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Cellular Dynamics of Small RNAs

Anita K. Hopper
Department of Biochemistry and Molecular Biology, Pennsylvania State University College of Medicine, Hershey, PA, USA

ABSTRACT This review highlights the unexpectedly complicated nuclear egress and nuclear import of small RNAs. Although nucleus/cytoplasm trafficking was thought to be restricted to snRNAs of many, but not all, eukaryotes, recent data indicate that such traffic may be more common than previously appreciated. First, in conflict with numerous previous reports, new information indicates that *Saccharomyces cerevisiae* snRNAs may cycle between the nucleus and the cytoplasm. Second, recent studies also provide evidence that other small RNAs that function exclusively in the nucleus–the budding yeast telomerase RNA and possibly small nucleolar RNAs–may exit to the cytoplasm, only to return to the nucleus. Third, nucleus/cytoplasm cycling of RNAs also occurs for RNAs that function solely in the cytoplasm, as it has been discovered that cytoplasmic tRNAs of budding yeast travel "retrograde" to the nucleus and, perhaps, back again to the cytoplasm to function in protein synthesis. Fourth, there is at least one example in ciliates of small double-stranded RNAs traveling multiple cycles between the cytoplasm and distinct nuclei to direct genome structure. This report discusses data that support or argue against nucleus/cytoplasm bidirectional movement for each category of small RNA and the possible roles that such movement may serve.

KEYWORDS snRNA, snoRNA, telomerase RNA, TLC1, tRNA, dsRNA, siRNA, miRNA

INTRODUCTION

Movement of macromolecules in and out of the nucleus occurs through aqueous channels that traverse the outer and inner nuclear membranes (ONM and INM, respectively). The channels are formed by nuclear pore complexes (NPCs), which join the ONM and INM. Both in lower and higher eukaryotes, NPCs are comprised of about 30 different proteins, called nucleoporins (Rout *et al.*, 2000; Cronshaw *et al.*, 2002). With the prominent exception of mRNAs (reviewed in Rodriguez *et al.*, 2004), transport of macromolecules through the NPC is generally mediated by a highly conserved mechanism consisting of the GTPase, Ran, Ran-regulatory proteins, and a family of Ran-binding proteins, the β importins. Members of the β importin family have the ability to specifically interact with particular cargoes and particular NPC proteins, moving with the cargo into or out of the nucleus. The β importin family is comprised of 14 members in budding yeast and ~20 in higher eukaryotes (reviewed in Gorlich & Kutay, 1999). The members are generally

Address correspondence to Anita K. Hopper, Department of Biochemistry and Molecular Biology, Pennsylvania State University College of Medicine, 500 University Drive, Hershey, PA 17033. E-mail: ahopper@psu.edu

referred to as importins if they move cargo from the cytoplasm to the nucleoplasm and as exportins if they move cargo from the nucleoplasm to the cytoplasm. However, it has been reported that some members can transport one specific cargo in one direction and another cargo in the opposite direction (*e.g.*, Yoshida & Blobel, 2001). Ran acts as a molecular switch regulating association or dissociation between a β importin family member and its cargo, dependent upon Ran's GTP or GDP bound state. The regulatory proteins determine whether Ran is associated with GTP (RanGEF) or GDP (RanGAP). Directionality across the nuclear pore is maintained by a gradient of RanGTP, which is high in the nucleus and low in the cytoplasm, due to the subcellular locations of RanGEF and RanGAP (Izaurralde *et al.*, 1997; Kalab *et al.*, 2002). RanGEF is located in the nucleoplasm, whereas RanGAP is located primarily in the cytoplasm (Aebi *et al.*, 1990; Hopper *et al.*, 1990; Ohtsubo *et al.*, 1991).

There are numerous types of small RNAs encoded by the nuclear genome, and they serve a multitude of functions in various subcellular compartments. Some function strictly within the nucleus, whereas others function elsewhere in the cell. Nuclear genome-encoded small RNAs (snRNAs) U1, U2, U4, U5 and U6 and the corresponding AT/AC snRNAs function solely in the nucleus at various steps of pre-mRNA intron removal (reviewed in Tarn & Steitz, 1997). Likewise, a host of other small RNAs (snoRNAs) located in the nucleolar compartment of the nucleus function in pre-rRNA processing and modification (reviewed in Kiss, 2002). Another example of a small nucleus-encoded RNA with a strictly nuclear role is the small RNA component of telomerase (TLC1 in budding yeast), which functions in telomere maintenance and biogenesis (reviewed in Cech, 2004). In contrast, tRNAs provide an example of small RNAs that appear not to have a biological role in the nucleus, as they participate in protein synthesis in the cytoplasm. However, there has been a controversial report of translation in the nucleus (Iborra *et al.*, 2001; Review: Dahlberg *et al.*, 2003). If verified, it would mean that some part of the cellular tRNA pool functions in the nucleus. Furthermore, in some organisms, tRNAs encoded by the nucleus are imported into mitochondria and function in protein synthesis within this organelle (reviewed in Hopper & Phizicky, 2003). Finally, small double-stranded RNAs (dsRNAs) are the most recently discovered category of RNA. Some members of this category function in the cytoplasm in post-transcriptional regulation of mRNA turnover and/or translation (reviewed in Tang, 2005), whereas other members function in the nucleus and regulate transcription and/or chromatin structure (reviewed in Matzke & Birchler, 2005; Bernstein & Allis, 2005). Whether a given dsRNA can function both in the nucleus and the cytoplasm is unknown.

As snRNA, snoRNA, and telomerase RNA are transcribed and function in the nucleus, one might have expected that they would never leave the nuclear compartment. Likewise, one would expect that tRNA movement in cells would be unidirectional–from the nucleus to the cytoplasm–because tRNAs are transcribed in the nucleus but function in the cytoplasm. However, in contradiction to the predictions, it has been established that U1, U2, U4, and U5 snRNAs have complicated cellular dynamics; they are exported to the cytoplasm where they undergo modification and associate with proteins, only to be re-imported back to the nucleus (Reviews: Kiss, 2004; Yong *et al.*, 2004). Surprisingly, complex nucleus/cytoplasm dynamics may not be restricted to snRNAs, as recent data indicate that other RNAs that function strictly in the nucleus may be exported to the cytoplasm and re-imported back into the nucleus. Moreover, it has been discovered recently that tRNAs in the cytoplasm can be imported into the nucleus–a process called "retrograde tRNA nuclear import" (Shaheen & Hopper, 2005; Takano *et al.*, 2005).

The focus here is threefold: (1) to assess the data indicating that small RNAs other than snRNAs that function exclusively in the nucleus may have a cytoplasmic phase; (2) to review the new developments documenting that resident cytoplasmic RNAs can be imported retrograde into the nucleus; and (3) to describe the nuclear/cytoplasmic dynamics of small dsRNAs; at least one member of this category of RNAs exhibits multiple cycles of nucleus egress and import. The review is organized to first discuss RNAs that function solely in the nucleus, followed by a discussion of RNA whose function is in the cytoplasm, and then by the dsRNA category of RNAs, some members of which function in the cytoplasm and some in the nucleus. Since the data in many of the recent publications rely upon a new application of yeast heterokaryon analysis, this methodology and its power/limitations are also assessed. Not discussed here are the RNAs that comprise the signal recognition particle, RNaseP, and U7 RNP, as there are limited published data concerning their movement among subcellular compartments. Also not elaborated upon

here are the complex intranuclear dynamics of resident nuclear RNAs; this subject has been thoroughly covered in recent reviews (Kiss, 2004; Lamond & Spector, 2003; Matera, 1999; Maser & DePinho, 2002).

snRNAs—PRECEDENT FOR A CYTOPLASMIC PHASE FOR RESIDENT NUCLEAR RNAS

Trafficking of U1, U2, U4 and U5 Between the Nucleus and the Cytoplasm

Cycling of U1, U2, U4, and U5 snRNAs between the nucleus and the cytoplasm is well established and extensively reviewed (reviewed in Kiss, 2004; Yong *et al.*, 2004). To set the stage for discussion of whether other RNAs also cycle between the nucleus and the cytoplasm, only the salient features of the complicated intracellular travels of U1, U2, U4, and U5 snRNAs are summarized (Figure 1).

U1, U2, U4, and U5 snRNAs are transcribed by RNA polymerase II (Pol II) into pre-snRNAs with extended 3′ termini. First, these pre-snRNAs acquire a 5′ monomethyl guanosine cap (^{7}mGpppG, "m^{7}G") and then the nuclear cap-binding complex (CBC) interacts with m^{7}G-modified snRNAs (Hamm & Mattaj, 1990; Izaurralde *et al.*, 1995). Subsequently, the CBC/snRNA ribonucleoprotein (RNP) complex interacts with PHAX, which acts as an adaptor for the cooperative addition of the exportin, CRM1, and RanGTP, both required for nuclear export (Ohno *et al.*, 2000). Once in the cytoplasm, hydrolysis of RanGTP to RanGDP and dephosphorylation of PHAX result in the release of the snRNAs (Ohno *et al.*, 2000). The SMN protein complex facilitates assembly of the RNA-binding Sm proteins to the cytoplasmic snRNAs (Reviewed in Terns & Terns, 2001; Yong *et al.*, 2004), followed by hypermodification of m^{7}G to m^{2,2,7}G ("TMG") catalyzed by Tgs1 (Hausmann & Shuman, 2005) and 3′ end maturation. The TMG moiety interacts with snurportin-1 (SPN1), which acts as an adaptor for importin-β, providing one of the two nuclear import pathways (Fischer & Lührmann, 1990; Huber *et al.*, 1998). The second import pathway is independent of TMG, but depends upon the Sm and SMN complexes; it also employs importin-β (Narayanan *et al.*, 2004).

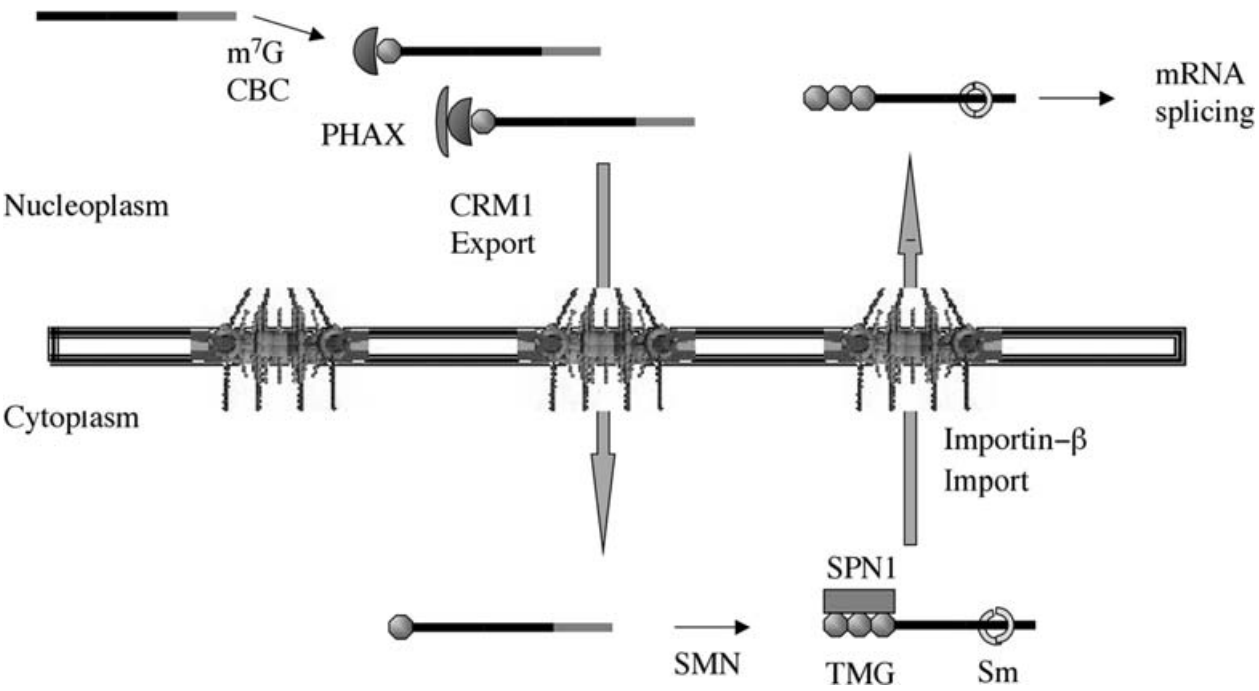

FIGURE 1 **Intracellular movements of U1, U2, U4, and U5 snRNAs between the nucleus and the cytoplasm. These RNAs are transcribed and partially processed in the nucleus. The m^{7}G modified 5′ end is recognized by the cap binding complex (CBC) and then the complex interacts with PHAX that acts as an adaptor for the exportin, CRM1. Once released in the cytoplasm the RNAs interact with the SMN complex followed by binding of the Sm proteins, 5′ cap modification to TMG and 3′ end processing. The modified snRNP associates with snoportin (SPN) and the resulting RNP is imported back into the nucleus by two importin-β dependent processes. Gray shaded bar, unprocessed 3′ terminus; small circles, m^{7}G (1 circle) or TMG (3 circles) 5′ modification; crescent shaped symbols, the cap-binding complex and PHAX as indicated.**

It has been proposed that the cytoplasmic phase for the snRNAs provides a proofreading step that prohibits nuclear accumulation of nonfunctional snRNAs and prevents Sm protein assembly on inappropriate RNAs (Yong *et al.*, 2004). If the cytoplasmic cycle for snRNAs serves an important proofreading function, then it should be conserved throughout eukaryotes. However, several lines of evidence have argued against a cytoplasmic phase for snRNPs in budding yeast. First, PHAX homologs are not apparent in the yeast genome; so, there is no candidate protein that would be involved in snRNA nuclear export (Cited in: Murphy *et al.*, 2004). Second, yeast also do not encode SPN1 and SMN homologs involved in snRNP nuclear import (Cited in: Murphy *et al.*, 2004; Yong *et al.*, 2004). Third, it has been reported that 5′ TMG formation and 3′ end trimming occur strictly in the nucleus in yeast, rather than in the cytoplasm as in higher eukaryotes (van Hoof *et al.*, 2000; Mouaikel *et al.*, 2002). Finally, snRNAs have not been detected in the cytoplasm in budding yeast (cited in Murphy *et al.*, 2004).

Despite the evidence against nucleus/cytoplasm bidirectional movement for snRNAs in budding yeast, recent reports indicate that yeast snRNAs may indeed cycle between the nucleus and the cytoplasm. First, the U1-binding protein, Prp40, which participates in pre-mRNA splicing, was shown to move between the nucleus and the cytoplasm. Furthermore, Prp40 possesses leucine-rich nuclear export motifs recognized by Crm1 that are necessary for its function in splicing (Murphy *et al.*, 2004). One interpretation of the data is that Prp40 functions to export U1 snRNA from the nucleus to the cytoplasm (Murphy *et al.*, 2004).

Second, Olson and Siliciano (2003) provided direct evidence that snRNAs in budding yeast do cycle between the nucleus and the cytoplasm. They used a *kar1*-based heterokaryon assay established for assessing protein nucleus/cytoplasm bidirectional movement in budding yeast (Flach *et al.*, 1994). The *kar1* mutant causes a defect in nuclear fusion following mating, resulting in mated heterokaryon zygotes that contain separate parental nuclei within a common cytoplasm. Because yeast have a closed mitosis, gene products encoded by one nucleus and located in that nucleus can gain entrance to second nucleus within the joint cytoplasm only if the gene products have the ability to exit the first nucleus. A large number of experiments have documented that DNA exchanges occur only rarely between the nuclei of heterokaryons and that soluble karyophilic proteins in one nucleus do not migrate to a second nucleus in the absence of nuclear export information (Dutcher, 1981; Sigurdson *et al.*, 1981; Flach *et al.*, 1994; Shaheen & Hopper, unpublished data).

Olson and Siliciano (2003) generated functional snRNA variants specifically detectable by fluorescence *in situ* hybridization (FISH). These variant snRNAs were provided to a haploid nucleus via a low copy plasmid, and the resulting cells were mated to a *kar1* haploid strain to generate heterokaryons. The subcellular dynamics of the tagged snRNAs were assessed using a modified version of the heterokaryon assay. About 50% of the heterokaryons demonstrated equal amounts of the snRNAs in each nucleus within ~2 h post mating, indicating movement from the first to the second nucleus. The authors provided convincing evidence that exit from one nucleus and entry to the second is not an artifact of "leaking" nuclei, because the process depends upon a functional exportin, Crm1 (Olson & Siliciano, 2003). However, because all of the various snRNA and snoRNAs tested (see below for further discussion of snoRNAs) demonstrated similar nucleus/cytoplasmic bidirectional movement, the authors suggested that "caution must be taken when testing nuclear envelope shuttling in yeast heterokaryons." Nevertheless, the ability of Prp40 and snRNAs to move between the nucleus and the cytoplasm in yeast haploids and heterokaryons leaves open the possibility that components of the yeast splicing machinery cycle between the nucleus and the cytoplasm, similarly to the higher eukaryotic counterparts. Further studies are necessary to support or refute this conclusion.

U6 snRNA Does Not Cycle Between the Nucleus and the Cytoplasm?

In contrast to U1, U2, U4, and U5 snRNAs, U6 snRNA appears not to exit the nucleus. Unlike the other snRNAs, U6 is transcribed by RNA polymerase III (Pol III). Also, unlike the other snRNAs, U6 does not possess Sm protein binding sites or a TMG cap; rather it possesses a methyphosphoryl G cap (Singh & Reddy, 1989). Although U6 snRNA that is injected into the cytoplasm of *Xenopus* oocytes is imported into the nucleus by a Ran-dependent process (Vankan *et al.*, 1990; Michaud & Goldfarb, 1992), data document that U6 snRNA transcribed in the nucleus is not exported to the cytoplasm. In particular, a catalytically active variant missing nuclear import information fails to accumu-

late in the cytoplasm after being injected into *Xenopus* oocyte nuclei (Vankan *et al.*, 1990). Assuming that the mutation did not alter putative nuclear export information, the data indicate that U6 RNA has no nuclear export potential. In support of this finding, variant U6 RNAs gain nuclear export ability when supplied with *cis*-acting nuclear export motifs (Terns *et al.*, 1993). On the other hand, budding yeast U6 snRNA moved simultaneously with U1 and U2 snRNAs in heterokaryons, although there are no published data to indicate that U6 nuclear egress is a Ran-dependent process (Olson & Siliciano, 2003). It has been suggested that U6's nuclear import capability in higher eukaryotes may serve to restore U6 snRNA nuclear pools after mitotic nuclear breakdown (Vankan *et al.*, 1990); however, since U6 snRNA is also imported into the nucleus in yeast and yeast has a closed mitosis, U6 snRNA nuclear import likely serves a different function, at least in budding yeast.

NO CYCLING OF NUCLEAR RESIDENT snoRNAs BETWEEN THE NUCLEUS AND THE CYTOPLASM?

Cells encode a plethora of small RNAs that are located in the nucleolus (snoRNAs) and function in pre-rRNA nucleolytic processing and nucleoside modifications. Curiously, this category of RNAs has different biogenesis pathways in different organisms. In plants, snoRNAs are transcribed by Pol III and U3 snoRNA possesses a γ monomethyl phosphate cap (Shimba *et al.*, 1992). In contrast, in vertebrates snoRNAs are transcribed by Pol II, either as individual transcripts or within introns of pre-mRNAs (Shimba *et al.*, 1992; Tycowski *et al.*, 1996), and pre-snoRNAs possess a m^7G cap that is modified to TMG on mature snoRNAs.

Given the similarities of vertebrate snoRNA biogenesis to snRNA biogenesis, it was anticipated, and reported (see citations in Peculis, 2001), that snoRNAs cycle between the nucleus and the cytoplasm. However, because snoRNAs lack motifs that bind Sm proteins, and binding Sm proteins, is important for their nuclear import, snoRNA nucleus/cytoplasm cycling was reinvestigated. It was learned that, in contrast to snRNAs, U3, U8, and U14 snoRNAs expressed in *Xenopus* oocytes could not be detected in the cytoplasm, unless vast excess of snoRNAs were injected into the nucleus or the cells possessed defects in the Ran cycle (Terns & Dahlberg, 1994; Terns *et al.*, 1995; Cheng *et al.*, 1995). Furthermore, and also in contrast to snRNAs, snoRNAs acquired the TMG modification in the nucleus, rather than the cytoplasm (Terns & Dahlberg, 1994). Subsequent studies employing HeLa and Ref52 cell cultures confirmed the results (Boulon *et al.*, 2004; Watkins *et al.*, 2004). So, even though snoRNAs and U1, U2, U4, and U5 snRNAs have a similar biogenesis pathway, their subcellular trafficking was reported to differ.

Studies of the role of CRM1 in snoRNA biogenesis showed that its inhibition by Leptomycin B (LMB) had no effect upon the maturation of U3 snoRNA, whereas the same regime had the anticipated inhibitory effect upon U1, U2, U4, and U5 pre-snRNA processing (Boulon *et al.*, 2004). Rather, CRM1 was important for subnuclear localization because snoRNAs failed to accumulate in the nucleolus when CRM1 was inhibited by LMB. How cells distinguish between m^7G-modified snRNAs that are exported to the cytoplasm and m^7G-modified snoRNAs that are apparently retained in the nucleus has been investigated. Boulon and coworkers (2004) and Watkins and associates (2004) showed that m^7G modified pre-U3 snoRNAs interact with PHAX and this interaction directs the pre-snoRNAs to Cajal bodies. TMG modification and 3′-end modification occur in the Cajal bodies and precede interaction of the matured snoRNA with CRM1. Rather than exporting the RNA to the cytoplasm, CRM1 appears to direct snoRNAs to the nucleolus (Boulon *et al.*, 2004). Thus, surprisingly, the same machinery necessary for U1, U2, U4, and U5 snRNA nuclear export may function in intranuclear movement of m^7G-modified snoRNAs.

Do the data for snoRNA biogenesis exclude a cytoplasmic phase for this category of RNAs? Previously, Cheng and colleagues (1995) showed that in the absence of a functional Ran cycle, part of the U3 snoRNA is cytoplasmic. Although an interpretation of the results is that RanGTP functions in U3 snoRNA nucleolar retention (Cheng *et al.*, 1995), a recent study provided no support for this finding (Narayanan *et al.*, 2003). Likewise, although the studies of the roles of PHAX and CRM1 in snoRNA biogenesis indicate that these proteins function in snoRNA intranuclear trafficking, both Boulon and colleagues (2004) and Watkins and coworkers (2004) considered the alternative possible interpretation that PHAX and CRM1 function in snoRNP nuclear export. If indeed all or part of the pool of this category of snoRNA exits the nucleus, then inhibition of the nuclear import machinery should cause their cytoplasmic accumulation. Employing RNAi methodology, Watkins and colleagues (2004)

reported that down-regulation of SMN causes reduced U3, but not U8 or U14, levels; however, the location of these snoRNAs upon RNAi treatment has not yet been published. Likewise, if snoRNAs cycle between the nucleus and the cytoplasm, snoRNAs introduced into the cytoplasm should be able to access the nuclear interior. It is known that U3 complex-associated proteins moves bidirectional between the nucleus and the cytoplasm (Leary *et al.*, 2004). However, studies of the capability of U3 snoRNA to be imported into the nucleus have led to contradictory conclusions. One report (Speckmann *et al.*, 1999) detected no significant import of U3 or U8 snoRNAs into *Xenopus* nuclei when these RNAs were injected into the cytoplasm, whereas others, also using the *Xenopus* system, reported that there is snoRNA nuclear import, albeit a lower rate and reduced efficiency compared to snRNAs (Baserga *et al.*, 1992; Peculis, 2001). It is possible that a nuclear import salvage pathway is able to retrieve snoRNAs that somehow escape the nuclear retention process (Terns *et al.*, 1995). Nevertheless, the question of whether U3, U8, and U14 snoRNAs cycle between the nucleus and the cytoplasm may warrant further investigation.

POSSIBLE CYCLING OF THE NUCLEAR RESIDENT RNA SUBUNIT OF TELOMERASE BETWEEN THE NUCLEUS AND THE CYTOPLASM

Telomerase is a RNP consisting of a single RNA (TLC1 in budding yeast) and multiple proteins. The RNA subunit serves as the template and DNA synthesis is catalyzed by the reverse transcriptase subunit of the RNP complex, TERT/Est2 (Lingner *et al.*, 1997; reviewed in Cech, 2004). In yeast, the other two proteins of the enzyme complex, Est1 and Est3, are required only *in vivo* for appropriate maintenance of telomere length (Lendvay *et al.*, 1996; review: Cech, 2004). Yeast and vertebrate telomerase RNA are transcribed by Pol II, but the ciliate RNA is transcribed by Pol III (reviewed in Collins, 1999). The vertebrate telomerase RNA contains H/ACA motifs typically found in snoRNAs that function in snoRNA biogenesis and subnuclear distribution (Mitchell *et al.*, 1999; Narayanan *et al.*, 1999; Lukowiak *et al.*, 2001). Like for snoRNAs, the vertebrate telomerase RNA appears to never exit the nucleus (Lukowiak *et al.*, 2001); rather, the H/ACA motif has been implicated in telomerase RNA nuclear retention (Lukowiak *et al.*, 2001). In contrast, budding yeast TLC1 possesses a TMG cap and associates with Sm proteins, so, its biogenesis resembles the biogenesis of snRNAs (Seto *et al.*, 1999). Since it has been thought that yeast snRNAs do not traffic between the nucleus and the cytoplasm, it was anticipated that the yeast telomerase RNA also would not exit the nucleus. However, two reports support the notion that budding yeast TLC1 may move between the nucleus and the cytoplasm.

Teixeira and colleagues (2002) employed the yeast heterokaryon assay to study the intracellular trafficking of TLC1. Because FISH technology is not sensitive enough to detect endogenous levels of TLC1, the authors used a plasmid encoding a *GAL1*-induced, over-expressed TLC1. Haploid cells containing the inducible *TLC1* gene were mated to haploid cells harboring a *kar1* mutation. For 10% to 60% of the heterokaryons, TLC1 RNA was present in all heterokaryon nuclei and not just the nucleus that contained the plasmid encoding TLC1 RNA. The data indicate that TLC1 can exit one nucleus and gain entrance to a second nucleus through the shared cytoplasm.

Two caveats complicate interpretation of the data: (1) the studies tracked considerably over-expressed RNA, and (2) the location of TLC1 was assessed 12 h. after heterokaryon formation. *A priori,* it is possible that over-expressed TLC1 could overwhelm a putative nuclear retention mechanism and diffuse out of the nucleus. However, unlike the ciliate or vertebrate telomerase RNAs that are quite small (150 and 450 nt., respectively), TLC1 from budding yeast is large (1.2 kb, ~400 kd) and very unlikely to exit the nucleus via diffusion because it greatly exceeds the size of the nuclear pore diffusion channel (<47 kd; Shulga *et al.*, 2000). Nevertheless, to address the caveat of over-production Teixeira and coworkers (2002) also generated a construct in which the TLC1 transcript was expressed in endogenous levels but was fused to another RNA encoding an ORF. This fusion transcript was translated and generated the protein encoded by the ORF. Since translation occurs only in the cytoplasm, production of the ORF protein required that fusion transcript to be exported to cytoplasm. One interpretation of the data is that TLC1 nuclear export can occur in the absence of its over-expression. However, the authors were unable to eliminate the alternative that the ORF-encoding part of the fusion transcript was responsible for directing the RNA to the cytoplasm. The second caveat concerns the interval between heterokaryon formation and FISH analysis,

as this length of time could be sufficient for TLC1 RNA that managed to "escape" from the nuclear interior to interact with newly synthesized TLC1-binding proteins and be imported in tow as RNPs. However, no cytoplasmic TLC1 pools were evident, indicating a rather efficient nuclear import process.

Nucleus/cytoplasmic bidirectional movement for *S. cerevisiae* TLC1 is supported by an independent study. Employing a synthetic genetic interaction screen for yeast mutants defective in telomere maintenance, Ferrezuelo and colleagues (2002) identified *mtr10*. Biochemical studies showed that *mtr10* mutant cells have short telomeres. A deletion of *MTR10* also resulted in low cellular levels of telomerase, similar to cells with defects in the telomerase protein subunits, Est1, Est2, and Est3. TLC1 processing was also altered in *mtr10*Δ cells. *MTR10* encodes 1 of the 14 members of the β importin family of Ran-binding proteins and it has a well established function in nuclear import of mRNA binding proteins (*e.g.*, Senger *et al.*, 1998). The authors eliminated the possibility that the phenotype of the *mtr10*Δ mutant cells was due to a defect in nuclear import of one of the known Mtr10 substrates, because mutation of the substrate did not cause defects in telomere metabolism. The authors also eliminated the possibility that low levels of telomerase resulted from defects in nuclear import of Sm proteins because *mtr10*Δ had normal levels of snRNAs that require nuclear import of Sm proteins for their stability. FISH analysis was employed to determine whether *mtr10*Δ altered the subcellular distribution of TLC1. Interestingly, whereas U3 snoRNA was located in the nucleus in both wild-type and *mtr10*Δ cells, *GAL1*-regulated over-expressed TLC1 was mislocated to the cytoplasm in *mtr10*Δ, but not in wild-type cells. The simplest interpretation of why TLC1 is mislocated to the cytoplasm is that it normally moves bidirectionally between the nucleus and the cytoplasm and that Mtr10 functions in its nuclear import. However, the authors were unable to eliminate the alternative interpretation that Mtr10 is responsible for import of a product needed for processing of TLC1 in the nucleus and in the absence of this processing step, TLC1 aberrantly exits the nucleus. Also, since in these experiments TLC1 was also over-expressed, it is possible that "escaped" TLC1 RNA can be re-imported as an RNP with newly synthesized Est proteins and that import of the RNP is an Mtr10-mediated process.

In combination, the heterokaryon studies that document TLC1 movement from one nucleus to another (Teixeira *et al.*, 2002), the ability of TLC1-ORF fusion transcripts to be translated (Teixeira *et al.*, 2002), and the location of TLC1 in the cytoplasm in *mtr10*Δ cells (Ferrezuelo *et al.*, 2002), provide tantalizing evidence that TLC1 traffics between the nucleus and the cytoplasm, at least in budding yeast (Figure 2). If this RNA indeed moves bidirectionally in and out of the nucleus, it will be important to discover its significance and to characterize mechanism responsible for its export to the

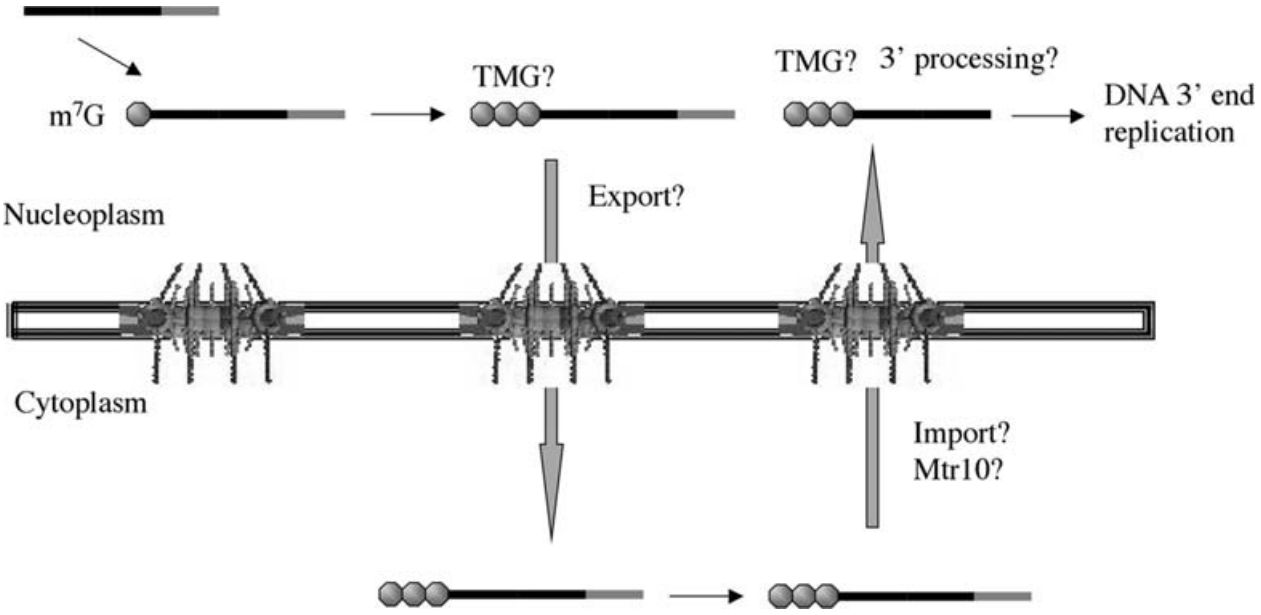

FIGURE 2 **Possible nucleus/cytoplasm dynamics for the yeast telomerase RNA subunit—TLC1. The mechanism of putative export to the cytoplasm is unknown. The importin Mtr10 is proposed to transport the RNA back into the nucleus. 3′ and possibly 5′, modifications may take place after the RNA returns to the nucleus. Symbols are as in Figure 1.**

cytoplasm. Along these lines, Boulon and colleagues (2004) demonstrated physical interaction of PHAX and telomerase RNA in HeLa cell extracts. As detailed above PHAX interaction with snRNAs mediates their Ran-dependent nuclear export; however, because PHAX interaction with U3, U8, and U13 snoRNA may function in their intranuclear dynamics rather than their nuclear export, the interactions are difficult to interpret. Future studies in yeast and higher eukaryotes are necessary to resolve whether telomerase RNA does indeed cycle between the nucleus and the cytoplasm.

tRNA NUCLEAR EXPORT AND IMPORT—PRECEDENT FOR NUCLEAR IMPORT FOR A RESIDENT CYTOPLASMIC RNA

Cytoplasmic tRNAs are transcribed in the nucleus where they undergo numerous post-transcriptional alterations. The tRNAs then exit the nucleus and undergo further modification and processing in the cytoplasm (reviewed in Hopper & Phizicky, 2003). Completely processed tRNAs acquire appropriate amino acids at their 3′ ends, catalyzed by 1 of 20 aminoacyl-tRNA synthetases, and the charged tRNAs deliver the amino acids to ribosomes where amino acids are assembled into proteins at the direction of mRNA codons. Given this simple "central dogma" view of the role of tRNA in gene expression, one would anticipate that the movement of tRNAs in cells is unidirectional, nucleus to cytoplasm, and that tRNA aminoacylation would be restricted to the cytoplasm. Both of these predictions are incorrect. Moreover, the subcellular organization of the tRNA biogenesis pathway is unexpectedly complex.

Complex Intranuclear and Cytoplasmic Organization of the tRNA Biogenesis Machinery

As transcribed, pre-tRNAs are oversized on the 5′ and 3′ termini and may contain an intron. Conversely, tRNA transcripts lack the numerous nucleoside modifications (>47 known nucleoside modifications in eukaryotes, 25 in *S. cerevisiae*; Rozenski *et al.*, 1999; Hopper & Phizicky, 2003) that reside on mature tRNAs, as well as the 3′ CCA sequence that is required for aminoacylation (reviewed in Hopper & Phizicky, 2003). Moreover, one tRNA, $tRNA^{His}$, acquires a post-transcriptional 5′ additional G (Gu *et al.*, 2003). In budding yeast at least 60 gene products, about 1% of the genome, are required to convert pre-tRNAs to mature functional molecules. In all eukaryotes many of the processing steps occur before tRNAs exit the nucleus to the cytoplasm. However, steps that occur in the nucleus in one organism may occur in the cytoplasm of another. For example, pre-tRNA splicing is clearly nuclear in vertebrates (De Robertis *et al.*, 1981; Lund & Dahlberg, 1998; Paushkin *et al.*, 2004), while this process occurs in the cytoplasm, on the surface of mitochondria, in yeast (Huh *et al.*, 2003; Yoshihisa *et al.*, 2003; Shaheen & Hopper, 2005).

The intranuclear locations of the tRNA transcription and processing machinery is highly organized in *S. cerevisiae*. Despite the fact that there are ~275 tRNA-encoding genes dispersed throughout the genome, their transcription is localized to the nucleolus (Thompson *et al.*, 2003; reviewed in Haeusler & Engelke, 2004). How this is achieved topologically is unknown, but it does put significant three-dimensional constraints on the genome. Removal of the 5′ leader sequence, catalyzed by RNase P, a 10-subunit RNP complex, also occurs in the nucleolus in yeast (Bertrand *et al.*, 1998), but perhaps not in vertebrate cells (reviewed in Xiao *et al.*, 2002). In contrast, for 19 proteins known to catalyze tRNA modifications steps in the nucleus (Huh *et al.*, 2003; Hopper & Phizicky, 2003), only one, Mod5, appears to be concentrated in the nucleolus (Tolerico *et al.*, 1999). Trm1 (Rose *et al.*, 1992; Rose *et al.*, 1995) and possibly Trm4 (Wu *et al.*, 1998) reside at the inner nuclear membrane, and the remaining 16 to 17 tRNA modification proteins appear to be dispersed throughout the nucleoplasm (Huh *et al.*, 2003). Nine, or possibly 10, of these proteins also have cytoplasmic pools, whereas 6, or possibly 7, modification proteins reside strictly in the cytoplasm. Cytoplasmic tRNA processing activities are also specifically located. For example, the four splicing endonuclease complex subunits are located on the cytoplasmic surface of mitochondria, whereas the enzymes that ligate the two tRNA halves together and remove the residual 2′ phosphate from the splice junction are located throughout the cytoplasm (Yoshihisa *et al.*, 2003; Huh *et al.*, 2003). Thus, for *S. cerevisiae*, tRNA processing activities reside in numerous subnuclear compartments–the nucleolus, the nucleoplasm, and the inner nuclear membrane–as well as in particular domains of the cytoplasm. What function(s) this elaborate organization serves and whether similar organizational patterns also exist in higher eukaryotes, remains to be discovered.

Parallel Pathways for tRNA Egress to the Cytoplasm

There is one known exportin (Los1 in yeast, Exportin-t [Xpo-t] in vertebrates, and PSD in plants) dedicated to transporting tRNA to the cytoplasm (Arts *et al.*, 1998a; Hellmuth *et al.*, 1998; Kutay *et al.*, 1998; Sarkar & Hopper, 1998; Hunter *et al.*, 2003; Park *et al.*, 2005). Xpo-t binds tRNA in the nucleus in the presence of RanGTP and it is able to interact with two distinct nucleopore proteins (Kuersten *et al.*, 2002). Vertebrate Xpo-t likely also serves a proofreading function because it preferentially interacts with tRNAs with correct tertiary structure and completely processed 5′ and 3′ termini (Arts *et al.*, 1998b; Lipowsky *et al.*, 1999). Interestingly, this exportin does not distinguish between intron-containing and spliced tRNAs (Arts *et al.*, 1998b; Lipowsky *et al.*, 1999). Upon movement of the heterotrimeric tRNA, RanGTP, and Xpo-t complex to the cytoplasm, RanGAP stimulates GTP hydrolysis, and the tRNA cargo is released from the complex into the cytoplasm (Figure 3).

Despite the simplicity and elegance of this nucleus-to-cytoplasm delivery mechanism, it fails to account for the egress of most tRNA to the cytosol for several organisms. As first demonstrated in yeast, the gene encoding this exportin, *LOS1*, is unessential (Hurt *et al.*, 1987); in fact, *los1*Δ cells are quite hardy. *LOS1* homologs also have been shown to be unessential in those organisms, *S. pombe* and *Arabidposis*, in which the gene has been ablated (Sazer, personal communication; Li & Chen, 2003), implicating an alternative Los1-independent tRNA nuclear egress pathway(s). Although no genetic studies of the necessity of the vertebrate Xpo-t have been reported, it has been thought to be responsible for the export of the vast majority of tRNA from the nucleus to the cytoplasm because its inhibition via antibody injection causes nuclear retention of nearly the entire tRNA pool (Arts *et al.*, 1998b; Lipowsky *et al.*, 1999). Nevertheless, vertebrates encode another exportin, Exp5, that is able to bind tRNA (Bohnsack *et al.*, 2002; Calado *et al.*, 2002) and may serve to export tRNA from the nucleus (Calado *et al.*, 2002). It has been reported that yeast possessing deletions of both the Xpo-t and the Exp5 homologs, Los1 and Msn5, respectively, accumulate more tRNA in the nucleus than either alone, perhaps implicating Msn5 in tRNA nucleus/cytoplasm dynamics in yeast (Takano *et al.*, 2005). However, because *msn5*Δ cells are reported not to accumulate tRNA in the nucleus, and *los1*Δ *msn5*Δ double mutants have no synergistic growth defects (Feng & Hopper, 2002; Takano *et al.*, 2005) it would appear

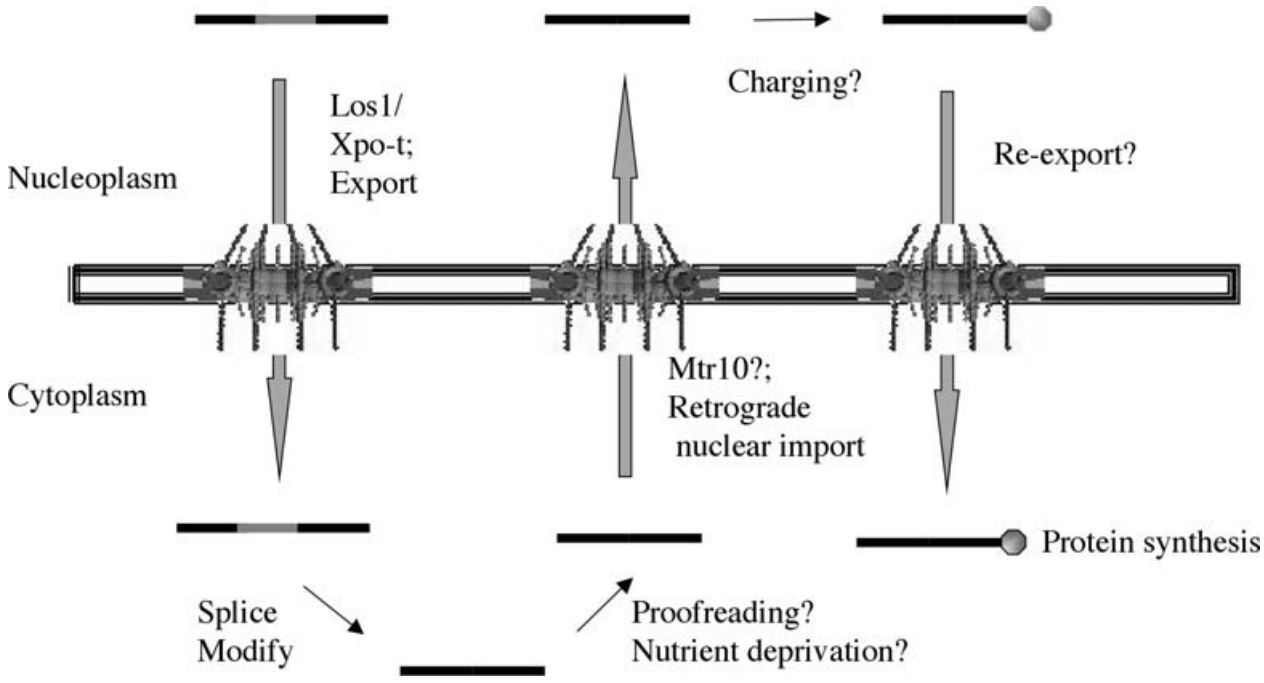

FIGURE 3 **tRNA in *S. cerevisiae* moves retrograde from the cytoplasm to the nucleus. Pre-tRNAs interact directly with the exportin, Los1, in the nucleus and are released into the cytoplasm. Further modification, including removal of introns occurs in the cytoplasm. Retrograde movement of the mature tRNAs to the nucleus under nutrient deprivation depends upon the importin, Mtr10. Likely, tRNAs are exported a second time to the cytoplasm under favorable growth conditions. *Gray shaded bar* = intron; *small circle* = amino acid.**

that Msn5 does not provide a major export pathway in budding yeast. Moreover, because Exp5 interacts more poorly with tRNA than does Xpo-t, it is also thought to provide only a minor nuclear export pathway for tRNA in vertebrates (Calado *et al.*, 2002). Consistent with this idea, it has been shown that deletion of the Exp5 homolog, HST, from the *Arabidopsis* genome causes no defect in tRNA nuclear export (Park *et al.*, 2005). Rather, Exp5 and HST bind dsRNAs, such as precursors to micro-RNAs (pre-miRNA), and serve as their nuclear export receptor (Lund *et al.*, 2004; Zeng & Cullen, 2005; Gwizdek *et al.*, 2004; Park *et al.*, 2005). Since the combination of *los1*Δ/PSD and *msn5*Δ/HST is not lethal in yeast or *Arabidopsis* (Takano *et al.*, 2005; Park *et al.*, 2005), the genetic data indicate the existence of additional unaccounted tRNA nuclear export pathways, at least in these organisms.

A Role for tRNA Aminoacylation in tRNA Nuclear Egress

Contrary to dogma, Lund & Dahlberg (1998) showed that tRNAs can be aminoacylated in the nuclear interior of *Xenopus* oocytes. Both genetic and biochemical studies showed that nuclear tRNA aminoacylation also occurs in budding yeast (Sarkar *et al.*, 1999; Steiner-Mosonyi & Mangroo, 2004). In vertebrates, nuclear tRNA aminoacylation is important, although not essential, for tRNA nuclear export (Lund & Dahlberg, 1998; Arts *et al.*, 1998b). Conditional mutations of genes encoding tyrosyl-tRNA synthetase, isoleucyl-tRNA synthetase, and methionyl-tRNA snthetase, each cause tRNA nuclear accumulation (Sarkar *et al.*, 1999), and inhibitors of isoleucyl-tRNA synthetase likewise cause tRNA nuclear accumulation (Grosshans *et al.*, 2000), indicating that nuclear tRNA aminoacylation is also important for tRNA nuclear export in budding yeast. Consistent with this finding, blocking the addition of the C, C, and A nucleotides that are requisite for aminoacylation or depletion of amino acids from growth media can cause tRNA nuclear accumulation (Sarkar *et al.*, 1999; Grosshans *et al.*, 2000; Feng & Hopper, 2002; Shaheen & Hopper, 2005; Takano *et al.*, 2005).

If indeed tRNAs can be aminoacylated in the nucleus as well as in the cytosol, then there should be nuclear pools of the aminoacyl-tRNA synthetases. It has been shown by both microscopic analyses and subcellular fraction studies that there are small pools of aminoacyl-tRNA synthetases in vertebrate nuclei (Popenko *et al.*, 1994; Nathanson & Deutscher, 2000). Likewise, a small pool of tyrosyl-tRNA synthetase, Tys1, co-fractionates with budding yeast nuclei (Azad *et al.*, 2001). Moreover, Tys1 possesses a functional nuclear localization sequence (NLS) that quantitatively delivered a passenger β-galactosidase to the nucleus (Azad *et al.*, 2001). Mutations of the NLS reduced the pool of Tys1 that co-fractionated with nuclei, but caused no significant defect in Tys1 catalytic activity. The NLS mutations resulted in increased nuclear tRNA pools, consistent with the hypothesis that nuclear pools of aminoacyl-tRNA synthetases are important for tRNA nucleus/cytoplasm distribution (Azad *et al.*, 2001).

Surprising Retrograde Traffic of tRNA from the Cytoplasm to the Nucleus

In vertebrate cells, pre-tRNA splicing occurs in the nucleus (De Robertis *et al.*, 1981; Lund & Dahlberg, 1998; Paushkin *et al.*, 2004), prior to tRNA end maturation (Lund & Dahlberg, 1998). In contrast, in budding yeast and, perhaps in *Arabidopsis*, splicing follows end processing (O'Connor & Peebles, 1991; Hiley *et al.*, 2005; Park *et al.*, 2005). Alteration of components of the Ran-dependent tRNA nuclear export pathway (RanGAP, RanGEF, or Los1p), as well as deletions of numerous nucleoporins, each cause accumulation of end-processed, intron-containing pre-tRNAs (Hopper *et al.*, 1978; Hopper *et al.*, 1980; Kadowaki *et al.*, 1994; Sharma *et al.*, 1996). The discovery that pre-tRNA splicing occurs in the cytoplasm in budding yeast (Yoshihisa *et al.*, 2003) provided an explanation for the defects in pre-tRNA intron removal that accompany alterations of the tRNA nuclear export machinery as pre-tRNAs maintained in the nucleus would be unable to access the cytosolic splicing endonuclease. However, not all mutations or physiological conditions that cause nuclear accumulation of tRNA in yeast result in defects in pre-tRNA intron removal. For example, inhibitors of aminoacylation, conditional mutations of aminoacyl-tRNA synthetases, Cca1, and the translation elongation factor EF1, or amino acid deprivation, each cause nuclear accumulation of spliced tRNA (Grosshans *et al.*, 2000; Feng & Hopper, 2002; Shaheen & Hopper, 2005). Splicing of pre-tRNAs in the cytoplasm does not provide an explanation for the presence of nuclear pools of spliced, mature tRNAs. If tRNA transport is unidirectional–nucleus to cytoplasm–nuclear accumulation of spliced tRNA is in

conflict with splicing occurring solely in the cytoplasm. Two possible scenarios could explain this enigma: either (1) the splicing machinery or (2) mature, spliced tRNAs move retrograde from the cytoplasm to the nucleus.

The subcellular location of a tagged version of a catalytic subunit of splicing endonuclease, Sen2-GFP (Huh *et al.*, 2003) in cells grown in rich media was compared to its location in cells depleted for amino acids or cells with *tys1-1*–both causing nuclear pools of spliced tRNAs (Sarkar *et al.*, 1999; Grosshans *et al.*, 2000; Azad *et al.*, 2001; Whitney *et al.*, in preparation). Under all conditions, detectable Sen2-GFP was associated with mitochondria, arguing against the first scenario that the pre-tRNA splicing complex is able to move from the cytoplasm to the nucleus (Shaheen & Hopper, 2005).

Two groups employed heterokaryon analysis to investigate the unorthodox alternative that nuclear pools result from retrograde movement of spliced tRNAs from the cytosol to the nucleus. Shaheen and Hopper (2005) provided one of the mating haploids with a gene expressed from a plasmid maintained in a single copy in yeast and regulated by its endogenous promoter from *D. discoideum*. This gene encodes an intronless suppressor tRNA, $tRNA^{Glu-D}$, known to participate in translation in yeast (Dingermann *et al.*, 1992). Takano and colleagues (2005) employed a tandem gene pair from *S. pombe* encoding intron-containing $tRNA^{Ser}_{UGA}$ and intronless $tRNA_i^{Met}$. Because tRNA is normally efficiently exported from the nucleus, and because efficient export would ablate detection of tRNA imported into the nucleus, heterokaryon analyses were conducted using mutations (*los1*Δ or *los1*Δ *msn5*Δ) or physiological conditions (amino acid deprivation) known to cause tRNA nuclear accumulation. If tRNA moves retrograde to the nucleus, then tRNA encoded by only one nucleus of a heterokaryon would be detected in both nuclei. In contrast, if tRNA movement is unidirectional, *i.e.*, strictly nucleus to the cytosol, tRNA would not be detected in the second nucleus. As assessed by FISH, tRNA signals were detected in both nuclei of the heterokaryons, supporting the hypothesis that tRNA encoded by one nucleus can gain access to a second nucleus and providing evidence for retrograde movement of tRNA from the cytoplasm to the nucleus (Shaheen & Hopper, 2005; Takano *et al.*, 2005).

Both groups also demonstrated retrograde movement of endogenous tRNAs in haploid yeast. Shaheen and Hopper (2005) took advantage of the fact that amino acid deprivation caused tRNA retrograde movement in the heterokaryon assay, and showed that endogenous tRNAs accumulate in the nucleus when wild-type haploid cells were deprived for amino acids. Takano and coworkers (2005) studied retrograde tRNA movement in cells in rich media, using a regimen established for assessing nuclear envelope permeability (Shulga *et al.*, 2000). This assay employs 2-deoxyglucose and sodium azide to equilibrate macromolecules across the nuclear membrane. Cells with defects in tRNA nuclear export (*los1*Δ *msn5*Δ) were treated with 2-deoxyglucose and sodium azide and then the location of cellular tRNA was monitored in re-fed cells in the absence of new tRNA synthesis. Transcription of new tRNAs was inhibited by thiolutin, a general inhibitor of all three RNA polymerases. tRNA accumulated in nuclei in the absence of new transcription, providing support for retrograde import from the cytoplasm (Takano *et al.*, 2005). Thus, the two groups provided evidence to support the paradigm-shifting idea that tRNAs move retrograde from the cytoplasm to the nucleus (Figure 3).

The results from both groups are in agreement that tRNA movement into nuclei is an active process. However, the role of the Ran cycle in tRNA retrograde movement to the nucleus is somewhat controversial. Nuclear accumulation of cytoplasmic tRNAs resulting from amino acid deprivation appeared to require a functional Ran cycle and the Mtr10 member of the β importin family (Shaheen & Hopper, 2005). However, a functional Ran cycle was not necessary for tRNA nuclear accumulation following 2-deoxyglucose, sodium azide, and thiolutin treatment (Takano *et al.*, 2005). Assuming both types of experiments faithfully report the retrograde process, it is possible that a functional Ran cycle is needed only for retrograde movement signaled by amino acid deprivation (Figure 3). The possible role of Mtr10 in tRNA nuclear import is interesting in light of its implicated role in nucleus/cytoplasm dynamics of telomerase RNA discussed above.

The ability of tRNA to enter the nucleus–"retrograde tRNA nuclear import"–reveals previously unknown complexity in its nucleus/cytosol dynamics. The purpose that this movement serves to cells is unknown. However, based upon the hypothesis that snRNAs move to the cytosol and then back to the nucleus so that they may be proofread in a different cellular compartment than that in which they function (Yong *et al.*, 2004), tRNA retrograde movement may likewise provide a proofreading role for tRNA processing steps

that occur in the cytoplasm, thereby preventing interaction of faulty tRNAs with the translation machinery (Shaheen & Hopper, 2005; Takano *et al.*, 2005). Alternatively, based upon the movement of mRNAs from ribosomes to P-bodies under nutrient deprivation (Teixeira *et al.*, 2005), retrograde tRNA nuclear import may serve a regulatory role by separating tRNAs from the translation machinery when active translation can not occur (Shaheen & Hopper, 2005). A model consistent with the evidence that aminoacylation affects tRNA nuclear export (Lund & Dahlberg, 1998; Sarkar *et al.*, 1999; Grosshans *et al.*, 2000; Azad *et al.*, 2001) suggests that tRNA aminoacylation regulates both tRNA retrograde import and re-export to the cytoplasm such that, upon appropriate signaling events, cytoplasmic tRNAs are delivered into the nucleus and are maintained there until the nutritional status is sufficient for nuclear tRNA aminoacylation can occur, providing a nutritional checkpoint prior to a second round of tRNA nuclear export (Figure 3).

Several burning questions remain to be answered: (1) Is tRNA retrograde movement constitutive and/or regulated? (2) What gene products are involved in the import and the putative nuclear retention processes? (3) Are tRNAs imported to the nucleus returned to the cytoplasm for translation or, instead, are they turned over by the nucleus-located tRNA degradation machinery (Kadaba *et al.*, 2004)? (4) Does tRNA retrograde movement occur in vertebrate cells? Since pre-tRNA splicing occurs in the nucleus in vertebrate cells, tRNAs may be proofread before leaving the nucleus, eliminating the need for import and a second round of export to assure that only mature functional tRNAs are in the same compartment with the translation machinery. On the other hand, since there is evidence for yeast that nuclear accumulation of cytoplasmic tRNAs responds to nutrient cues, tRNA retrograde movement might provide a new level of regulation of gene expression in response to nutritional deprivation for all eukaryotes.

SMALL DOUBLE-STRANDED RNAs FUNCTION IN THE CYTOPLASM AND THE NUCLEUS

Since the discoveries of small double-stranded RNAs (dsRNAs) in the 1990s, these RNAs have been implicated in numerous processes, including mRNA turnover, mRNA translation, regulation of transcription, chromatin structure and silencing, and genome stability. There are two categories of endogenous small dsRNAs: (1) small interfering RNAs (siRNA) and (2) micro RNAs (miRNA). Endogenous siRNAs are generally encoded by two complementary RNAs that can perfectly base pair with their targets, whereas miRNAs are encoded by self-complementary hairpin structures able to form interrupted base pairs with their targets. RNA-induced silencing complexes (RISC) use one strand of the dsRNAs to inactivate particular mRNAs by complementary base pairing, either by targeting the mRNAs for turnover or by inhibiting target mRNA translation, likely within cytoplasmic P-bodies (Bagga *et al.*, 2005; Liu *et al.*, 2005; Pillai *et al.*, 2005; Sen & Blau, 2005). Other siRNAs assemble into RNA-induced silencing complexes (RITS) that are recruited to chromatin and function in transcription silencing, and/or genome stability. The biogenesis (reviewed in Tomari & Zamore, 2005; Sontheimer, 2005) and biological roles of small dsRNAs (reviewed in Bartel, 2004; Pasquinelli *et al.*, 2005; Bernstein & Allis, 2005) have been extensively reviewed. Here, only their nucleus/cytoplasmic dynamics are explored.

miRNAs are encoded in the nucleus, but generally function in the cytoplasm. The mechanism for their nuclear egress has recently been described. In vertebrates the genes encoding miRNAs are transcribed into self-complementary hairpin initial precursor RNAs (pri-miRNAs) that undergo a two-step maturation process. In the nucleus, pri-miRNAs are processed to ~70 nucleotide double-stranded hairpin precursor miRNAs (pre-miRNAs) catalyzed by an RNase III-like enzyme, Drosha (Lee *et al.*, 2003). Pre-miRNAs directly interact with Exp5, an exportin member of the β importin family, in the presence of RanGTP. Subsequently, the heterotrimeric complex is exported from the nucleus to the cytoplasm (Lund *et al.*, 2004; Yi *et al.*, 2003; Bohnsack *et al.*, 2004). In the cytoplasm pre-miRNAs are processed to ~22-nucleotide double-stranded mature and functional microRNAs (miRNAs) by Dicer, another member of the RNase III category of endonucleases (Figure 4, left). This two-step biogenesis process involving two separate subcellular compartments appears to be conserved because there are Drosha homologs in mammals, *Xenopus*, *C. elegans*, and *Drosophila*, although not in *Arabidopsis* or *S. pombe* (Review: Murchison & Hannon, 2004). Nevertheless, in *Arabidopsis*, the steady state level of most, but not all, miRNAs is dependent upon the Exp5 homolog, *HASTY* (*HST*) (Park *et al.*,

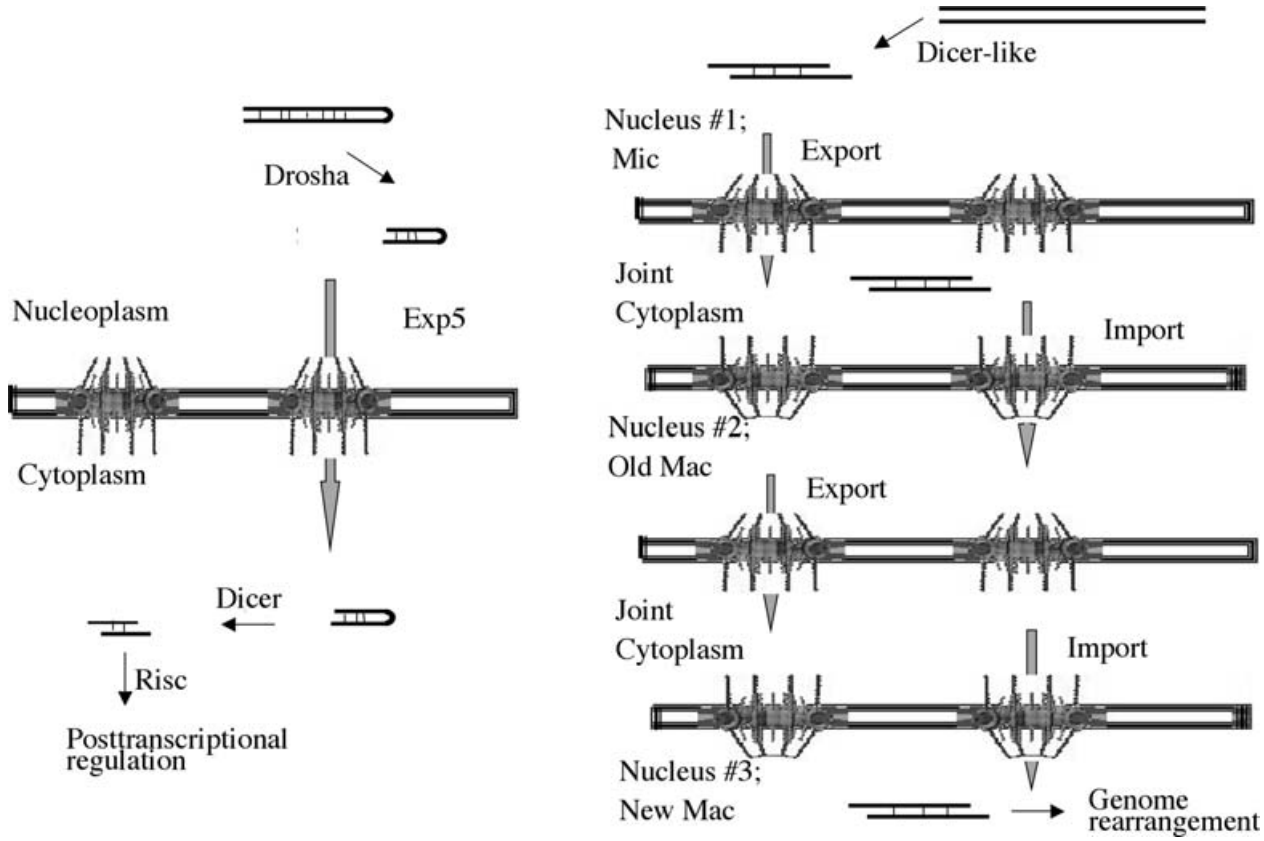

FIGURE 4 **Intracellular dynamics of dsRNAs. Left: miRNAs. Initial precursor miRNAs are processed by Drosha to ~70 nucleotide hairpins. The resulting pre-miRNAs interact directly with the exportin, Exp5, and are transported to the cytoplasm. In the cytoplasm, they are processed further to 21-nucleotide mature dsRNA by Dicer before one strand enters the Risc complex. Right: scnRNA, an example of siRNAs that undergo several cycles between the nucleus and the cytoplasm. ScnRNAs generated by action of a Dicer-like enzyme in the nucleus are transported to the cytoplasm by an unknown mechanism. The RNAs are imported to the old macronucleus, also by an unknown mechanism, where they scan the genome for deleted DNA sequences; RNAs with no homolog are exported to the cytoplasm and imported to the new macronucleus where they specify deletion of the appropriate genomic sequences.**

2005), perhaps indicative of nuclear export, despite the absence of Drosha.

Some siRNAs are recruited to sites on chromatin (Noma *et al.*, 2004; Reviewed in Martienssen *et al.*, 2005; Bernstein & Allis, 2005) where they may guide DNA rearrangements and/or transcriptional silencing, processes requiring transcription of the target DNA by Pol II (Schramke *et al.*, 2005; Kato *et al.*, 2005). Whether the siRNAs that function in the nucleus are first processed in the nucleus and then in the cytoplasm, like miRNAs that assemble into RISC complexes, or whether they are solely processed in the nucleus and assemble into RITS complexes in the nucleus, is not completely resolved. However, since deletion of the gene encoding the HST exportin from *Arabidopsis* appears to have no effect upon the steady state level of several tested endogenous siRNAs involved in post-transcriptional gene silencing (Park *et al.*, 2005), siRNA nuclear export and re-import may not occur in plants. In contrast, there is at least one example in *Tetrahymena* where specialized siRNAs (siRNA-like scan, scnRNAs) are thought to travel from the original micronucleus to the cytoplasm, to a second nucleus–the old macronucleus, and eventually to a third nucleus–the new macronucleus, to influence epigenetic DNA elimination in conjugating cells (Figure 4, right; Mochizuki & Gorovsky, 2005). Many important questions remain: (1) How are dsRNAs are imported into nuclei? (2) Do the same dsRNAs that participate in chromatin alteration also participate in posttranscriptional events? (3) How do cells distinguish between dsRNAs that function in the cytoplasm and those that function in the nucleus?

SUMMARY AND PERSPECTIVES

The subcellular dynamics of small RNAs encoded by the nucleus are unexpectedly complex. Some small RNAs that function solely in the nucleus, such as snRNAs, are processed in the cytoplasm before they return to the nucleus (Figure 1). It is not clear whether

this seemingly complicated life style for snRNAs is conserved among all eukaryotes, but some recent tantalizing evidence for budding yeast suggest that it may be. It is also not clear whether other types of small RNAs that function in the nucleus sample the cytoplasm during their biogenesis, but new studies in budding yeast for telomerase RNA (Figure 2), and in vertebrate cells for snoRNAs, suggest that these RNAs may also sample the cytoplasm. In budding yeast tRNAs undergo very intricate cellular dynamics (Figure 3). Pre-tRNAs exit the nucleus and complete their processing in the cytoplasm. Some or all of the processed cytoplasmic tRNAs move retrograde back to the nucleus and likely return once again to the cytoplasm via a re-export process. Whether the retrograde process is regulated is unknown; it is also unknown whether it is conserved among other eukaryotes. Small dsRNAs may also have complex nucleus/cytoplasmic dynamics. There is one documented case for members of this group (scnRNAs) traveling from one nucleus to the cytoplasm and then to a second and third nucleus (Figure 4); it is unknown whether other dsRNAs sample the cytoplasm before functioning in the nucleus. Future experiments must address the details of the mechanism(s) for each category of traveling RNA, determine whether the travels are conserved among eukaryotes, and elucidate the functional significance that such seemingly complicated cell biology serves.

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