNA genes at the NPC is dynamic, being observed primarily in the M-phase of cell cycle, when tRNA gene transcription is elevated. Docking of tRNA genes to the NPC is also thought to expedite the exit of nascent tRNAs from the nucleus [64]. However, docking of tRNA genes to the NPC appears to contradict previous studies of tRNA gene clustering in the nucleolus where tRNA transcription and pre-tRNA 5'-end maturation occur [65].

The limiting nuclear pools of Los1 in yeast cells also appear to coordinate tRNA transcription and primary tRNA nuclear export. Maf1, an evolutionarily conserved negative regulator of the DNA-dependent RNA polymerase for transcription of tRNA genes, Pol III, is inactivated by phosphorylation under favorable growth conditions leading to production of pre-tRNAs, which are exported out of the nucleus by Los1. In contrast, pre-tRNAs accumulate in nuclei of *maf1* cells, irrespective of whether the mutant cells are grown in glucose or non-fermentative carbon sources. It has been proposed that in the absence of Maf1, derepression of transcription occurs, which thereby saturates the limiting amounts of nuclear Los1 for tRNA nuclear export [66]. The data document interplay between transcription and primary nuclear export [67].

2.4. tRNA quality control and primary nuclear export

Before exiting the nucleus, pre-tRNAs undergo processing at their 5' and 3' ends as well as numerous nucleoside modifications. The 5' processing enzyme RNase P is localized in the nucleolus where clustering of tRNA genes and tRNA gene transcription occur [65 [Review 68] 69,70]. Following 3'-end trimming, addition of the CCA trinucleotide, catalyzed by Cca1, at the 3' end of all tRNAs completes the tRNA end-maturation steps [71]. Pre-tRNA must be processed accurately at the 5' and 3' ends in order to bind Los1. Various biochemical studies, as well as a 3.2 Å resolution structure of *Schizosaccharomyces pombe* Xpot in complex with tRNA and RanGTP, demonstrated that the tRNA exportin binds the appropriately structured tRNA backbone (the D and the TΨC arm of the tRNA tertiary structure) with mature 5' and 3' ends [21–26]. The evolutionarily conserved binding mode of Xpot to structures that are common to all tRNA species provides an explanation as to how Exportin-t can recognize both spliced and unspliced tRNAs and yet can distinguish between aberrantly end-processed tRNAs from tRNAs with matured ends, thus coupling quality control to tRNA nuclear export. However, tRNA nuclear export by Los1 is not completely error-free as over-expressed Los1 causes accumulation of aberrant, spliced pre-tRNAs with unprocessed ends in the cytoplasm [72]. The data indicate that there is competition between tRNA end processing and tRNA nuclear export by Los1 and thereby may provide an explanation as to why Los1 is present in limiting amounts in the nucleus [60]. Mex67-Mtr2

levels may also be limiting for tRNA export [37], however, the fidelity of such Los1-independent tRNA export pathways has not been investigated.

Numerous nucleotides of pre-tRNAs are also modified in the nucleus and some of the modifications are important for tRNA structure [Reviews [7,73,74]]. Competition between the tRNA modification and tRNA nuclear export, may also lead to precocious nuclear export of unprocessed, hypomodified tRNAs to the cytoplasm [72]. Therefore, there is a delicate balance between processing and modification of pre-tRNAs and primary nuclear export to maximize the fidelity of tRNA in the cytoplasm.

Pre-tRNA 5' and 3' end processing and nuclear modifications are regulated by stress, which, in turn, influences the amount of pre-tRNAs suitable for nuclear export. For example, the human La protein, which plays an evolutionarily conserved role in tRNA 3' end processing, is regulated by stress. Upon DNA damage, the predominant nuclear localization of phosphorylated La is altered as it becomes de-phosphorylated and unable to associate with nuclear pre-tRNAs [75,76]. Recent studies document accumulation of pre-tRNAs with aberrant ends upon temperature and carbon source stress in budding yeast [77]. Reprogramming of tRNA modifications also occurs upon stress [Review [43]]. The observation that nuclear localized tRNA pseudouridine synthetase, *PUS1*, has negative synthetic interactions with *LOS1* led to the hypothesis that certain tRNA modifications and Los1-mediated tRNA nuclear export are coupled [78]. Moreover, human Exportin-t has a 10 fold higher affinity for binding tRNAs isolated from cultured cells, than in vitro transcribed tRNAs, demonstrating the requirement of at least certain modifications for efficient binding [17]. The interplay between pre-tRNA modification in the nucleus and tRNA nuclear export by Los1 is complicated. On one hand, the absence of certain combinations of modifications alters the tRNA structure and Los1 binds only appropriately structured tRNAs, thereby rendering Los1-mediated tRNA nuclear export dependent on tRNA modifications [7,73,74]. On the other hand, Los1 recognizes 42 different tRNA species, each containing a unique array of nucleotide modifications, making it unlikely that Los1 closely monitors individual tRNA modification status. The fact that over-expression of Los1 led to increased levels of hypomodified tRNAs in the cytoplasm [72], supports the notion that Los1 can interact with hypomodified tRNAs in the nucleus and export them to the cytoplasm. The data again document that there is a delicate balance between tRNA modification in the nucleus and Los1-mediated tRNA nuclear export.

2.5. Substrate specificity in primary pre-tRNA export

Since Los1 binds to appropriately structured pre-tRNAs with processed ends, it was predicted that Los1/Exportin-t would bind all tRNAs with equal affinities, resulting in nuclear export of all tRNAs with equal efficiencies. However, it was reported that human Exportin-t binds different tRNA species with different affinities [79]. Moreover, a recent study showed that primary nuclear export of some intron-containing pre-tRNAs is highly dependent upon Los1, whereas the nuclear export of other tRNAs is apparently somewhat Los1-independent [37]. As there may be substrate specificity among the tRNA nuclear exporters, their regulation under particular environmental conditions such as stress could

influence the cytoplasmic pools of particular tRNAs and hence possibly influence the proteome under various conditions.

3. Retrograde tRNA nuclear import

Although tRNA nuclear-cytoplasmic traffic was once thought to be a one way process, nucleus to cytoplasm, it is now known that there is bidirectional traffic between the nucleus and the cytoplasm. In budding yeast the discovery of pre-tRNA intron splicing in the cytoplasm at the surface of the mitochondrial outer membrane [10,11], and accumulation of spliced tRNA in the nucleus under various conditions [80–83] led to the prediction and subsequent documentation that tRNAs can move from the cytoplasm to the nucleus [84,85]. The Fassati lab also reported tRNA import into the nucleus while studying the mechanism of HIV reverse transcription complex entry into the nucleus in plasma membrane-permeabilized HeLa cells [86]. Since these discoveries, the process of tRNA re-entry back into the nucleus, termed “retrograde tRNA nuclear import” has been reported under various cellular conditions for numerous eukaryotic species ranging from budding yeast to humans, documenting evolutionary conservation [86–91]. Here, we first summarize the current evidence for retrograde tRNA nuclear import. Subsequently, we discuss the proteins implicated in this process and their role in constitutive vs. regulated tRNA retrograde nuclear import as well as the physiological functions of this process.

3.1. Evidence for evolutionary conservation of retrograde tRNA nuclear import

The first reports of retrograde tRNA nuclear import were independently shown by three labs spanning across three continents. Using the yeast strain *S. cerevisiae*, the Yoshihisa and Hopper labs demonstrated that in tRNA export-defective *los1* heterokaryons with mature “foreign” cytoplasmic *S. pombe* tRNA^{Met} [84] or *D. discoideum* tRNA^{Glu} (tRNA^{Glu-D}) [85], encoded by one nucleus of the heterokaryon accumulated in both nuclei via the shared cytoplasm. These results indicate cytoplasmic to nuclear tRNA trafficking. Using the same strategy, Shaheen and Hopper [85] also demonstrated tRNA trafficking from the cytoplasm to the nucleus in cells transiently deprived of amino acids. Much like the deletion of *Los1*, amino acid deprivation also causes nuclear tRNA accumulation [80,82] and thus serves as an alternate method to visualize nuclear pools of previously cytoplasmic tRNAs. A second set of experiments from Yoshihisa’s group demonstrated retrograde tRNA nuclear import by treatment of the export-defective *los1 msn5* strain with the RNA polymerase inhibitor, thiolutin. Since the production of newly transcribed tRNAs was blocked, the presence of tRNA^{Pro}_{UGG} and tRNA^{Tyr}_{GUA} in the nucleus indicated that these mature nuclear tRNAs were previously localized in the cytoplasm [84]. The results of these experiments provide evidence of tRNA movement from the cytoplasm to the nucleus in *S. cerevisiae*.

Since intron splicing occurs in the cytoplasm of budding yeast cells, the question remained as to whether organisms for which splicing occurs in the nucleus can also import tRNA from the cytoplasm. Remarkably, the retrograde tRNA nuclear import process is not limited to yeast. The first study to demonstrate this was performed in digitonin-permeabilized HeLa cells. tRNA was found to traffic from the cytoplasm to the nucleus in an energy- and temperature-dependent process [86]. Further, in amino acid deprived rat hepatoma cells,

tRNA^{Lys}_{CUU} accumulated in the nucleus even in the presence of the transcription inhibitor actinomycin D to block de novo RNA synthesis, indicating that the tRNAs had originated from the cytoplasm [92]. Other stressors have also been shown to elicit retrograde tRNA import in mammalian cells. For example, HeLa cells exposed to heat stress demonstrated not only tRNA^{Met}_I and tRNA^{Met}_e retrograde trafficking to the nucleus, but also accumulation of these tRNA in nuclear tRNA granules [88,91]. Collectively, these results demonstrate that retrograde tRNA nuclear import occurs even in species that splice pre-tRNAs in the nucleus and it is subject to nutrient availability and other stresses.

3.2. Is retrograde tRNA nuclear import constitutive or regulated?

The question remains as to whether the retrograde tRNA nuclear import process is constitutive, with tRNA trafficking from the cytoplasm to the nucleus regardless of the cellular status, and/or whether the retrograde import process is regulated, with conditions of cellular and environmental stress triggering tRNA movement from the cytoplasm to the nucleus. Furthermore, an increase in retrograde import may not be the only mechanism for nuclear accumulation of previously cytoplasmic tRNA, as a decrease in tRNA re-export could result in a similar effect. Like the retrograde import process, the tRNA re-export step may also function in a constitutive and/or regulated manner. We present the evidence for constitutive and regulated retrograde tRNA import (and re-export, discussed later) and illustrate multiple models for the regulation, or lack thereof, of these processes (Fig. 2).

In order to assess constitutive import of tRNAs into the nucleus, Murthi et al. [47] altered the localization of an endogenously cytoplasmic tRNA modifying enzyme, Trm7, to the nucleus, NLS-Trm7. Trm7 catalyzes 2'-O-methylation of C₃₂ (Cm₃₂) and displays specificity solely for spliced tRNA [93,94]. Thus, in cells expressing only NLS-Trm7, any spliced tRNA species with the Cm₃₂ modification would have been first spliced at the mitochondrial membrane before being modified in the nucleus. Detection of Cm₃₂ modifications on spliced tRNA under standard fed conditions indicates that the retrograde nuclear import of tRNA is constitutive.

Additional support for constitutive retrograde tRNA import by the Suzuki lab showed that retrograde tRNA nuclear import is needed for proper modification of tRNA^{Phe}_{GAA} in *S.*

cerevisiae [95]. tRNA^{Phe}_{GAA} is unique as it is the only tRNA to contain the wybutosine (yW) modification, located at nucleotide position 37. The yW modification stabilizes anticodon-codon pairing and prevents -1 frameshifting [96]. The first step of wybutosination requires the nuclear-localized modification enzyme, Trm5, which catalyzes methylation of G₃₇ to m¹G₃₇. Since substrates for Trm5 include only those tRNAs that have previously undergone cytoplasmic intron splicing and Trm5 is localized only in the nucleus [93,94], tRNA with a m¹G₃₇ modification is indicative of cytoplasmic to nuclear transport. The remaining steps of wybutosine modification are carried out in the cytoplasm, catalyzed in sequential steps by Tyw1-Tyw4. Therefore, for wybutosine formation in tRNA^{Phe}_{GAA}, the pre-tRNA must first be exported out of the nucleus into the cytoplasm for intron splicing, constitutively imported back into the nucleus for Trm5-catalyzed m¹G₃₇ modification, and finally constitutively re-exported to the cytoplasm for the final steps of wybutosination. Although the wybutosine

modification only occurs on tRNA^{Phe}_{GAA}, the Trm5-catalyzed m¹G₃₇ modification is found on 7 tRNA species in *S. cerevisiae* (3 intron-less tRNAs and 4 intron-containing tRNAs). Additionally, in a pathway analogous to wybutosination of yeast tRNA^{Phe}, tRNA^{Tyr} in *Trypanosoma brucei* requires retrograde nuclear import following cytoplasmic intron splicing for queuosine modification of position 34 of the anticodon [97]. Therefore, constitutive retrograde import and re-export is necessary for the proper maturation of several tRNAs.

Several lines of evidence also provide support for regulated tRNA nuclear import. For example, many stress conditions are known to induce nuclear accumulation of previously cytoplasmic tRNA, such as deprivation for amino acids [80,82,85], glucose [46] and phosphate [98]. tRNA^{Tyr} accumulated in the nucleus of *S. cerevisiae* cells as early as 10 min after amino acid or glucose deprivation, as evidenced by FISH analysis [46]. This process is reversible, as the nuclear pools of tRNA are no longer detected upon re-addition of amino acids or glucose, even when transcription is inhibited. A similar phenomenon was observed upon gradual glucose deprivation, with nuclear accumulation of tRNA detected as cells entered late log phase into early stationary phase. The nuclear pools of tRNA observed under conditions of glucose starvation are indeed due to retrograde import of cytoplasmic tRNA and not a defect in export of newly synthesized tRNA, since tRNA expressed by only one nucleus of a heterokaryon accumulate in both nuclei via the shared cytoplasm upon glucose deprivation [46]. This was similarly demonstrated in cells starved for amino acids [85] and phosphate [98]. Evidence of amino acid deprivation-induced nuclear accumulation of tRNA was also observed in other species of *Saccharomyces*, specifically *S. paradoxus* and *S. bayanus*, but not in the yeast species *Kluyveromyces lactis* [99]. Vertebrate cells also demonstrate starvation and stress-induced tRNA nuclear accumulation [86,91,92]. The nuclear tRNA pools that accumulate upon starvation may play a role in preparing cells for the lack of nutrients, rather than being the result of the deprivation itself, since the nuclear accumulation of tRNA occurs on a similar time scale as other nutrient deprivation-sensitive signaling events, specifically translation inhibition and the formation of P-bodies [46,100]. Interestingly, tRNA nuclear-cytoplasmic distribution was found to be coordinated with the early stages of P-body formation/translation repression as a strain defective in glucose starvation-induced P body formation (*dhh1 pat1*) also failed to accumulate tRNA within the nucleus [100].

3.3. Proteins involved in retrograde tRNA nuclear import

3.3.1. Mtr10—Like most RNA and protein nuclear-cytoplasmic trafficking processes, retrograde tRNA nuclear import was found to be energy-dependent [84], reported to utilize both the RanGTPase [85] and Ran-independent pathways [84]. The Ran-dependent mechanism was first investigated by employing budding yeast heterokaryons and the *rna1-1* mutation; *rna1-1* encodes a ts RanGAP that blocks nuclear import and export of proteins [101] and nuclear export of tRNA [80], upon incubation at the non-permissive temperature. For studies of tRNA nuclear import, the majority of tRNA^{Glu-D} in *rna1-1* + *rna1-1* heterokaryons was localized in only one nucleus of the heterokaryons, whether the cells were in the fed or starved states [85]. This is in contrast to the cytoplasmic or bi-nuclear localization of tRNA^{Glu-D} in fed and amino acid-starved *RNA1* heterokaryons, respectively.

These data support a role for the RanGTPase pathway in tRNA nuclear import, since newly transcribed tRNA^{Glu-D} would be trapped in the tRNA^{Glu-D}-synthesizing nucleus and cytoplasmic tRNA^{Glu-D} pools would be unable to enter either nucleus. Conversely, the Yoshihisa lab found that although energy is required for the import of both intron-containing and intronless tRNA, this process is Ran-independent [84]. Surprisingly, this opposing finding was also demonstrated in *ma1-1* cells; however, reasons for this contradiction are not currently apparent. Overall, both Ran-dependent and Ran-independent pathways apparently play roles in retrograde tRNA nuclear import.

To identify the protein(s) mediating Ran-dependent tRNA nuclear import, deletion strains of each of the unessential members of the importin- β family were screened for defects in nuclear accumulation of tRNA^{Tyr}, tRNA^{His}, and tRNA^{Met} upon amino acid deprivation [85]. Of the 9 β -importin family members screened, only deletion of *MTR10* prohibited tRNA nuclear accumulation upon amino acid starvation, implicating a role for Mtr10, either directly or indirectly, in the retrograde tRNA nuclear import process. The findings were supported by data from Takano et al. [102] in which the absence of tRNA nuclear accumulation was evidenced in *MTR10* shut-off strains. Since the *mtr10* strain is severely growth-defective, these latter experiments minimize the potential for acquisition of adaptive mutations in the cells, and thus further verify the role of Mtr10 in this process.

The finding that Mtr10 is involved in tRNA retrograde import is not surprising given that it is a karyopherin in the β -importin family. Mtr10 is conserved, with orthologues existing in humans (Transportin 3 (Tnp3)/Transportin-SR2 and its alternative splice form Transportin-SR) [103,104], *Drosophila* (dTRN-SR) [105] and plants (MOS14) [106]. Previous studies demonstrated that Mtr10 serves as a nuclear importer for the mRNA nuclear export protein, Npl3 [107], as well as an importer for a RNA subunit of telomerase, Tlc1 [108].

Although data support a role for Mtr10 in tRNA nuclear import, direct interaction between Mtr10 and tRNA has not been detected. Using co-immunoprecipitation assays followed by RT-PCR, interaction between Mtr10 and tRNA was unable to be detected in either RanGTP- or RanGDP-locked mutants, despite the fact that RanGDP-dependent interaction of Mtr10 with its known import cargo, Npl3, was detected in the same experiment [25]. Interestingly, the human homolog of Mtr10, Tnp3, directly interacts with viral tRNA and capsid proteins in the presence of RanGTP and can export viral tRNA from the nucleus in a RanGTP-dependent manner [109]. Together, the findings that retrograde tRNA nuclear import can be both Ran-independent and Ran-dependent, along with a lack of direct evidence of Mtr10 binding to tRNA, suggest that Mtr10 and Ran may not be directly involved in the import of tRNA through the nuclear pore, but rather may regulate the localization of other proteins involved directly in the import process [85].

3.3.2. Ssa2—The chaperone protein Ssa2 was identified as a likely tRNA nuclear importer [102]. Ssa2 is a constitutively expressed protein of the Hsp70 heat-shock family. Unlike Mtr10, Ssa2 was identified as a tRNA-binding protein by a biochemical approach using tRNA immobilized on agarose via its 3' end. In addition to binding tRNA in vitro, Ssa2 was also found to be involved in nuclear tRNA import in vivo. Cells deficient in Ssa2 displayed an inhibition in tRNA nuclear accumulation under conditions of nutrient deprivation, but not

under normal growth conditions [102]. This is consistent with the regulated shuttling of Ssa2 protein between the cytoplasm and nucleus depending on cellular conditions [110]. Additionally, cells deficient in the Ssa2 co-chaperones Sis1 and Ydj1, two members of the DnaJ/Hsp40 family that function as ATPase activators and assist in substrate selection [111], are also needed for the nuclear accumulation of tRNA [102].

Ssa2 interacts with tRNA via its nucleotide binding (NTB) domain, displaying a preference for tRNA variants with more relaxed acceptor stem structures, suggesting the NTB domain of Ssa2 has a chaperone-like activity for tRNA. Much like many β -importin proteins, Ssa2 also interacts with the nuclear pore complex, specifically the nucleoporin, Nup116 [102]. Together, these findings demonstrate that Ssa2 is likely a tRNA nuclear importer, at least under conditions of nutrient starvation. Furthermore, Ssa2 acts in parallel to Mtr10, since a mutant defective in both proteins displayed an additive effect as compared to strains defective in only one of these proteins [102].

3.4. Function of retrograde tRNA nuclear import

Evolutionary conservation of the retrograde tRNA nuclear import process suggests that shuttling between the nucleus and cytoplasm plays an important role in cellular physiology. A key function of retrograde tRNA import into the nucleus is to allow for proper modification and maturation of several tRNAs [95,97]; however, there are additional functions for this process.

Cytoplasmic to nuclear tRNA transport has also been identified to play a role in tRNA quality control. Unlike tRNA export, in which the majority of exporters display substrate specificity for end-matured tRNAs with the ability to be aminoacylated [20–25,46,85], the retrograde import process does not exhibit such stringent substrate specificity. In yeast, a wide variety of tRNA substrates can undergo retrograde import, such as endogenous and exogenous tRNAs, aminoacylated or deacylated tRNAs, and CCA-less tRNAs [46,82,84–86], suggesting the nuclear import process may function in tRNA quality control. Accordingly, two types of cytoplasmic aberrant tRNAs (5',3' end-extended, spliced tRNA and hypomodified tRNA), are imported back into the nucleus for repair or degradation [72].

More global cellular processes, such as translation regulation [45] are also impacted by retrograde tRNA nuclear import. Evidence for nuclear accumulation of tRNA under conditions of nutrient starvation suggests that retrograde import may serve to deplete available pools of cytoplasmic tRNA by sequestering them in the nucleus, thus inhibiting translation. Although nuclear import or re-export of tRNAs did not alter global mRNA translation efficiency in cells deficient in Mtr10 or Msn5, the import and re-export process did alter the translation of enzymes involved in the biosynthesis of methionine, arginine and leucine [45]. This may function as a way to regulate growth conditions, since Leu (via the TOR pathway) and Met are very strong growth promoters, and Arg is needed to make more of the transcription machinery.

Cell cycle control is another process affected by the retrograde tRNA nuclear import process [87]. Under environmentally unfavorable conditions, females in the brine shrimp species *Artemia*, release encysted embryos that enter a state of obligatory dormancy known as

diapause. The formation of diapause requires gene expression of *Ar-Larp*, a gene encoding a nuclear-localized La-related RNA-binding protein, which mediates cell cycle arrest. In *Artemia*, Ar-Larp directly binds both pre-tRNA and mature tRNA in the nucleus, suggesting the tRNA-Ar-Larp interaction may mediate cell cycle arrest in diapause. Accordingly, HeLa cells over-expressing Ar-Larp displayed a similar interaction with tRNA and this protein-tRNA interaction elicited the nuclear accumulation of tRNAs. The Ar-Larp-interacting tRNAs were previously localized in the cytoplasm and not newly synthesized tRNA, since this effect was evident even in the presence of the transcription inhibitor, actinomycin D. Overall, the data suggest Ar-Larp functions directly in the retrograde movement of tRNA from the cytoplasm to the nucleus, and both Ar-Larp and tRNA play a role in cell cycle arrest.

The retrograde tRNA import pathway is also important for regulation of retroviral replication. The human immunodeficiency virus-1 (HIV-1), utilizes the retrograde tRNA import pathway as one mechanism to transport the HIV-1 reverse transcription and pre-integration complexes into the nucleus in non-dividing host cells [86]. These complexes interact with select species of tRNA via the anticodon loop. Additionally, they display specificity for tRNA with defective 3' CCA ends, which may be imported into the nucleus as a tRNA quality control mechanism.

3.5. Remaining questions

Retrograde tRNA nuclear import is an evolutionarily conserved process that has important roles in regulating cell physiology. However, several important questions remain. First, although Mtr10 plays a role in the retrograde tRNA nuclear import process, there is currently no evidence for an interaction between Mtr10 and tRNA. Thus, it is unknown whether Mtr10 functions directly or indirectly in retrograde tRNA nuclear import. Second, it remains to be determined whether there are proteins other than Mtr10 and Ssa2 that are involved in cytoplasm to nucleus tRNA trafficking, and more specifically how these proteins may affect constitutive and/or regulated tRNA nuclear import. Third, if additional proteins are involved in the import process, the interplay between these proteins will need to be addressed. In the tRNA nuclear export process, for example, there are multiple exporters that display tRNA substrate specificities in terms of both the processing status of the tRNA and the species of tRNA. Thus it is likely that all tRNAs are not exported equally. It remains to be determined whether there is a similar situation for the retrograde tRNA import process. Fourth, the role of various cellular conditions on the import of tRNA from the cytoplasm needs to be explored. Consistent with the role of Ssa2 in amino acid starvation-induced tRNA import, it is possible that different importers are activated based on the stress conditions. Lastly, it is unknown whether tRNA fragments are imported into the nucleus. Many of the cellular stresses that induce retrograde tRNA nuclear import also induce tRNA fragmentation as well as the nuclear-cytoplasmic distribution of tRNA within the cell [Reviews] [43,112, [Review 113]]. These tRNA fragments are important in the regulation of the translation machinery in the cytoplasm. Whether tRNA fragments are imported into the nucleus is not yet known, but it is interesting to speculate that they could play an important role in processes such as tRNA quality control and translation efficiency.

Ideally, the development of a fluorescent tRNA tracker would revolutionize the field of tRNA biology. Specifically, a tRNA tracker could allow for identification of real-time subcellular movements of tRNA. However, due to the complex and essential tertiary structure of tRNA and the lack of a 3' UTR as in mRNA, tagging tRNA in a way that does not affect its function is currently challenging. In conclusion, despite the wealth of information about the retrograde tRNA nuclear import process, a lot of work remains. Undoubtedly, the development of new technologies and assays will provide a complete picture of the retrograde tRNA nuclear import process and the full range of physiological functions impacted by trafficking of tRNA into the nucleus.

4. tRNA re-export

4.1. Multiple tRNA re-export pathways

tRNAs that are shuttled back to the nucleus either constitutively, or under certain stresses by the retrograde import process, are exported back to the nucleus under favorable conditions to execute cytoplasmic protein synthesis [46]. This tRNA nuclear re-export step constitutes the third tRNA nuclear-cytoplasmic shuttling step.

Biochemical studies demonstrated that Los1 binds to spliced tRNAs transcribed from yeast intron-containing tRNA genes, revealing the role of Los1 in the tRNA nuclear re-export step [22,25]. Binding of Los1 to spliced tRNA is conserved from yeast to vertebrates and is independent of the aminoacylation status of the bound, spliced tRNA [17,21,25,26,114]. Los1-independent pathways for tRNA nuclear re-export likely exist because *LOS1* and its orthologues are unessential in all tested organisms. Multiple studies suggest that at least one of the Los1-independent tRNA nuclear re-export pathways is aminoacylation-dependent. First, tRNAs can be aminoacylated in the nucleus by nuclear pools of tRNA aminoacyl synthetases [23,80–82] and although Los1 binding to tRNA is independent of tRNA aminoacylation status [25], interference with tRNA charging, either by amino-acid deprivation or inhibition of specific tRNA synthetases leads to tRNA nuclear export defects [23,80,82]. Moreover, over-expression of Cca1, which catalyzes the requisite 3' C, C, and A addition, essential for aminoacylation, corrects the nuclear export, but not the pre-tRNA splicing defects of *los1* cells [115]. Finally, mutations in *TEF1/TEF2*, encoding the translation factor, eEF1A, which has high specificity for charged tRNAs, leads to nuclear accumulation of spliced tRNAs [48,82].

The β -importin Msn5 is involved in tRNA re-export in yeast. Budding yeast Msn5 and its orthologues, Exportin-5 in vertebrates and HASTY in plants, serve different functions in different organisms [32–34,116–121]. Vertebrate Exportin-5 and plant HASTY bind directly to nuclear pre-microRNAs and function to export these dsRNAs to the cytoplasm. Vertebrate Exportin-5 also binds tRNA and is reported to serve a minor role in tRNA nuclear export [32]. In contrast, budding yeast Msn5 binds particular phosphorylated proteins and functions in their nuclear export [Review [122]]. Yeast Msn5 also functions in tRNA re-export via a nuclear export complex composed of Msn5, charged spliced tRNA, eEF1A, and RanGTP [25]. Since Msn5 does not efficiently bind intron-containing tRNAs and *msn5* cells do not accumulate intron-containing pre-tRNA [25,47], Msn5 preferentially functions in tRNA nuclear re-export, rather than in the initial tRNA nuclear export step. Though *los1 msn5*

double mutant cells have a larger nuclear pool of tRNAs than either mutant alone, the double mutant cells are viable [47,84], indicating there are likely additional Los1- and Msn5-independent tRNA nuclear re-exporters.

Though Los1 binds aminoacylated as well as uncharged tRNAs, in vivo co-IP studies indicate that Msn5 preferentially interacts with aminoacylated tRNAs [25]. However, contrary to these in vivo data, two in vitro studies showed that Msn5 and its orthologues bind short duplex RNAs and uncharged tRNAs [34,118]. The contradiction between the in vitro and in vivo studies may be explained by the involvement of an additional protein that imparts specificity to Msn5 for binding aminoacylated tRNAs for re-export in vivo. eEF1A (yeast Tef1/2) is a likely candidate to serve this function in vivo. First, yeast cells deleted for *TEF1* and *TEF2* and possessing an ectopic *ts tef2-1* accumulate spliced tRNAs in the nucleus [82], indicating the involvement of Tef1/2 in tRNA re-export. Second, the subcellular distribution of GFP-tagged Tef1 is dependent upon Msn5 [47]. Third, eEF1A binds aminoacylated tRNAs with several orders of magnitude higher than uncharged tRNAs [Review [123]]. Finally, Msn5, but not Los1, interacts with Tef1/2 in vivo in a Ran-dependent manner, demonstrating the specificity of the Tef1/2 interaction with Msn5 [25]. However, it is unclear how the heterotrimeric Msn5-GTP, Tef1/2, aa-tRNA complex assembles. A structural study of human Exportin-5 predicted binding of Exportin-5 to the 3' end of tRNA [124]. Since eEF1A also binds to the 3' end of aminoacylated tRNAs [125,126], concomitant binding of Msn5 and Tef1/2 to the same tRNA structure for the formation of the ternary Msn5-tRNA-eEF1A export complex seems unlikely. In vivo co-IP assays of Msn5 in cells depleted of Tef1/2 revealed the requirement of Tef1/2 to assemble and/or stabilize Msn5-tRNA export complexes [25]. Moreover, treatment of the co-IP fractions with RNase demonstrated that Msn5 can interact with Tef1/2 in the absence of detectable tRNA [25]. This is unsurprising as Msn5 is known to bind various phosphorylated proteins [Review [122]]. Combined, the data favor a model in which Tef1/2, binds aminoacylated tRNAs and interacts with Msn5 via protein-protein interactions [25]. However, further studies are required to assess whether Msn5 interacts directly with tRNA or Tef1/2 and/or whether tRNA binding to Tef1/2 induces a conformational change that stabilizes Tef1/2 interactions with Msn5-RanGTP.

The predicted mechanisms of tRNA recognition by Los1 and Msn5, along with Tef1/2, ensure only tRNAs with processed ends and thus capable of being aminoacylated, are selected for tRNA nuclear re-export, hence coupling tRNA proof-reading with nuclear export. Moreover, the observation that eEF1A competes with the Rapid tRNA Decay machinery for specific tRNA substrates demonstrates the involvement of eEF1A in tRNA quality control [127,128]. Also, as the aminoacyl tRNA synthetases likely monitor matured 3' ends along with internal sequences in the tRNA, nuclear tRNA aminoacylation provides an additional quality control step before nuclear re-export [Review [43]].

Three additional proteins involved in tRNA nuclear export in yeast have recently been identified - Mex67, Mtr2 and Crm1. In vivo biochemical pull down studies in yeast demonstrated that Mex67 binds the spliced form of intron-containing tRNA^{Ile}_{UAU}, suggesting a possible role for Mex67 in tRNA nuclear re-export [37]. There are no reports regarding the

ability of Crm1 to bind tRNA; so, its role in tRNA nuclear re-export remains unknown. Finally, Lord et al., reported that cells with deletion of the nucleoporin Nup100 accumulates specific, spliced tRNAs in the nucleus of yeast by a mechanism independent of Los1 or Msn5 [63]. Hence, there exist multiple pathways to re-export tRNAs from the nucleus to the cytoplasm.

4.2. Constitutive and regulated re-export

In budding yeast some tRNA modifications occur in the nucleus post-splicing in the cytoplasm, such as Trm5 mediated methylation of G₃₇ [93,94]. In order for these fully mature tRNAs to be available for protein synthesis, they must undergo at least one cycle of nuclear import and re-export to the cytoplasm in budding yeast. The re-export step can also occur under regulated conditions, as the re-exporter Msn5 binds poorly to uncharged tRNA upon amino acid starvation [25]. Murthi et al. [47] further demonstrated that fed and amino-acid starved cells have equal levels of Cm₃₂ modification. This suggests that the observed nuclear accumulation of tRNA under amino acid deprivation is not mediated by an increase in tRNA import, but rather regulated at the level of tRNA nuclear re-export. Recent studies have unraveled complex mechanisms regulating tRNA nuclear re-export under different environmental conditions. Whether the newly discovered tRNA nuclear exporters, Mex67-Mtr2 and Crm1 regulate the nuclear tRNA re-export process remains unknown; thus, this section focuses on Los1 and Msn5-mediated regulation of tRNA nuclear re-export.

Retrograde tRNA nuclear accumulation under stress is rapid and reversible [46]. The aminoacylation status of the tRNA cargoes could provide one regulatory mechanism for nuclear re-export of tRNAs. Four independent studies support this hypothesis. First, a recent biochemical study in yeast showed that though Los1 can bind both charged and uncharged tRNAs, Msn5 preferentially binds charged tRNAs for efficient re-export [25]. Second, tRNA with defective 3' termini and hence deficient in charging, accumulate in the nucleus of permeabilized vertebrate cells [86]. Third, nuclear export of the cognate tRNA^{Met} was affected in cells with charging defective methionyl-tRNA synthetase, but the export of non-cognate tRNAs were not affected [80]. Finally, depletion of Thg1, an enzyme critical for charging of tRNA^{His}, accumulated cognate tRNA^{His} in the nucleus but not non-cognate tRNA^{Tyr} [129].

Unlike vertebrate cells, yeast produce all the 20 amino acids, unless they harbor mutations in particular amino acid biosynthesis pathways. The necessity of tRNA aminoacylation for tRNA export led to the prediction, that cells auxotrophic for a particular amino acid would accumulate only that cognate, spliced but uncharged tRNA in the nucleus, when deprived of all amino acids. However, this prediction has not been met. By employing a yeast strain prototrophic for methionine, but auxotrophic for leucine, Whitney et al., showed that, as expected, tRNA^{Leu} mostly became uncharged upon amino acid deprivation and tRNA^{Met} remained charged. Surprisingly, both charged tRNA^{Met} and uncharged tRNA^{Leu} accumulated in the nucleus during amino acid deprivation, indicating tRNA aminoacylation alone is not sufficient for tRNA nuclear re-export [46]. Also, in human foreskin fibroblast cells when Xpo-t was depleted, accumulation of similar levels of tRNA^{Lys} was observed, whether the cells were fed or deprived of amino-acids [90]. Therefore, aminoacylation-

independent as well as aminoacylation-dependent mechanisms regulate tRNA nuclear re-export upon amino acid deprivation.

Upon glucose deprivation, tRNA is rapidly (10 min) imported from the cytoplasm into the nucleus and then the imported tRNAs are rapidly (10 min) re-exported upon glucose re-feeding [46]. The mechanism by which tRNA nuclear re-export responds to glucose deprivation is quite different than to amino acid deprivation. In yeast, under optimal growth conditions, Los1 and Msn5 are predominantly nuclear and they remain so upon amino acid deprivation. Interestingly, within 15 min of glucose deprivation, the predominantly nuclear pools of Los1 and Msn5 become largely cytoplasmic [48], likely due to collapse of the RanGTP nuclear/cytoplasmic gradient [48]. Thus, yeast sense various nutrients in their environment and employ nutrient-specific mechanisms to regulate tRNA nuclear re-export.

4.3. Modes of regulation for retrograde tRNA nuclear import vs. re-export

Several possibilities exist for the role of constitutive and/or regulated tRNA import and the contribution of the re-export step in the nuclear accumulation of tRNA (Fig. 2). First, the evidence for retrograde tRNA import and re-export occurring constitutively under optimal cellular conditions are strongly supported (Fig. 2A). However, under conditions of stress, the import and re-export processes may be further activated. A possibility is that under stress, one process remains constitutive and the other is regulated by the stress conditions. The data from Murthi et al. [47] for example, suggest that the import of tRNA is constitutive, whereas the re-export step is regulated (Fig. 2B). It is possible that under different stress conditions the reverse could be true (Fig. 2C). Perhaps a more accurate model is that import and re-export occur as a coordinated combination of constitutive and regulated processes depending on the cellular conditions (Fig. 2D).

It is interesting to speculate that constitutive import and re-export may serve to continuously monitor the tRNA pool, whereas regulated import and re-export may allow the cell to modulate tRNA availability under conditions of stress. A combination of these processes may provide a mechanism for the cell to fine-tune its response to the dynamic conditions of life. Unfortunately, it is difficult to distinguish constitutive from regulated retrograde tRNA import and re-export. For the tRNA import process, it is necessary that previously cytoplasmic tRNA can be distinguished from newly synthesized tRNA. Since splicing in yeast occurs in the cytoplasm, the presence of spliced tRNA in the nucleus is evidence of tRNA import into the nucleus. However, isolation of yeast nuclei is a challenging task. Nuclear isolation in vertebrate cells on the other hand, is routinely performed. However, splicing in vertebrates occurs in the nucleus. Therefore, it is difficult to track whether tRNA have been trafficked to the cytoplasm before nuclear re-entry. There is a similar issue in distinguishing constitutive from regulated re-export. Overall, further studies are needed to tease apart the contributions that constitutive and regulated import and re-export play in the dynamics of tRNA trafficking and cellular physiology.

Due to the limitations of the current methods, some of these questions are challenging to answer. For example, identification of additional tRNA importers requires the development of an assay that allows for efficient genome-wide screening. Another limitation is that current methodology allows tracking of tRNA for only a single cycle of tRNA movement

from the cytoplasm to the nucleus. However, it is possible that the key functions of retrograde tRNA nuclear import as well as the long lifespan of a tRNA allows for multiple cycles of import and re-export.

5. Cytoplasmic splicing of intron-containing tRNAs

5.1. Pre-tRNA splicing is essential and conserved among eukaryotes

Among eukaryotes, there is phylogenetic divergence regarding the percentage of tRNA genes that contain introns, ranging from 3.9% in sea urchins, to 5% in *Drosophila*, *C. elegans*, mouse and human, to 22% in yeast *S. cerevisiae*, and to 92% in the pathogenic yeast *Cryptococcus neoformans* [130–132]. In some organisms, such as *S. cerevisiae*, if one gene encoding an isoaccepting tRNA contains an intron, then each of the reiterated genes encoding the same tRNA family member will also contain an intron, generally of identical or near identical sequence. In contrast, for other organisms, such as humans, generally only a subset of a given tRNA family will contain an intron. Just the same, removal of introns by tRNA splicing is essential in all studied eukaryotes because all members of at least one tRNA family necessary to decode the genetic code requires splicing. For example, all 5 gene copies of human tRNA^{Ile}_{UAU} contain introns (<http://gttnadb.ucsc.edu/>) [130].

The first step of pre-tRNA splicing is the endonucleolytic removal of introns from pre-tRNAs, catalyzed by the tRNA splicing endonucleases (SEN) complex [133]. In yeast and vertebrates, the SEN complex is a heterotetramer with four protein subunits: Sen2, Sen15, Sen34 and Sen54 (TSEN in human) [8,9]. Each gene encoding SEN subunits is essential in yeast. Likewise, *TSEN2* and *TSEN54* are scored as essential genes in multiple human haploid cancer cell lines by three independent genome-wide screens to identify essential human genes; in contrast, human *TSEN15* and *TSEN34* are scored as essential genes in some, but not all cell lines [29–31]. Sen2 and Sen34 are well conserved whereas Sen15 and Sen54 are poorly conserved. Sen2 and Sen34 are the catalytic subunits of the SEN complex and are responsible for cleavage at the 5' and 3' splice sites, respectively. Structural studies showed that Sen2 and Sen34 form a composite active site for catalysis [134]. The functions of Sen15 and Sen54 remain unknown but they are thought to help establish the cleavage sites on the pre-tRNA. By yeast two hybrid, *S. cerevisiae* Sen2 and Sen54 interact and Sen15 and Sen34 interact [8]. However, exactly how the SEN subunits form a heterotetramer remains unknown.

After endonucleolytic cleavage by the SEN subunits, the two tRNA half molecules (5' exon and 3' exon) are ligated by evolutionarily distinct mechanisms. For budding yeast, the ligation step is carried out by the essential tRNA ligase Trl1 that has cyclic dephosphatase, 5' kinase, and ligase activities; Trl1 catalyzes phosphorylation of the 5' end of the 3' exon and ligates the modified exons together [8,135]. Ligation by Trl1 generates a residual 2' phosphate at the splice junction which is then removed by the 2' phosphotransferase Tpt1 [136,137]. In contrast, in vertebrate cells, the 5' and 3' exons are directly ligated by a protein complex RtcB [138 [Review 139]]. However, a “yeast-like” pathway also exists and the RNA kinase Clp1 has been shown to play an important role [140].

5.2. Nuclear vs. cytoplasmic localization of tRNA splicing endonucleases

Although the function of tRNA splicing endonucleases is conserved in eukaryotes, its subcellular distribution varies. In vertebrates, pre-tRNA splicing occurs in the nucleoplasm [23,141] and the tRNA splicing endonucleases are located in the nucleus [9]. Surprisingly, the *S. cerevisiae* tRNA splicing endonucleases are not in the nucleus, but rather peripherally associated with the cytoplasmic surface of the mitochondrial outer membrane [10,11,142] (Fig. 3).

The cytoplasmic localization of tRNA splicing endonucleases have been reported for both *Saccharomyces cerevisiae* and *Trypanosoma brucei* [10,11,143]. However, the endogenous subcellular localization of tRNA splicing endonuclease has only been studied in budding yeast. In *los1* mutants, yeast cells accumulate intron-containing tRNAs in the nucleus [83] because intron-containing tRNAs are not efficiently exported from the nucleus to cytoplasm, where they can interact with the SEN complex. Genetic data from other organisms suggests that the cytoplasmic localization of tRNA splicing endonucleases may be a common theme. For example, for *S. pombe* and *A. thaliana*, deletion of *XpoT* or *PAUSED*, respectively, results in accumulation of end-mature intron-containing tRNAs [18,33], perhaps easiest explained if tRNAs restricted to the nucleus are unable to interact with a cytoplasmic SEN complex.

Although these genetic data lead to the prediction that the tRNA splicing endonucleases of *S. pombe* and *A. thaliana* should be located in the cytoplasm, other studies of the subcellular localization of tRNA splicing endonuclease subunits in *S. pombe* and *A. thaliana* suggest that this may not be the case. A genome-wide protein localization study in *S. pombe* [144], reported that Sen2 locates to some unknown cytoplasmic foci, Sen15 locates to the nucleus and cytoplasm, and Sen54 is on mitochondria. This study was conducted employing over-expression with the inducible *nmt1* promoter. Englert et al. [145] investigated the subcellular localization of plant tRNA splicing endonucleases *A. thaliana* Sen1 and Sen2 that were transiently expressed via a strong constitutive 35S Ω promoter in *Allium* epidermal and *Vicia* guard cells. The authors reported that AtSen1 and AtSen2 are located in the nucleus and the cytoplasm. Similarly, in *T. brucei*, TbSen34 over-expressed by tetracycline-induction localizes to cytoplasmic puncta [143]. Determination of protein localization via over-expression may not accurately report the subcellular location of SEN subunits as the SEN complex is a heterotetramer and over-expressed single subunits may not achieve the same location as endogenous proteins. Recently Yang et al., reported that in *A. thaliana*, the tRNA ligase (encoded by *AtRNL*) is localized in the cytoplasm and excluded from the nucleus when endogenously expressed [146]. Their data support the notion that tRNA splicing may be cytoplasmic in *A. thaliana* [146]. However, due to the low expression levels of SEN subunits in yeast (a few hundred copies per cell) [147,148], it may be challenging to determine the subcellular location of endogenous levels of SEN subunits in *S. pombe* and *A. thaliana*. Thus, the phylogenic distribution of the SEN complex among various eukaryotic organisms awaits further study.

5.3. Novel essential functions of the SEN complex on the mitochondrial surface

The differences in subcellular distributions of the tRNA splicing endonuclease between yeast and vertebrates suggest that the SEN complex may have other functions in addition to pre-tRNA splicing. To test whether yeast cells can splice tRNAs in the nucleus, Dhungel et al. [149] re-localized the SEN subunits to the nucleus by providing each subunit with a nuclear localization signal. Yeast cells with both nuclear and cytoplasmic tRNA splicing endonucleases grow as well as wild-type, suggesting that nuclear tRNA splicing in yeast is not harmful. Yeast cells with only nuclear tRNA splicing endonuclease can splice intron-containing tRNAs efficiently, but they cannot grow if the mitochondrial-localized SEN is inactivated or depleted [149]. The data indicate that yeast cells cannot grow without mitochondrial-localized tRNA splicing endonuclease, even if tRNAs are efficiently spliced. The data led to the notion that there may be putative, novel, and essential functions for the SEN complex on the mitochondrial surface [149].

Results from two studies in yeast suggest that the SEN complexes may play a role in pre-ribosomal RNA (pre-rRNA) processing. With only nuclear tRNA splicing endonuclease, yeast cells have aberrant pre-rRNA processing, even though pre-tRNA splicing is efficient in the nucleus [149]. In addition, cells depleted for Sen34 showed a delay in the processing of 35S and 27S rRNA, resulting in a reduction of mature 18S and 25S rRNAs, indicating that Sen34 depletion decreases the kinetics of rRNA processing [150]. One enticing, but untested, possibility is that the tRNA splicing endonuclease may affect pre-rRNA processing via its function in processing small nucleolar RNAs in the cytoplasm prior to import into the nucleus for pre-rRNA processing steps.

Recent findings from yeast, mouse, flies, and worms showed that several post-transcriptional RNA processing steps occur on the mitochondrial surface, including steps affecting tRNA, mRNA, and piRNA (PIWI-interacting RNAs) biogenesis (Fig. 3). The yeast tRNA splicing endonuclease also catalyzes a particular step in mRNA biogenesis on the mitochondrial surface. It cleaves the mitochondria-localized mRNA encoding Cbp1 (cytochrome *b* mRNA processing 1) [151]. Cleavage of this unessential *CBP1* mRNA requires the mitochondrial localization of the SEN complex and two *CBP1* mRNA cis-elements: the mitochondrial targeting signal and the stem-loop 652–726-nt region [151]. The authors proposed that endonucleolytic cleavage of Cbp1 mRNA occurs co-translationally, when *CBP1* mRNA is localized to mitochondria via its mitochondrial targeting signal [151]. It is unknown whether additional mRNAs are processed by SEN.

Also, in yeast a particular tRNA modification step likely occurs on the surface of mitochondria. *N*⁶-threonylcarbamoyladenine (t⁶A) is a universally conserved, essential tRNA modification [Review 152,153]. It is responsible for decoding ANN codons in all three domains of life and it serves an important role in promoting decoding efficiency. Miyauchi et al. reported that t⁶A is a hydrolyzed form of the modification and that in vivo this modification exists in a cyclized form, ct⁶A. Cyclization is catalyzed by Tcd1 and Tcd2 [154]. Tcd1 and Tcd2 are located on the mitochondrial surface [142] and are required for respiratory growth [154]. Therefore, it is likely that this particular step in tRNA modification occurs on the mitochondrial surface. Likewise, tRNA G₃₄UN queuosine formation in mouse

is catalyzed by a heteromeric transglycosylase located on the mitochondrial outer membrane (Fig. 3) [155].

The outer surface of mitochondria also serves as a site for biogenesis of piRNAs. piRNAs are a class of small RNAs that can form piRNA-induced silencing complexes to repress transposons and maintain germline genome integrity [Review 156]. piRNAs can also function in the regulation of cellular genes in various organisms [156]. Essential processing steps of piRNA occur on the surface of mitochondria in mouse, flies and silkworm [157–159]. Specifically, Zucchini (zuc) in *D. melanogaster* that is required for piRNA processing is located to mitochondria [160]. The mammalian homolog of zuc, MitoPLD, also localizes to mitochondria. In silkworm *Bombyx mori*, two piRNA processing enzymes, BmPAPI and Trimmer, also locate to mitochondria [159,161]. The essential processing steps of piRNAs include 5' and 3' processing and have been extensively reviewed [162–164]. Recently, Honda et al. reported the mechanism of biogenesis of a special class of piRNAs that are derived from tRNAs (td-piRNAs) in *B. mori* BmN4 cells [165]. Td-piRNAs are derived primarily from the 5' halves of tRNA^{Asp}_{GUC} and tRNA^{His}_{GUG}. These tRNA halves are specifically and abundantly expressed in sex hormone-dependent breast and prostate cancer cells [166]. These two td-piRNAs are processed in the canonical piRNA biogenesis pathway, and their 3' end maturation is catalyzed by Trimmer, with the help of BmPAPI, both of which are located to mitochondria [165] (Fig. 3). The identity and molecular mechanism of the endonuclease that underlies the anticodon loop cleavage of only specific tRNA species in BmN4 cells is unknown, but is thought to be similar to endonuclease ANG in mammalian cells [165].

5.4. Why is the mitochondrial cytoplasmic surface involved in RNA processing?

We consider 4 possible roles for the location of RNA processing activities to the cytoplasmic surface of mitochondria. First, it is possible that the mitochondrial localization of the RNA processing enzymes evolved for moonlighting functions. For example, in addition to tRNA splicing, the SEN complex also splices *CBP1* mRNA on the mitochondrial surface for the co-translational import of Cbp1 protein [151]. It is possible that the RNA processing enzymes located on the mitochondrial surface have unidentified moonlighting functions that may be involved in mitochondria composition and/or function.

A second possibility is that the RNA processing enzymes are located on the mitochondria to maintain substrate specificity to the mitochondrial surface. For example, structural and biochemical studies showed that purified mouse piRNA processing enzyme Zucchini (mZuc) functions as a backbone-non-specific, single strand-specific nuclease for either RNA or DNA substrates, with similar binding affinity for ssDNA and ssRNA [167]. Perhaps the substrate specificity of mZuc for pre-piRNA is achieved by limiting its access to those RNAs able to locate to the mitochondrial surface.

A third possibility is that the mitochondrial surface serves as an efficient platform for assembly of RNA processing enzymes. For example, the yeast SEN subunits exist in only a few hundred copies in each cell [147,148], and therefore they may not be able to “find each other” and efficiently form a heterotetramer in the dilute cytoplasm. However, delivery of

the SEN subunits to the mitochondrial surface could increase their local concentrations and perhaps enhance heterotetramerization, possibly with the help of other mitochondrial outer membrane proteins.

Fourth, it is possible that RNA processing is coupled with mitochondrial metabolism. For example, by locating the post-transcriptional RNA processing events to the surface of mitochondria, cells may be able to down-regulate RNA processing when there is insufficient energy produced by mitochondria.

5.5. How do RNA processing enzymes locate to the mitochondrial surface?

Wu et al. conducted a genome-wide screen in *S. cerevisiae* and discovered 55 genes previously not known to be involved in tRNA splicing [35]. Among these were two genes encoding mitochondrial outer membrane proteins, Tom70 and Sam37. *tom70* and *sam37* mutant cells accumulate end-processed, intron-containing tRNAs [35]. Tom70 and Sam37 function in importing proteins into the mitochondria and localizing other proteins to the mitochondrial surface [168]. Tom70 is a subunit of the translocase of the outer mitochondrial membrane (TOM) complex, which is composed of the subunits Tom5, Tom6, Tom7, Tom20, Tom22, Tom40, and Tom70 [169,170]. Tom70 is an integral membrane protein with cytosolic domains that function as receptors for incoming proteins. Sam37 is a subunit of the sorting and assembly machinery (SAM) complex, which is composed of the subunits Sam35, Sam37, Sam50, and Mdm10. Similar to Tom70, Sam37 also has a cytosolic domain but unlike Tom70, it is not well conserved beyond fungi [171,172].

Although *TOM70* and *SAM37* are not essential for yeast viability, they are essential for the respiratory metabolism of yeast [173,174]. It is possible that pre-tRNA splicing requires efficient respiratory metabolism and therefore *tom70* or *sam37* cells accumulate intron-containing tRNAs because they are not spliced efficiently. This possibility could be tested by examining the efficiency of intron-containing tRNA splicing in other mutants that have an absence of respiratory metabolism. Another possibility is that the deletion of *TOM70* or *SAM37* causes defects in tRNA nuclear export through an unknown feedback mechanism; thus, tRNAs cannot reach the cytoplasm to be spliced on the surface of mitochondria. This possibility can be tested by examining if *tom70* or *sam37* cells accumulate intron-containing tRNAs in the nucleus using FISH. Because Tom70 and Sam37 are both mitochondrial outer membrane proteins with cytosolic domains [171,172], and the SEN complex is located on the surface of mitochondria, peripherally associated with the mitochondrial outer membrane [10,11], it is also possible that Tom70 and Sam37 interact with SEN complex subunits directly or indirectly. Therefore, Tom70 and Sam37 may be involved in the localization, assembly, stability and/or function of the SEN complex proteins. These possibilities and their verification may help to gain an understanding of how the mitochondrial surface is involved in tRNA processing, and perhaps processing of other RNA species like mRNA and piRNAs.

6. Summary and perspectives

tRNA nuclear-cytoplasmic dynamics in eukaryotic cells was previously thought to be unidirectional export of tRNAs from the nucleus to the cytoplasm for protein translation.

However, it is now clear that there are evolutionarily conserved and regulated bidirectional movements of tRNAs between the nucleus and the cytoplasm. The subcellular distributions of tRNA serve unanticipated functions in diverse processes, including cellular stress response, DNA repair and cell proliferation, tRNA quality control, and even HIV replication. It is also now clear that there are multiple proteins functioning in parallel for each step of tRNA trafficking. The response of the individual exporters and importers to stress conditions and their potential substrate specificity may uncover a new way to regulate gene expression at the level of tRNA availability for translation in the cytoplasm. Future studies are required to determine which of the newly discovered tRNA export and import pathways uncovered in budding yeast are conserved among plants and vertebrates. Moreover, in budding yeast, tRNAs must access the mitochondrial surface for splicing by the mitochondrial located SEN complex and for a particular modification. Although pre-tRNA splicing on mitochondria does not occur in vertebrate cells, it is also now clear that several other RNA processing steps occur on the outer surface of mitochondria rather than in the nucleus. The reasons for the selection of this organellar surface for RNA processing remain a mystery for future discoveries.

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References

1. Guy MP, Shaw M, Weiner CL, Hobson L, Stark Z, Rose K, Kalscheuer VM, Gecz J, Phizicky EM. Defects in tRNA anticodon loop 2'-O-methylation are implicated in nonsyndromic X-linked intellectual disability due to mutations in FTSJ1. *Hum Mutat.* 2015; 36:1176–1187. [PubMed: 26310293]
2. Shaheen R, Abdel-Salam GMH, Guy MP, Alomar R, Abdel-Hamid MS, Afifi HH, Ismail SI, Emam BA, Phizicky EM, Alkuraya FS. Mutation in WDR4 impairs tRNA m(7)G46 methylation and causes a distinct form of microcephalic primordial dwarfism. *Genome Biol.* 2015; 16:210. [PubMed: 26416026]
3. Kirchner S, Ignatova Z. Emerging roles of tRNA in adaptive translation, signalling dynamics and disease. *Nat Rev Genet.* 2014; 16:98–112. [PubMed: 25534324]
4. Salinas-Giegé T, Giegé R, Giegé P. tRNA biology in mitochondria. *Int J Mol Sci.* 2015; 16:4518–4559. [PubMed: 25734984]
5. O'Connor JP, Peebles CL. In vivo pre-tRNA processing in *Saccharomyces cerevisiae*. *Mol Cell Biol.* 1991; 11:425–439. [PubMed: 1986237]
6. Ohira T, Suzuki T. Precursors of tRNAs are stabilized by methylguanosine cap structures. *Nat Chem Biol.* 2016; 12:648–655. [PubMed: 27348091]
7. Phizicky EM, Hopper AK. tRNA biology charges to the front. *Genes Dev.* 2010; 24:1832–1860. [PubMed: 20810645]
8. Trotta CR, Miao F, Arn EA, Stevens SW, Ho CK, Rauhut R, Abelson JN. The yeast tRNA splicing endonuclease: a tetrameric enzyme with two active site subunits homologous to the archaeal tRNA endonucleases. *Cell.* 1997; 89:849–858. [PubMed: 9200603]
9. Paushkin SV, Patel M, Furia BS, Peltz SW, Trotta CR. Identification of a human endonuclease complex reveals a link between tRNA splicing and pre-mRNA 3' end formation. *Cell.* 2004; 117:311–321. [PubMed: 15109492]
10. Yoshihisa T, Yunoki-Esaki K, Ohshima C, Tanaka N, Endo T. Possibility of cytoplasmic pre-tRNA splicing: the yeast tRNA splicing endonuclease mainly localizes on the mitochondria. *Mol Biol Cell.* 2003; 14:3266–3279. [PubMed: 12925762]

11. Yoshihisa T, Ohshima C, Yunoki-Esaki K, Endo T. Cytoplasmic splicing of tRNA in *Saccharomyces cerevisiae*. *Genes Cells*. 2007; 12:285–297. [PubMed: 17352735]
12. Zasloff M. tRNA transport from the nucleus in a eukaryotic cell: carrier-mediated translocation process. *Proc Natl Acad Sci*. 1983; 80:6436–6440. [PubMed: 6579529]
13. Terry LJ, Shows EB, Wentz SR. Crossing the nuclear envelope: hierarchical regulation of nucleocytoplasmic transport. *Science*. 2007; 318:1412–1416. [PubMed: 18048681]
14. Wentz SR, Rout MP. The nuclear pore complex and nuclear transport. *Cold Spring Harb Perspect Biol*. 2010; 2
15. Hopper AK, Schultz LD, Shapiro RA. Processing of intervening sequences: a new yeast mutant which fails to excise intervening sequences from precursor tRNAs. *Cell*. 1980; 19:741–751. [PubMed: 7363329]
16. Arts GJ, Fornerod M, Mattaj LW. Identification of a nuclear export receptor for tRNA. *Curr Biol*. 1998; 8:305–314. [PubMed: 9512417]
17. Kutay U, Lipowsky G, Izaurralde E, Bischoff FR, Schwarzmaier P, Hartmann E, Görlich D. Identification of tRNA-specific nuclear export receptor. *Mol Cell*. 1998; 1:359–369. [PubMed: 9660920]
18. Cherkasova V, Maury LL, Bacikova D, Pridham K, Bähler J, Marais RJ. Altered nuclear tRNA metabolism in *La*-deleted *Schizosaccharomyces pombe* is accompanied by a nutritional stress response involving Atf1p and Pcr1p that is suppressible by Xpo-t/Los1p. *Mol Biol Cell*. 2012; 23:480–491. [PubMed: 22160596]
19. Hunter CA, Aukerman MJ, Sun H, Fokina M, Poethig RS. PAUSED encodes the *Arabidopsis* exportin-t orthologues. *Plant Physiol*. 2003; 132:2135–2143. [PubMed: 12913168]
20. Hurt DJ, Wang SS, Lin YH, Hopper AK. Cloning and characterization of LOS1, a *Saccharomyces cerevisiae* gene that affects tRNA splicing. *Mol Cell Biol*. 1987; 7:1208–1216. [PubMed: 3031485]
21. Arts GJ, Kuersten S, Romby P, Ehresmann B, Mattaj LW. The role of exportin-t in selective nuclear export of mature tRNAs. *EMBO J*. 1998; 17:7430–7441. [PubMed: 9857198]
22. Hellmuth K, Lau DM, Bischoff FR, Künzler M, Hurt E, Simos G. Yeast Los1p has properties of an exportin-like nucleocytoplasmic transport factor for tRNA. *Mol Cell Biol*. 1998; 18:6374–6386. [PubMed: 9774653]
23. Lund E, Dahlberg JE. Proofreading and aminoacylation of tRNAs before export from the nucleus. *Science*. 1998; 282:2082–2085. [PubMed: 9851929]
24. Cook AG, Fukuhara N, Jinek M, Conti E. Structures of the tRNA export factor in the nuclear and cytosolic states. *Nature*. 2009; 461:60–65. [PubMed: 19680239]
25. Huang HY, Hopper AK. In vivo biochemical analyses reveal distinct roles of β -importins and eEF1A in tRNA subcellular traffic. *Genes Dev*. 2015; 29:772–783. [PubMed: 25838545]
26. Lipowsky G, Bischoff FR, Izaurralde E, Kutay U, Schäfer S, Gross HJ, Beier H, Görlich D. Coordination of tRNA nuclear export with processing of tRNA. *RNA*. 1999; 5:539–549. [PubMed: 10199570]
27. Li J, Chen X. PAUSED, a putative exportin-t, acts pleiotropically in *Arabidopsis* development but is dispensable for viability. *Plant Physiol*. 2003; 132:1913–1924. [PubMed: 12913148]
28. Lippai M, Tirian L, Boros I, Mihaly J, Erdelyi M, Belec I, Mathe E, Posfai J, Nagy A, Udvardy A, Paraskova E, Görlich D, Szabad J. The Ketel gene encodes a *Drosophila* homologue of importin- β . *Genetics*. 2000; 156:1889–1900. [PubMed: 11102382]
29. Blomen VA, Májek P, Jae LT, Bigenzahn JW, Nieuwenhuis J, Staring J, Sacco R, van Diemen FR, Olk N, Stukalov A, Marceau C, Janssen H, Carette JE, Bennett KL, Colinge J, Superti-Furga G, Brummelkamp TR. Gene essentiality and synthetic lethality in haploid human cells. *Science*. 2015; 350:1092–1096. [PubMed: 26472760]
30. Hart T, Chandrasekhar M, Aregger M, Steinhart Z, Brown KR, MacLeod G, Mis M, Zimmermann M, Fradet-Turcotte A, Sun S, Mero P, Dirks P, Sidhu S, Roth FP, Rissland OS, Durocher D, Angers S, Moffat J. High-resolution CRISPR screens reveal fitness genes and genotype-specific cancer liabilities. *Cell*. 2015; 163:1515–1526. [PubMed: 26627737]

31. Wang T, Birsoy K, Hughes NW, Krupczak KM, Post Y, Wei JJ, Lander ES, Sabatini DM. Identification and characterization of essential genes in the human genome. *Science*. 2015; 350:1096–1101. [PubMed: 26472758]
32. Calado A, Treichel N, Müller EC, Otto A, Kutay U. Exportin-5-mediated nuclear export of eukaryotic elongation factor 1A and tRNA. *EMBO J*. 2002; 21:6216–6224. [PubMed: 12426393]
33. Park MY, Wu G, Gonzalez-Sulser A, Vaucheret H, Poethig RS. Nuclear processing and export of microRNAs in Arabidopsis. *Proc Natl Acad Sci*. 2005; 102:3691–3696. [PubMed: 15738428]
34. Shibata S, Sasaki M, Miki T, Shimamoto A, Furuichi Y, Katahira J, Yoneda Y. Exportin-5 orthologues are functionally divergent among species. *Nucleic Acids Res*. 2006; 34:4711–4721. [PubMed: 16963774]
35. Wu J, Bao A, Chatterjee K, Wan Y, Hopper AK. Genome-wide screen uncovers novel pathways for tRNA processing and nuclear–cytoplasmic dynamics. *Genes Dev*. 2015; 29:2633–2644. [PubMed: 26680305]
36. Hutten S, Kehlenbach RH. CRM1-mediated nuclear export: to the pore and beyond. *Trends Cell Biol*. 2007; 17:193–201. [PubMed: 17317185]
37. Chatterjee, K., Majumder, S., Wan, Y., Shah, V., Wu, J., Huang, HY., Hopper, AK. Sharing the load: Mex67-Mtr2 co-functions with Los1 in primary tRNA nuclear export. *Genes Dev*. 2017. <http://dx.doi.org/10.1101/gad.305904.117>
38. Okamura M, Inose H, Masuda S. RNA export through the NPC in eukaryotes. *Genes (Basel)*. 2015; 6:124–149. [PubMed: 25802992]
39. Katahira J, Sträßer K, Podtelejnikov A, Mann M, Jung JU, Hurt E. The Mex67p-mediated nuclear mRNA export pathway is conserved from yeast to human. *EMBO J*. 1999; 18:2593–2609. [PubMed: 10228171]
40. Ossareh-Nazari B, Maison C, Black BE, Lévesque L, Paschal BM, Dargemont C. RanGTP-binding protein NXT1 facilitates nuclear export of different classes of RNA in vitro. *Mol Cell Biol*. 2000; 20:4562–4571. [PubMed: 10848583]
41. Kodiha M, Crompton N, Shrivastava S, Umar R, Stochaj U. Traffic control at the nuclear pore. *Nucleus*. 2010; 1:237–244. [PubMed: 21327069]
42. Cautain B, Hill R, De Pedro N, Link W. Components and regulation of nuclear transport processes. *FEBS J*. 2015; 282:445–462. [PubMed: 25429850]
43. Huang H-Y, Hopper A. Multiple layers of stress-induced regulation in tRNA biology. *Life*. 2016; 6:16.
44. Holcik M, Sonenberg N. Translational control in stress and apoptosis. *Nat Rev Mol Cell Biol*. 2005; 6:318–327. [PubMed: 15803138]
45. Chu H-Y, Hopper AK. Genome-wide investigation of the role of the tRNA nuclear-cytoplasmic trafficking pathway in regulation of the yeast *Saccharomyces cerevisiae* transcriptome and proteome. *Mol Cell Biol*. 2013; 33:4241–4254. [PubMed: 23979602]
46. Whitney ML, Hurto RL, Shaheen HH, Hopper AK. Rapid and reversible nuclear accumulation of cytoplasmic tRNA in response to nutrient availability. *Mol Biol Cell*. 2007; 18:2678–2686. [PubMed: 17475781]
47. Murthi A, Shaheen HH, Huang H-Y, Preston MA, Lai T-P, Phizicky EM, Hopper AK. Regulation of tRNA bidirectional nuclear-cytoplasmic trafficking in *Saccharomyces cerevisiae*. *Mol Biol Cell*. 2010; 21:639–649. [PubMed: 20032305]
48. Huang H-Y, Hopper AK. Separate responses of karyopherins to glucose and amino acid availability regulate nucleocytoplasmic transport. *Mol Biol Cell*. 2014; 25:2840–2852. [PubMed: 25057022]
49. Quan X, Yu J, Bussey H, Stochaj U. The localization of nuclear exporters of the importin- β family is regulated by Snf1 kinase, nutrient supply and stress. *Biochim Biophys Acta*. 2007; 1773:1052–1061. [PubMed: 17544521]
50. Izaurralde E. A novel family of nuclear transport receptors mediates the export of messenger RNA to the cytoplasm. *Eur J Cell Biol*. 2002; 81:577–584. [PubMed: 12498157]
51. Kelly SM, Corbett AH. Messenger RNA export from the nucleus: a series of molecular wardrobe changes. *Traffic*. 2009; 10:1199–1208. [PubMed: 19552647]
52. Stewart M. Nuclear export of mRNA. *Trends Biochem Sci*. 2010; 35:609–617. [PubMed: 20719516]

53. Pasquinelli AE, Ernst RK, Lund E, Grimm C, Zapp ML, Rekosh D, Hammar skjöld ML, Dahlberg JE. The constitutive transport element (CTE) of Mason-Pfizer monkey virus (MPMV) accesses a cellular mRNA export pathway. *EMBO J.* 1997; 16:7500–7510. [PubMed: 9405378]
54. Ernst RK, Bray M, Rekosh D, Hammar skjöld ML. Secondary structure and mutational analysis of the Mason-Pfizer monkey virus RNA constitutive transport element. *RNA.* 1997; 3:210–222. [PubMed: 9042947]
55. Aibara S, Katahira J, Valkov E, Stewart M. The principal mRNA nuclear export factor NXF1:NXT1 forms a symmetric binding platform that facilitates export of retroviral CTE-RNA. *Nucleic Acids Res.* 2015; 43:1883–1893. [PubMed: 25628361]
56. Faza MB, Chang Y, Occhipinti L, Kemmler S, Panse VG. Role of Mex67-Mtr2 in the nuclear export of 40S pre-ribosomes. *PLoS Genet.* 2012; 8
57. Zander G, Hackmann A, Bender L, Becker D, Lingner T, Salinas G, Krebber H. mRNA quality control is bypassed for immediate export of stress-responsive transcripts. *Nature.* 2016; 540:593–596.
58. Bond U. Stressed out! Effects of environmental stress on mRNA metabolism. *FEMS Yeast Res.* 2006; 6:160–170. [PubMed: 16487339]
59. Krebber H, Taura T, Lee MS, Silver PA. Uncoupling of hnRNP Npl3p from mRNAs during the stress-induced block in mRNA export. *Genes Dev.* 1999; 13:1994–2004. [PubMed: 10444597]
60. Ghavidel A, Kislinger T, Pogoutse O, Sopko R, Jurisica I, Emili A. Impaired tRNA nuclear export links DNA damage and cell-cycle checkpoint. *Cell.* 2007; 131:915–926. [PubMed: 18045534]
61. Qiu H, Hu C, Anderson J, Björk GR, Sarkar S, Hopper AK, Hinnebusch AG. Defects in tRNA processing and nuclear export induce GCN4 translation independently of phosphorylation of the alpha subunit of eukaryotic translation initiation factor 2. *Mol Cell Biol.* 2000; 20:2505–2516. [PubMed: 10713174]
62. Hinnebusch AG, Natarajan K. Gcn4p, a master regulator of gene expression, is controlled at multiple levels by diverse signals of starvation and stress. *Eukaryot Cell.* 2002; 1:22–32. [PubMed: 12455968]
63. Lord CL, Ospovat O, Wente SR. Nup100 regulates *Saccharomyces cerevisiae* replicative life span by mediating the nuclear export of specific tRNAs. *RNA.* 2017; 23:365–377. [PubMed: 27932586]
64. Chen M, Gartenberg MR. Coordination of tRNA transcription with export at nuclear pore complexes in budding yeast. *Genes Dev.* 2014; 28:959–970. [PubMed: 24788517]
65. Haeusler RA, Pratt-Hyatt M, Good PD, Gipson TA, Engelke DR. Clustering of yeast tRNA genes is mediated by specific association of condensin with tRNA gene transcription complexes. *Genes Dev.* 2008; 22:2204–2214. [PubMed: 18708579]
66. Karkusiewicz I, Turowski TW, Graczyk D, Towpik J, Dhungel N, Hopper AK, Boguta M. Maf1 protein, repressor of RNA polymerase III indirectly affects tRNA processing. *J Biol Chem.* 2011; 286:39478–39488. [PubMed: 21940626]
67. Boguta M. Maf1, a general negative regulator of RNA polymerase III in yeast. *Biochim Biophys Acta Gene Regul Mech.* 2013; 1829:376–384.
68. Hopper AK. Transfer RNA post-transcriptional processing, turnover, and subcellular dynamics in the yeast *Saccharomyces cerevisiae*. *Genetics.* 2013; 194:43–67. [PubMed: 23633143]
69. Thompson M, Haeusler RA, Good PD, Engelke DR. Nucleolar clustering of dispersed tRNA genes. *Science.* 2003; 302:1399–1401. [PubMed: 14631041]
70. Duan Z, Andronescu M, Schutz K, McIlwain S, Kim YJ, Lee C, Shendure J, Fields S, Blau CA, Noble WS. A three-dimensional model of the yeast genome. *Nature.* 2010; 465:363–367. [PubMed: 20436457]
71. Aebi M, Kirchner G, Chen JY, Vijayraghavan U, Jacobson A, Martin NC, Abelson J. Isolation of a temperature-sensitive mutant with an altered tRNA nucleotidyltransferase and cloning of the gene encoding tRNA nucleotidyltransferase in the yeast *Saccharomyces cerevisiae*. *J Biol Chem.* 1990; 265:16216–16220. [PubMed: 2204621]
72. Kramer EB, Hopper AK. Retrograde transfer RNA nuclear import provides a new level of tRNA quality control in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A.* 2013; 110:21042–21047. [PubMed: 24297920]

73. Phizicky EM, Alfonzo JD. Do all modifications benefit all tRNAs? FEBS Lett. 2010; 584:265–271. [PubMed: 19931536]
74. Lorenz C, Lünse C, Mörl M. tRNA modifications: impact on structure and thermal adaptation. Biomol Ther. 2017; 7:35.
75. Rutjes SA, Utz PJ, van der Heijden A, Broekhuis C, van Venrooij WJ, Pruijn GJ. The La (SS-B) autoantigen, a key protein in RNA biogenesis, is de-phosphorylated and cleaved early during apoptosis. Cell Death Differ. 1999; 6:976–986. [PubMed: 10556975]
76. Intine RV, Tenenbaum SA, Sakulich AL, Keene JD, Maraia RJ. Differential phosphorylation and subcellular localization of La RNPs associated with precursor tRNAs and translation-related mRNAs. Mol Cell. 2003; 12:1301–1307. [PubMed: 14636586]
77. Foretek D, Wu J, Hopper AK, Boguta M. Control of *Saccharomyces cerevisiae* pre-tRNA processing by environmental conditions. RNA. 2016;339–349. [PubMed: 26729922]
78. Simos G, Tekotte H, Grosjean H, Segref A, Sharma K, Tollervy D, Hurt EC. Nuclear pore proteins are involved in the biogenesis of functional tRNA. EMBO J. 1996; 15:2270–2284. [PubMed: 8641292]
79. Li S, Sprinzl M. Interaction of immobilized human exportin-t with calf liver tRNA. RNA Biol. 2006; 3:145–149. [PubMed: 17224653]
80. Sarkar S, Azad AK, Hopper AK. Nuclear tRNA aminoacylation and its role in nuclear export of endogenous tRNAs in *Saccharomyces cerevisiae*. Proc Natl Acad Sci U S A. 1999; 96:14366–14371. [PubMed: 10588711]
81. Azad AK, Stanford DR, Sarkar S, Hopper AK. Role of nuclear pools of aminoacyl-tRNA synthetases in tRNA nuclear export. Mol Biol Cell. 2001; 12:1381–1392. [PubMed: 11359929]
82. Grosshans H, Hurt E, Simos G. An aminoacylation-dependent nuclear tRNA export pathway in yeast. Genes Dev. 2000; 14:830–840. [PubMed: 10766739]
83. Sarkar S, Hopper AK. tRNA nuclear export in *Saccharomyces cerevisiae*: in situ hybridization analysis. Mol Biol Cell. 1998; 9:3041–3055. [PubMed: 9802895]
84. Takano A, Endo T, Yoshihisa T. tRNA actively shuttles between the nucleus and cytosol in yeast. Science. 2005; 309:140–142. [PubMed: 15905365]
85. Shaheen HH, Hopper AK. Retrograde movement of tRNAs from the cytoplasm to the nucleus in *Saccharomyces cerevisiae*. Proc Natl Acad Sci U S A. 2005; 102:11290–11295. [PubMed: 16040803]
86. Zaitseva L, Myers R, Fassati A. tRNAs promote nuclear import of HIV-1 in-tracellular reverse transcription complexes. PLoS Biol. 2006; 4:1689–1706.
87. Chen D-F, Lin C, Wang H-L, Zhang L, Dai L, Jia S-N, Zhou R, Li R, Yang J-S, Yang F, Clegg JS, Nagasawa H, Yang W-J. An La-related protein controls cell cycle arrest by nuclear retrograde transport of tRNAs during diapause formation in *Artemia*. BMC Biol. 2016; 14:16. [PubMed: 26941127]
88. Watanabe K, Miyagawa R, Tomikawa C, Mizuno R, Takahashi A, Hori H, Ijiri K. Degradation of initiator tRNA met by Xrn1/2 via its accumulation in the nucleus of heat-treated HeLa cells. Nucleic Acids Res. 2013; 41:4671–4685. [PubMed: 23471000]
89. Barhoom S, Kaur J, Cooperman BS, Smorodinsky NI, Smilansky Z, Ehrlich M, Elroy-Stein O. Quantitative single cell monitoring of protein synthesis at subcellular resolution using fluorescently labeled tRNA. Nucleic Acids Res. 2011; 39:e129. [PubMed: 21795382]
90. Huynh LN, Thangavel M, Chen T, Cottrell R, Mitchell JM, Praetorius-Ibba M. Linking tRNA localization with activation of nutritional stress responses. Cell Cycle. 2010; 9:3112–3118. [PubMed: 20714220]
91. Miyagawa R, Mizuno R, Watanabe K, Ijiri K. Formation of tRNA granules in the nucleus of heat-induced human cells. Biochem Biophys Res Commun. 2012; 418:149–155. [PubMed: 22244871]
92. Shaheen HH, Horetsky RL, Kimball SR, Murthi A, Jefferson LS, Hopper AK. Retrograde nuclear accumulation of cytoplasmic tRNA in rat hepatoma cells in response to amino acid deprivation. Proc Natl Acad Sci. 2007; 104:8845–8850. [PubMed: 17502605]
93. Jiang HQ, Motorin Y, Jin YX, Grosjean H. Pleiotropic effects of intron removal on base modification pattern of yeast tRNA^{Phe}: an in vitro study. Nucleic Acids Res. 1997; 25:2694–2701. [PubMed: 9207014]

94. Pintard L, Lecoite F, Bujnicki JM, Bonnerot C, Grosjean H, Lapeyre B. Trm7p catalyses the formation of two 2'-O-methylriboses in yeast tRNA anticodon loop. *EMBO J.* 2002; 21:1811–1820. [PubMed: 11927565]
95. Ohira T, Suzuki T. Retrograde nuclear import of tRNA precursors is required for modified base biogenesis in yeast. *Proc Natl Acad Sci U S A.* 2011; 108:10502–10507. [PubMed: 21670254]
96. Waas WF, Druzina Z, Hanan M, Schimmel P. Role of a tRNA base modification and its precursors in frameshifting in eukaryotes. *J Biol Chem.* 2007; 282:26026–26034. [PubMed: 17623669]
97. Kessler AC, Kulkarni SS, Paulines MJ, Rubio MAT, Limbach PA, Paris Z, Alfonzo JD. Retrograde nuclear transport from the cytoplasm is required for tRNA^{Tyr} maturation in *T. brucei*. *RNA Biol.* 2017; 0:1–9.
98. Hurto RL, Tong AHY, Boone C, Hopper AK. Inorganic phosphate deprivation causes tRNA nuclear accumulation via retrograde transport in *Saccharomyces cerevisiae*. *Genetics.* 2007; 176:841–852. [PubMed: 17409072]
99. Chafe SC, Pierce JB, Eswara MBK, McGuire AT, Mangroo D. Nutrient stress does not cause retrograde transport of cytoplasmic tRNA to the nucleus in evolutionarily diverse organisms. *Mol Biol Cell.* 2011; 22:1091–1103. [PubMed: 21289100]
100. Hurto RL, Hopper AK. P-body components, Dhh1 and Pat1, are involved in tRNA nuclear-cytoplasmic dynamics. *RNA.* 2011; 17:912–924. [PubMed: 21398402]
101. Corbett AH, Koepf DM, Schlenstedt G, Lee MS, Hopper AK, Silver PA. Rna1p, a Ran/TC4 GTPase activating protein, is required for nuclear import. *J Cell Biol.* 1995; 130:1017–1026. [PubMed: 7657689]
102. Takano A, Kajita T, Mochizuki M, Endo T, Yoshihisa T. Cytosolic Hsp70 and co-chaperones constitute a novel system for tRNA import into the nucleus. *elife.* 2015
103. Kataoka N, Bachorik JL, Dreyfuss G. Transportin-SR, a nuclear import receptor for SR proteins. *J Cell Biol.* 1999; 145:1145–1152. [PubMed: 10366588]
104. Lai MC, Lin RI, Tam WY. Transportin-SR2 mediates nuclear import of phosphorylated SR proteins. *Proc Natl Acad Sci U S A.* 2001; 98:10154–10159. [PubMed: 11517331]
105. Allemand E, Dokudovskaya S, Bordonné R, Tazi J. A conserved *Drosophila* transportin-serine/arginine-rich (SR) protein permits nuclear import of *Drosophila* SR protein splicing factors and their antagonist repressor splicing factor 1. *Mol Biol Cell.* 2002; 13:2436–2447. [PubMed: 12134081]
106. Xu S, Zhang Z, Jing B, Gannon P, Ding J, Xu F, Li X, Zhang Y. Transportin-SR is required for proper splicing of resistance genes and plant immunity. *PLoS Genet.* 2011; 7:e1002159. [PubMed: 21738492]
107. Senger B, Simos G, Bischoff FR, Podtelejnikov A, Mann M, Hurt E. Mtr10p functions as a nuclear import receptor for the mRNA-binding protein Npl3p. *EMBO J.* 1998; 17:2196–2207. [PubMed: 9545233]
108. Ferrezuelo F, Steiner B, Aldea M, Fitcher B. Biogenesis of yeast telomerase depends on the importin Mtr10. *Mol Cell Biol.* 2002; 22:6046–6055. [PubMed: 12167699]
109. Zhou L, Sokolskaja E, Jolly C, James W, Cowley SA, Fassati A. Transportin 3 promotes a nuclear maturation step required for efficient HIV-1 integration. *PLoS Pathog.* 2011; 7
110. Shulga N, James P, Craig EA, Goldfarb DS. A nuclear export signal prevents *Saccharomyces cerevisiae* Hsp70 Ssb1p from stimulating nuclear localization signal-directed nuclear transport. *J Biol Chem.* 1999; 274:16501–16507. [PubMed: 10347213]
111. Kampinga HH, Craig EA. The HSP70 chaperone machinery: J proteins as drivers of functional specificity. *Nat Rev Mol Cell Biol.* 2010; 11:579–592. [PubMed: 20651708]
112. Thompson DM, Parker R. Stressing out over tRNA cleavage. *Cell.* 2009; 138:215–219. [PubMed: 19632169]
113. Saikia M, Krokowski D, Guan BJ, Ivanov P, Parisien M, Hu F, Anderson P, Pan T, Hatzoglou M. Genome-wide identification and quantitative analysis of cleaved tRNA fragments induced by cellular stress. *J Biol Chem.* 2012; 287:42708–42725. [PubMed: 23086926]
114. Kuersten S, Arts G-J, Walther TC, Englmeier L, Mattaj IW. Steady-state nuclear localization of exportin-t involves RanGTP binding and two distinct nuclear pore complex interaction domains. *Mol Cell Biol.* 2002; 22:5708–5720. [PubMed: 12138183]

115. Feng W, Hopper AK. A Los1p-independent pathway for nuclear export of intronless tRNAs in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci*. 2002; 99:5412–5417. [PubMed: 11959996]
116. Mingot JM, Vega S, Cano A, Portillo F, Nieto MA. eEF1A mediates the nuclear export of SNAG-containing proteins via the exportin5-aminoacyl-tRNA complex. *Cell Rep*. 2013; 5:727–737. [PubMed: 24209753]
117. Johnson AW, Lund E, Dahlberg J. Nuclear export of ribosomal subunits. *Trends Biochem*. 2002; 27:580–585.
118. Lund E, Güttinger S, Calado A, Dahlberg JE, Kutay U. Nuclear export of microRNA precursors. *Science*. 2004; 303:95–98. [PubMed: 14631048]
119. Kim VN. MicroRNA precursors in motion: exportin-5 mediates their nuclear export. *Trends Cell Biol*. 2004; 14:156–159. [PubMed: 15134074]
120. Bohnsack MT. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. *RNA*. 2004; 10:185–191. [PubMed: 14730017]
121. Yi R, Qin Y, Macara IG, Cullen BR. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev*. 2003; 17:3011–3016. [PubMed: 14681208]
122. Hopper AK. Nucleocytoplasmic transport: inside out regulation. *Curr Biol*. 1999; 9:803–806.
123. Noble CG, Song H. Structural studies of elongation and release factors. *Cell Mol Life Sci*. 2008; 65:1335–1346. [PubMed: 18213444]
124. Okada C, Yamashita E, Lee SJ, Shibata S, Katahira J, Nakagawa A, Yoneda Y, Tsukihara T. A high-resolution structure of the pre-microRNA nuclear export machinery. *Science*. 2009; 326:1275–1279. [PubMed: 19965479]
125. Nissen P, Kjeldgaard M, Thirup S, Polekhina G, Reshetnikova L, Clark BF, Nyborg J. Crystal structure of the ternary complex of Phe-tRNA^{Phe}, EF-Tu, and a GTP analog. *Science*. 1995; 270:1464–1472. [PubMed: 7491491]
126. Nissen P, Kjeldgaard M, Thirup S, Clark BFC, Nyborg J. The ternary complex of aminoacylated tRNA and EF-Tu-GTP recognition of a bond and a fold. *Biochimie*. 1996; 78:921–933. [PubMed: 9150869]
127. Dewe JM, Whipple JM, Chernyakov I, Jaramillo LN, Phizicky EM. The yeast rapid tRNA decay pathway competes with elongation factor 1A for substrate tRNAs and acts on tRNAs lacking one or more of several modifications. *RNA*. 2012; 18:1886–1896. [PubMed: 22895820]
128. Turowski TW, Karkusiewicz I, Kowal J, Boguta M. Maf1-mediated repression of RNA polymerase III transcription inhibits tRNA degradation via RTD pathway. *RNA*. 2012; 18:1823–1832. [PubMed: 22919049]
129. Gu W, Hurto RL, Hopper AK, Grayhack EJ, Phizicky EM. Depletion of *Saccharomyces cerevisiae* tRNA(His) guanylyltransferase Thg1p leads to uncharged tRNA^{His} with additional m(5)C. *Mol Cell Biol*. 2005; 25:8191–8201. [PubMed: 16135808]
130. Jühling F, Mörl M, Hartmann RK, Sprinzl M, Stadler PF, Pütz J. tRNAdb 2009: compilation of tRNA sequences and tRNA genes. *Nucleic Acids Res*. 2009; 37:D159–D162. [PubMed: 18957446]
131. Yoshihisa T. Handling tRNA introns, archaeal way and eukaryotic way. *Front Genet*. 2014; 5:213. [PubMed: 25071838]
132. Chan PP, Lowe TM. GtRNAdb: a database of transfer RNA genes detected in genomic sequence. *Nucleic Acids Res*. 2009; 37
133. Knapp G, Beckmann JS, Johnson PF, Fuhrman SA, Abelson J. Transcription and processing of intervening sequences in yeast tRNA genes. *Cell*. 1978; 14:221–236. [PubMed: 352537]
134. Trotta CR, Paushkin SV, Patel M, Li H, Peltz SW. Cleavage of pre-tRNAs by the splicing endonuclease requires a composite active site. *Nature*. 2006; 441:375–377. [PubMed: 16710424]
135. Phizicky EM, Consaul SA, Nehrke KW, Abelson J. Yeast tRNA ligase mutants are nonviable and accumulate tRNA splicing intermediates. *J Biol Chem*. 1992; 267:4577–4582. [PubMed: 1537841]
136. Spinelli SL, Consaul SA, Phizicky EM. A conditional lethal yeast phosphotransferase (tpt1) mutant accumulates tRNAs with a 2'-phosphate and an under-modified base at the splice junction. *RNA*. 1997; 3:1388–1400. [PubMed: 9404890]

137. Culver GM, McCraith SM, Consaul SA, Stanford DR, Phizicky EM. A 2'-phosphotransferase implicated in tRNA splicing is essential in *Saccharomyces cerevisiae*. *J Biol Chem*. 1997; 272:13203–13210. [PubMed: 9148937]
138. Popow J, Englert M, Weitzer S, Schleiffer A, Mierzwa B, Mechtler K, Trowitzsch S, Will CL, Luhrmann R, Soll D, Martinez J. HSPC117 is the essential subunit of a human tRNA splicing ligase complex. *Science*. 2011; 331:760–764. [PubMed: 21311021]
139. Popow J, Schleiffer A, Martinez J. Diversity and roles of (t)RNA ligases. *Cell Mol Life Sci*. 2012; 69:2657–2670. [PubMed: 22426497]
140. Mair B, Popow J, Mechtler K, Weitzer S, Martinez J. Intron excision from precursor tRNA molecules in mammalian cells requires ATP hydrolysis and phosphorylation of tRNA-splicing endonuclease components. *Biochem Soc Trans*. 2013; 41:831–837. [PubMed: 23863140]
141. Melton DA, De Robertis EM, Cortese R. Order and intracellular location of the events involved in the maturation of a spliced tRNA. *Nature*. 1980; 284:143–148. [PubMed: 6987526]
142. Huh WKK, Falvo JV, Gerke LC, Carroll AS, Howson RW, Weissman JS, O'Shea EK. Global analysis of protein localization in budding yeast. *Nature*. 2003; 425:686–691. [PubMed: 14562095]
143. Lopes RRS, de G, Silveira O, Eitler R, Vidal RS, Kessler A, Hinger S, Paris Z, Alfonzo JD, Polycarpo C. The essential function of the *Trypanosoma brucei* Trl1 homolog in procyclic cells is maturation of the intron-containing tRNA^{Tyr}. *RNA*. 2016; 22:1190–1199. [PubMed: 27284166]
144. Matsuyama A, Arai R, Yashiroda Y, Shirai A, Kamata A, Sekido S, Kobayashi Y, Hashimoto A, Hamamoto M, Hiraoka Y, Horinouchi S, Yoshida M. ORFeome cloning and global analysis of protein localization in the fission yeast *Schizosaccharomyces pombe*. *Nat Biotechnol*. 2006; 24:841–847. [PubMed: 16823372]
145. Englert M, Latz A, Becker D, Gimple O, Beier H, Akama K. Plant pre-tRNA splicing enzymes are targeted to multiple cellular compartments. *Biochimie*. 2007; 89:1351–1365. [PubMed: 17698277]
146. Yang K-J, Guo L, Hou X-L, Gong H-Q, Liu C-M. *ZYGOTE-ARREST 3* that encodes the tRNA ligase is essential for zygote division in *Arabidopsis*. *J Integr Plant Biol*. 2017; 59:680–692. [PubMed: 28631407]
147. Ghaemmaghami S, Huh W-K, Bower K, Howson RW, Belle A, Dephoure N, O'Shea EK, Weissman JS. Global analysis of protein expression in yeast. *Nature*. 2003; 425:737–741. [PubMed: 14562106]
148. Kulak NA, Pichler G, Paron I, Nagaraj N, Mann M. Minimal, encapsulated proteomic-sample processing applied to copy-number estimation in eukaryotic cells. *Nat Methods*. 2014; 11:319–324. [PubMed: 24487582]
149. Dhungel N, Hopper AK. Beyond tRNA cleavage: novel essential function for yeast tRNA splicing endonuclease unrelated to tRNA processing. *Genes Dev*. 2012; 26:503–514. [PubMed: 22391451]
150. Volta V, Ceci M, Emery B, Bachi A, Petfalski E, Tollervey D, Linder P, Marchisio PC, Piatti S, Biffo S. Sen34p depletion blocks tRNA splicing in vivo and delays rRNA processing. *Biochem Biophys Res Commun*. 2005; 337:89–94. [PubMed: 16188229]
151. Tsuboi T, Yamazaki R, Nobuta R, Ikeuchi K, Makino S, Ohtaki A, Suzuki Y, Yoshihisa T, Trotta C, Inada T. The tRNA splicing endonuclease complex cleaves the mitochondria-localized Cbp1 mRNA. *J Biol Chem*. 2015; 290:16021–16030. [PubMed: 25971974]
152. El Yacoubi B, Bailly M, de Crécy-Lagard V. Biosynthesis and function of post-transcriptional modifications of transfer RNAs. *Annu Rev Genet*. 2012; 46:69–95. [PubMed: 22905870]
153. Schweizer MP, Chheda GB, Baczynskyj L, Hall RH. Aminoacyl nucleosides VII N-(purin-6-ylcarbonyl)threonine. A new component of transfer ribonucleic acid. *Biochemistry*. 1969; 8:3283–3289. [PubMed: 4897334]
154. Miyauchi K, Kimura S, Suzuki T. A cyclic form of N6-threonylcarbonyl-adenosine as a widely distributed tRNA hypermodification. *Nat Chem Biol*. 2012; 9:105–111. [PubMed: 23242255]
155. Boland C, Hayes P, Santa-Maria I, Nishimura S, Kelly VP. Queuosine formation in eukaryotic tRNA occurs via a mitochondrial-localized heteromeric transglycosylase. *J Biol Chem*. 2009; 284:18218–18227. [PubMed: 19414587]

156. Iwasaki YW, Siomi MC, Siomi H. PIWI-Interacting RNA: its biogenesis and functions. *Annu Rev Biochem.* 2015; 84:405–433. [PubMed: 25747396]
157. Watanabe T, Chuma S, Yamamoto Y, Kuramochi-Miyagawa S, Totoki Y, Toyoda A, Hoki Y, Fujiyama A, Shibata T, Sado T, Noce T, Nakano T, Nakatsuji N, Lin H, Sasaki H. MITOPLD is a mitochondrial protein essential for nuage formation and piRNA biogenesis in the mouse germline. *Dev Cell.* 2011; 20:364–375. [PubMed: 21397847]
158. Huang H, Gao Q, Peng X, Choi SY, Sarma K, Ren H, Morris AJ, Frohman MA. PiRNA-associated germline nuage formation and spermatogenesis require MitoPLD profusogenic mitochondrial-surface lipid signaling. *Dev Cell.* 2011; 20:376–387. [PubMed: 21397848]
159. Izumi N, Shoji K, Sakaguchi Y, Honda S, Kirino Y, Suzuki T, Katsuma S, Tomari Y. Identification and functional analysis of the pre-piRNA 3' trimmer in silkworms. *Cell.* 2016; 164:962–973. [PubMed: 26919431]
160. Saito K, Ishizu H, Komai M, Kotani H, Kawamura Y, Nishida KM, Siomi H, Siomi MC. Roles for the Yb body components Armitage and Yb in primary piRNA biogenesis in *Drosophila*. *Genes Dev.* 2010; 24:2493–2498. [PubMed: 20966047]
161. Honda S, Kirino Y, Maragkakis M, Alexiou P, Ohtaki A, Murali R, Mourelatos Z, Kirino Y. Mitochondrial protein BmPAPI modulates the length of mature piRNAs. *RNA.* 2013; 19:1405–1418. [PubMed: 23970546]
162. Aravin AA, Chan DC. PiRNAs meet mitochondria. *Dev Cell.* 2011; 20:287–288. [PubMed: 21397839]
163. Weick E-M, Miska EA. piRNAs: from biogenesis to function. *Development.* 2014; 141:3458–3471. [PubMed: 25183868]
164. Czech B, Hannon GJ. A happy 3' ending to the piRNA maturation story. *Cell.* 2016; 164:838–840. [PubMed: 26919421]
165. Honda S, Kawamura T, Loher P, Morichika K, Rigoutsos I, Kirino Y. The biogenesis pathway of tRNA-derived piRNAs in Bombyx germ cells. *Nucleic Acids Res.* 2017; 45:9108–9120. [PubMed: 28645172]
166. Honda S, Loher P, Shigematsu M, Palazzo JP, Suzuki R, Imoto I, Rigoutsos I, Kirino Y. Sex hormone-dependent tRNA halves enhance cell proliferation in breast and prostate cancers. *Proc Natl Acad Sci U S A.* 2015; 112:E3816–25. [PubMed: 26124144]
167. Ipsaro JJ, Haase AD, Knott SR, Joshua-Tor L, Hannon GJ. The structural biochemistry of Zucchini implicates it as a nuclease in piRNA biogenesis. *Nature.* 2012; 491:279–283. [PubMed: 23064227]
168. Stojanovski D, Bohnert M, Pfanner N, van der Laan M. Mechanisms of protein sorting in mitochondria. *Cold Spring Harb Perspect Biol.* 2012; 4:a011320. [PubMed: 23028120]
169. Moczek M, Dietmeier K, Söllner T, Segui B, Steger HF, Neupert W, Pfanner N. Identification of the mitochondrial receptor complex in *Saccharomyces cerevisiae*. *FEBS Lett.* 1992; 310:265–268. [PubMed: 1327874]
170. Dekker PJ, Ryan MT, Brix J, Müller H, Hönliger A, Pfanner N. Preprotein translocase of the outer mitochondrial membrane: molecular dissection and assembly of the general import pore complex. *Mol Cell Biol.* 1998; 18:6515–6524. [PubMed: 9774667]
171. Gratzer S, Lithgow T, Bauer RE, Lamping E, Paltauf F, Kohlwein SD, Haucke V, Junne T, Schatz G, Horst M. Mas37p, a novel receptor subunit for protein import into mitochondria. *J Cell Biol.* 1995; 129:25–34. [PubMed: 7698990]
172. Wiedemann N, Kozjak V, Prinz T, Ryan MT, Meisinger C, Pfanner N, Truscott KN. Biogenesis of yeast mitochondrial cytochrome c: a unique relationship to the TOM machinery. *J Mol Biol.* 2003; 327:465–474. [PubMed: 12628251]
173. Riezman H, Hase T, van Loon AP, Grivell LA, Suda K, Schatz G. Import of proteins into mitochondria: a 70 kilodalton outer membrane protein with a large carboxy-terminal deletion is still transported to the outer membrane. *EMBO J.* 1983; 2:2161–2168. [PubMed: 6321150]
174. Lithgow T, Junne T, Suda K, Gratzer S, Schatz G. The mitochondrial outer membrane protein Mas22p is essential for protein import and viability of yeast. *Proc Natl Acad Sci U S A.* 1994; 91:11973–11977. [PubMed: 7991567]

Fig. 1.

tRNA nuclear-cytoplasmic dynamics in eukaryotic cells. Nuclear-cytoplasmic shuttling of tRNA, encoded from an intron-containing tRNA gene is illustrated. Purple circles represent sequences of the initial transcript that are removed by the 5' and 3' processing enzymes. Yellow circles represent intron sequences located after tRNA nucleotide 37. Blue and red circles are transcribed sequences that remain after processing in the mature tRNA; red circles indicate the anticodon. Green circles depict the added CCA trinucleotide. For simplicity, modified nucleotides are not depicted. Note that the order of splicing relative to 5' and 3' processing differs between yeast and vertebrate cells. In vertebrates (right), tRNA splicing takes place in the nucleus, whereas in budding yeast (left) pre-tRNA splicing takes place on the outer mitochondrial membrane. Both intron-containing and intronless newly synthesized pre-tRNAs are escorted out of the nucleus by the primary tRNA nuclear export process (red). The proteins involved are marked in red; solid red arrows denote proteins with a verified role in the primary tRNA nuclear export; dashed red arrows denote possible role in the process. Red question mark denotes yet unidentified pathways in the primary tRNA nuclear export process. After primary nuclear export and post-splicing in yeast, the nuclear cytoplasmic pathways converge in yeast and vertebrates. For both groups, spliced tRNAs are charged by aminoacyl tRNA synthetases. The tRNAs are then imported back to the nucleus by the retrograde tRNA import process (blue). The proteins involved in the process are indicated by solid and dashed blue arrows as described above. The blue question mark denotes yet unidentified pathways. In the third step of tRNA subcellular trafficking, the imported nuclear tRNAs are exported out of the nucleus by the tRNA re-export step. The proteins participating in the process are indicated in green. Since the roles of vertebrate orthologues of Mex67-Mtr2 and Crm1 in this process are unknown, they are indicated by a green question mark.

Fig. 2.

Potential pathways for retrograde tRNA nuclear import and re-export under optimal and stress conditions. (A) Under optimal cellular conditions, tRNA is imported into the nucleus and re-exported out of the nucleus constitutively. Under stress conditions, tRNA can accumulate in the nucleus by one of three pathways: (B) a block in tRNA nuclear re-export, (C) an increase in tRNA nuclear import, or (D) a combination of increased tRNA nuclear import and decreased tRNA nuclear re-export. The thin arrows indicate a constitutive process. The thicker pointed and flat-ended arrows indicate a regulated process.

Fig. 3.

Summary of post-transcriptional processing of RNAs on the mitochondrial surface. In yeast *Saccharomyces cerevisiae*, intron-containing tRNA splicing occurs on the mitochondrial surface by tRNA splicing endonuclease (SEN) complex. The SEN complex also cleaves unessential *CBP1* mRNA for the co-translational mitochondrial import of Cbp1 protein. Formation of cyclic N6-threonylcarbamoyladenosine (ct⁶A₃₇) from t⁶A₃₇ is catalyzed by Tcd1/2 on the surface of the mitochondria. In metazoan, piRNA 5' processing is carried out by endonucleases Zuc (*D. melanogaster*) or MitoPLD (*M. musculus*) on the mitochondrial surface. The 3' trimming of piRNA in silkworm (*B. mori*) requires two mitochondria-localized proteins, Trimmer (exonuclease) and Papi. Mouse Q34 modification of tRNAs is catalyzed by the heteromeric transglycosylase (TGT/Qv1) located on the mitochondrial surface. OM, outer mitochondrial membrane; IM, inner mitochondrial membrane.