Proofreading and Aminoacylation of tRNAs Before Export from the Nucleus

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After synthesis and processing in the nucleus, mature transfer RNAs (tRNAs) are exported to the cytoplasm in a Ran·guanosine triphosphate–dependent manner. Export of defective or immature tRNAs is avoided by monitoring both structure and function of tRNAs in the nucleus, and only tRNAs with mature 5′ and 3′ ends are exported. All tRNAs examined can be aminoacylated in nuclei of *Xenopus* oocytes, thereby providing a possible mechanism for functional proofreading of newly made tRNAs. Inhibition of aminoacylation of a specific tRNA retards its appearance in the cytoplasm, indicating that nuclear aminoacylation promotes efficient export.

In eukaryotic cells, most RNAs are processed in the nucleus. Nuclear maturation of tRNAs involves base modification, processing of the 5′ and 3′ ends, and in some cases splicing of tRNA precursors (pre-tRNAs), with enzymes such as ribonuclease P (RNase P), tRNA nucleotidyl transferase, and tRNA splicing endonuclease and ligase (*1*–*5*). In addition, low amounts of factors that normally interact with tRNAs during protein synthesis in the cytoplasm have been identified in the nucleus, including aminoacyl-tRNA synthetases (*5*) and translation elongation factor EF-1α (*6*).

A complex involved in tRNA export contains tRNA, the tRNA export receptor exportin-t, and the guanosine 5′-triphosphate (GTP)–bound form of Ran (Ran·GTP) (*7*), a GTPase required for nucleocytoplasmic transport of both RNAs and proteins (*8*, *9*), but no cargo-specific adapter protein. In *X. laevis* oocytes, a single pathway appears to function for export of all tRNAs (*7*, *10*); in *Saccharomyces cerevisiae* there may be more than one pathway, because cells lacking the homologous tRNA export receptor (Los1p) are viable (*11*, *12*).

Only mature tRNAs are found in the cytoplasm (*1*, *2*), and defective tRNAs, which might disrupt translation, appear to be excluded. We show here that in *X. laevis* oocytes the absence of nonfunctional tRNAs from the cytoplasm can be explained by kinetic differences in maturation steps and by monitoring of mature tRNAs for their abilities to be aminoacylated, before export from the nucleus.

To determine if processing enzymes contribute to the proofreading of tRNA transcripts, precursors of *X. laevis* $tRNA^{Tyr}$ (*13*) containing an intron (intervening sequence, IVS) and extra sequences at the 5′ and 3′ ends (5′, 3′ ext.) were injected into nuclei of *X. laevis* oocytes (*14*). Wild-type transcripts were rapidly matured to tRNA (Fig. 1A, lanes 2 and 3), with the transient accumulation in the nucleus of a heterogeneous population of processing intermediates that were spliced (ΔIVS) but not end-matured (5′, 3′ ext.) (*15*). In contrast, injection of high, nonphysiological amounts of the same precursor led to generation of a different processing intermediate (Fig. 1B, lanes 2 and 3) that was fully matured at its 5′ and 3′ ends but not spliced (+IVS) (*15*). A comparable dose-dependent switch in processing pathways was observed when a DNA template for $tRNA^{Tyr}$ was injected (compare lanes 1 of Fig. 1, C and D); low to moderate amounts of $tRNA^{Tyr}$ genes produced exclusively spliced intermediates (Fig. 1C), whereas high amounts led to accumulation of the intron-containing intermediate (Fig. 1D). The change in the types of intermediates that accumulated in response to different amounts of pre-tRNA shows that excess substrate saturates splicing more easily than it saturates end maturation.

In contrast to the intron-containing intermediate (Fig. 1, B and D), the spliced but end-immature intermediates were not exported (Fig. 1, A and C), indicating that end maturation but not splicing is a prerequisite for export. When lower, more physiological

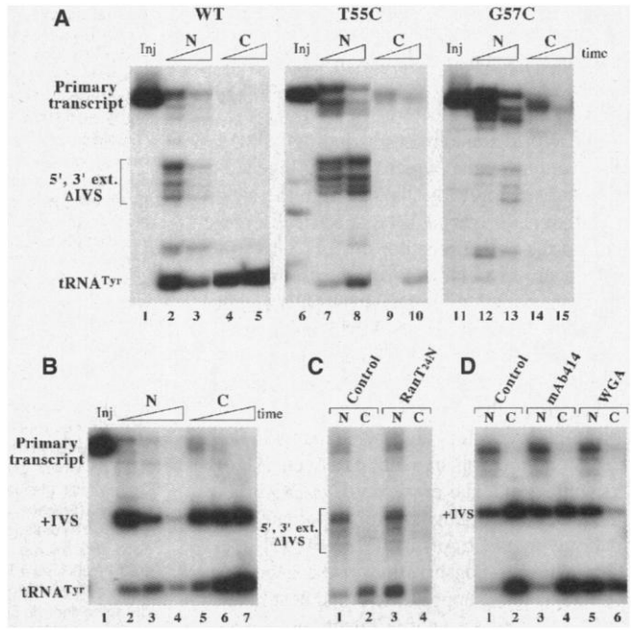

Fig. 1. Structural requirements for nuclear maturation and export of $tRNA^{Tyr}$. (**A**) Disruption of conserved tertiary interactions within $tRNA^{Tyr}$ interferes with both processing and export. Low amounts of ^{32}P-labeled RNAs corresponding to wild-type (WT) or mutant (T55C and G57C) precursors of *X. laevis* $tRNA^{Tyr}$ were injected into nuclei of *Xenopus* oocytes (*14*), and 1 (lanes 2, 4, 7, 9, 12, and 14) and 2 hours (lanes 3, 5, 8, 10, 13, and 15) later the intracellular distributions of the injected primary pre-tRNA transcripts (lanes 1, 6, and 11), the spliced (ΔIVS) but not end-matured (5′, 3′ ext.) processing intermediates, and the mature tRNA ($tRNA^{Tyr}$), were determined by analysis of total nuclear (N) and cytoplasmic (C) RNAs. The small amounts of injected precursor RNAs in the cytoplasm probably resulted from inefficient retention (*22*) shortly after injection. (**B**) Saturation of the tRNA splicing machinery leads to nuclear accumulation and export of nonspliced, end-matured $tRNA^{Tyr}$. High amounts of wild-type primary transcript were injected into oocyte nuclei, and export of unspliced (+IVS) and mature $tRNA^{Tyr}$ was monitored at 0.75 (lanes 2 and 5), 1.5 (lanes 3 and 6), and 4 hours (lanes 4 and 7) after injection. (**C**) Disruption of the RanGTPase system blocks export but not processing of $tRNA^{Tyr}$. Low amounts of the gene for *X. laevis* $tRNA^{Tyr}$ (*14*) were injected into control oocytes (lanes 1 and 2) or oocytes preinjected with RanT24N, and export and processing of the newly made $tRNA^{Tyr}$ transcripts were monitored 4.5 hours later. The processing intermediates are fully spliced but end-immature (*18*), as observed in (A) upon injection of low amounts of WT pre-$tRNA^{Tyr}$. (**D**) Nonspliced $tRNA^{Tyr}$ is exported via the tRNA pathway. High amounts of the gene for *X. laevis* $tRNA^{Tyr}$ (*14*) were injected with [α-^{32}P]GTP into nuclei of control oocytes (lanes 1 and 2) or oocytes preinjected with anti-nucleoporin (mAb 414, lanes 3 and 4) or wheat germ agglutinin (lanes 5 and 6), and export and processing of the newly made $tRNA^{Tyr}$ transcripts were analyzed 20 hours later.

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amounts of precursor are present in oocyte nuclei, splicing is an early event that occurs before end maturation, thereby ensuring that processing intermediates are not normally exported. Previous studies with very high amounts of $tRNA^{Tyr}$ template DNA reported accumulation and export of the unspliced intermediate, leading to the inappropriate conclusion that splicing is normally the last step in the processing pathway (*16*).

The unspliced tRNA intermediate appears to be exported by the pathway used for mature tRNA, because both types of molecules responded similarly to export inhibitors. Export was resistant to an antibody to nucleoporins [monoclonal antibody (mAb) 414] (*17*) (Fig. 1D, lanes 3 and 4), but was competed by coinjected $tRNA^{Phe}$ (*18*) and was sensitive to injection of the lectin wheat germ agglutinin (*17*) (lanes 5 and 6). Exportin-t–mediated export of an unspliced intermediate has recently been observed by others (*10*).

Although depletion of nuclear Ran·GTP by injection of the dominant-negative mutant RanT24N or the RanGTPase-activating protein RanGAP (*9, 19*) blocked export of the mature tRNA, as shown by others (*9*), it had no effect on splicing or end maturation of pre-$tRNA^{Tyr}$ (Fig. 1C, compare lanes 1 and 3) (*15, 18*). In yeast cells disruption of the RanGTPase system leads to rapid accumulation of unspliced intermediates (*20*), perhaps reflecting tighter coupling between export and tRNA splicing in those cells.

The influence of tertiary structure on maturation and export of $tRNA^{Tyr}$ was assessed by injection of precursors having mutations that would disrupt conserved interactions between loops I and III (the D- and T-ψ-C-G loops, respectively). The precursor with a T55C mutation or with either C or T at position 57 (normally G in $tRNA^{TyrC}$) (*13*) was processed much more slowly than wild-type (Fig. 1A, lanes 7, 8, 12, and 13), and the unprocessed mutant precursors were unstable, providing evidence for proofreading during tRNA maturation (*18, 21*). Those few mutant tRNAs that were processed to the mature form were exported poorly (lanes 7 to 10) (*21*). Also, the binding affinity of exportin-t in vitro is reported to be higher for wild-type tRNA than for end-immature or mutated tRNAs (*7, 10*), indicating a role of exportin-t in export cargo selection.

The requirement in tRNA export for maturation of both 5′ and 3′ ends was demonstrated by the export behavior of several variants of the human initiator tRNA, $tRNA_i^{Met}$ (*14*). $tRNA_i^{Met}$ with three extra nucleotides at its 5′ end and the mature 3′ end appeared in the cytoplasm only after processing of the 5′ end (Fig. 2A). $tRNA_i^{Met}$ lacking the 3′-terminal adenosine was processed rapidly at the 5′ end but was not exported until the 3′ end had also been matured (Fig. 2B); likewise, when generation of the 3′ terminal CCA by tRNA–nucleotidyl transferase was not possible (because of the lack of unpaired nucleotides at the 3′ end), the transcript remained in the nucleus (Fig. 2C) (*22*). As expected, a transcript having three extra nucleotides at its 5′ end but lacking three (CCA) at the 3′ end (*23*) was processed at both ends before export, yielding a molecule with the same length and mobility as the precursor (Fig. 2D).

The requirements for both correct folding and mature ends of tRNAs raised the possibility that nuclear tRNAs might be subject to "functional proofreading" by aminoacylation before export. To test for aminoacylation of nuclear tRNAs, potential aminoacyl-tRNA linkages were stabilized by isolation and electrophoresis of $tRNA_i^{Met}$ under acidic conditions (*24*); brief incubation at pH 9 (which causes deacylation) permitted comparison with markers of acylated and deacylated forms of cytoplasmic $tRNA_i^{Met}$ (Fig. 3A, lanes 4 and 5). Within 20 min of injection a

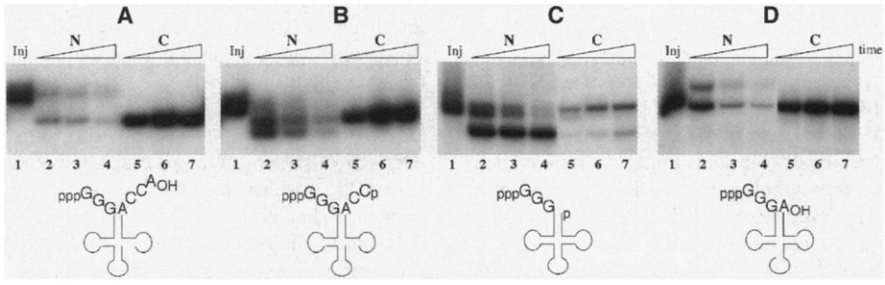

Fig. 2. Requirements for both 5′ and 3′ end maturation before tRNA export. ^{32}P-labeled RNAs corresponding to human $tRNA_i^{Met}$ with the indicated 5′ and 3′ terminal sequences (*14*) were injected into oocyte nuclei, and the intracellular distributions of the RNAs were analyzed at 45, 100, and 220 min (**A** and **B**) and at 30, 60, and 120 min (**C** and **D**). The $tRNA_i^{Met}$ injected in (A) and (D) were primary transcripts synthesized in vitro, whereas the RNAs injected in (B) and (C) were generated by periodate oxidation and β-elimination (*14*) of the RNAs used in (A) and (D), respectively. In all cases, the major cytoplasmic forms correspond to fully mature $tRNA_i^{Met}$, and the small amount of this form in (C) (lanes 5 to 7) is due to heterogeneity of the 3′ ends of the injected RNA (*18*).

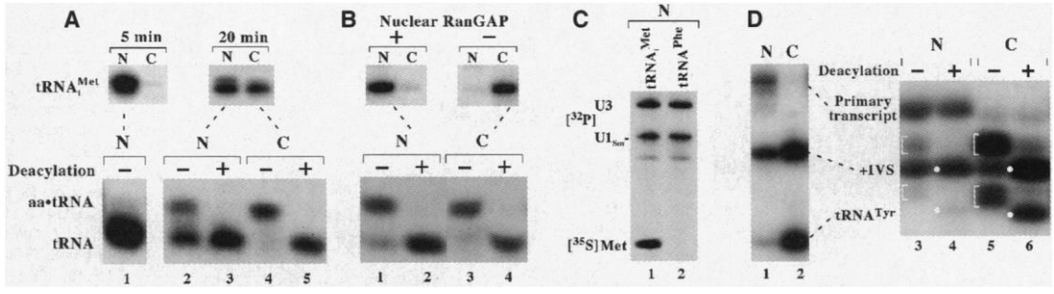

Fig. 3. Aminoacylation of tRNA within the nucleus. (**A**) Aminoacylation of $tRNA_i^{Met}$ occurs in the nucleus. ^{32}P-labeled pre-$tRNA_i^{Met}$ (Fig. 2A) was injected into oocyte nuclei, and the intracellular RNA distributions were monitored 5 and 20 min later (upper panels). The state of aminoacylation (lower panels) was assessed by isolation and analysis of RNAs under acidic conditions (*14, 24*) without (−) or with (+) prior deacylation. (**B**) Nuclear aminoacylation of $tRNA_i^{Met}$ occurs independently of export. Export (upper panels) and the extents of aminoacylation (lower panel) of $tRNA_i^{Met}$ were monitored 1.5 hours after nuclear injection into oocytes that had been depleted of Ran·GTP (*14*) by prior intranuclear injection of RanGAP protein (+) or control oocytes (−). (**C**) Nuclear aminoacylation of $tRNA_i^{Met}$ is specific. Mixtures of unlabeled human $tRNA_i^{Met}$ (lane 1) or yeast $tRNA^{Phe}$ (lane 2) (4 to 5 pmol/oocyte) and ^{32}P-labeled control RNAs were injected into nuclei of oocytes depleted of Ran·GTP and labeled with ^{35}S-methionine (*14*). Five hours later, nuclear RNAs were isolated and analyzed under acidic conditions. (**D**) $tRNA^{Tyr}$ also is aminoacylated in the nucleus. Newly made nuclear and cytoplasmic $tRNA^{Tyr}$ transcripts (Fig. 1D, lanes 1 and 2) were isolated under acidic conditions and analyzed in both neutral (lanes 1 and 2) and acidic gels (lanes 3 to 6). Aminoacylated forms of $tRNA^{Tyr}$ and end-mature but unspliced pre-$tRNA^{Tyr}$ (lanes 3 and 5) are indicated by lower and upper brackets, respectively, whereas nonacylated forms (lanes 4 and 6) are indicated by dots. Differences in the extents of aminoacylation were confirmed by assaying for resistance to periodate oxidation (*18*).

new form of nuclear $tRNA_i^{Met}$ accumulated that comigrated with cytoplasmic aminoacylated $tRNA_i^{Met}$ (lane 2). Like the acylated cytoplasmic $tRNA_i^{Met}$, the nuclear RNA was resistant to periodate oxidation unless first deacylated at pH 9 (*18*), thereby confirming the presence of a blocking group at the 3′ end, presumably an amino acid.

To test if the aminoacylated $tRNA_i^{Met}$ in the nuclear fraction represented molecules that had been exported but remained associated with the outside of the nucleus, transport was blocked by depletion of the nuclear pool of Ran GTP (compare with Fig. 1D) (*9*, *19*). When tRNA export was inhibited in this manner, essentially all of the nuclear $tRNA_i^{Met}$ migrated as the aminoacylated form (Fig. 3B, lanes 1 and 2), demonstrating that it was aminoacylated within the nucleus.

The specificity of nuclear aminoacylation was examined by injection of unlabeled $tRNA_i^{Met}$ or $tRNA^{Phe}$ into oocyte nuclei containing ^{35}S-methionine, under conditions where tRNA export was blocked. Only the tRNA of nuclei receiving $tRNA_i^{Met}$ was labeled by the ^{35}S-methionine (Fig. 3C), and this label was released from the $tRNA_i^{Met}$ by incubation under deacylation conditions (*18*). Thus, nuclear $tRNA_i^{Met}$ was aminoacylated with its cognate amino acid.

Other tRNAs also can be aminoacylated in the nucleus. Both the mature and the unspliced forms of $tRNA^{Tyr}$ were aminoacylated in the nucleus, although aminoacylation was more extensive for the mature tRNA (Fig. 3D, lanes 3 and 4) (*25*); in the cytoplasm, both forms were almost fully charged (lane 5). Injection of pxt210 DNA (*26*) resulted in the nuclear accumulation of *X. laevis* tRNAs specific for alanine, asparagine, leucine, lysine, methionine, tyrosine, and phenylalanine. By both the deacylation and periodate sensitivity assays, all seven nuclear tRNAs appeared to be aminoacylated (*18*). Thus it is very likely that all tRNAs can be aminoacylated with their cognate amino acids before export from the nucleus.

Confirmation that the mobility shift of the nuclear $tRNA^{Tyr}$ is due to aminoacylation was obtained through the use of tyrosyl sulfamoyl adenosine (Tyr-AMS), a strong competitive inhibitor of the requisite intermediate, tyrosyl-AMP (Tyr-AMP) (*27*). Tyr-AMS blocked aminoacylation of $tRNA^{Tyr}$ (both nuclear and cytoplasmic) but not $tRNA_i^{Met}$, as assayed by the absence or presence of mobility shifts upon incubation under deacylation conditions (Fig. 4A, left panel; compare lanes 3 and 4, and lanes 7 and 8; right panel, lanes 2 and 4). As expected, protein synthesis was strongly inhibited in oocytes treated with Tyr-AMS or the asparaginyl-AMP analog Asn-AMS, reflecting the absence of specific charged tRNAs (*18*).

The effect of nuclear aminoacylation on $tRNA^{Tyr}$ export was monitored with Tyr-AMS. In the absence of aminoacylation, export of $tRNA^{Tyr}$ was significantly retarded (Fig. 4B, top panel, compare lanes 2 to 5 with lanes 6 to 9), but export of $tRNA_i^{Met}$ (middle panel) and $U1_{Sm^-}$ RNA (bottom panel) was not affected; the control oocytes received cycloheximide, to account for nonspecific effects due to inhibition of protein synthesis. In a similar series of experiments, Asn-AMS specifically blocked aminoacylation of nuclear and cytoplasmic Asn-$tRNA^{Asn}$ and interfered with the export of $tRNA^{Asn}$, but had no effect on aminoacylation and export of $tRNA^{Tyr}$ (*18*). Thus, nuclear aminoacylation affects the rate of export of several, and perhaps all, tRNAs (*28*).

Although aminoacylation is important for tRNA export, it is not essential under the conditions used here, because uncharged tRNAs can be exported, albeit more slowly. Even with the lowest amounts of tRNAs injected, the concentration in the nucleus far exceeds that of endogenous nuclear tRNAs, possibly driving formation of tRNA-containing complexes that normally might not be stable. Aminoacylation greatly strengthens the binding of tRNAs to the translation factor EF-1α (*29*), and charging could serve a similar function in promoting the association of nuclear tRNAs with exportin-t (plus Ran·GTP); artificially high levels of nuclear tRNA would diminish but not abolish the importance of this effect. Similarly, the high amounts of intron-containing processing intermediates that accumulate when splicing is saturated (Fig. 1B) favor their inappropriate export.

Proofreading of transcripts before export prevents precursors from being prematurely separated from nuclear processing enzymes. In oocyte nuclei, splicing of pre-tRNAs normally occurs faster than end maturation (Fig. 1A), thereby ensuring that unspliced intermediates are not exported. Moreover, export of any end-mature but unspliced tRNA is reduced because export is coupled to nuclear aminoacylation (Fig. 4) and unspliced tRNAs are inefficiently charged (Fig. 3D) (*1*). Such coupling may account for the absence of unspliced tRNAs from the cytoplasm in yeast, where splicing and end maturation can occur in either order (*30*).

Unspliced mRNAs are generally retained in the nucleus, and it is likely that other RNAs are also subject to proofreading before export, by mechanisms that are yet to be defined. The presence of aminoacylated tRNA in the nucleus supports proposals that some mRNAs may undergo functional monitoring before or during export from the nucleus, as a step in nonsense-mediated decay (*31*); similarly, newly made ribosomes may be subjected to a functional quality control check before export.

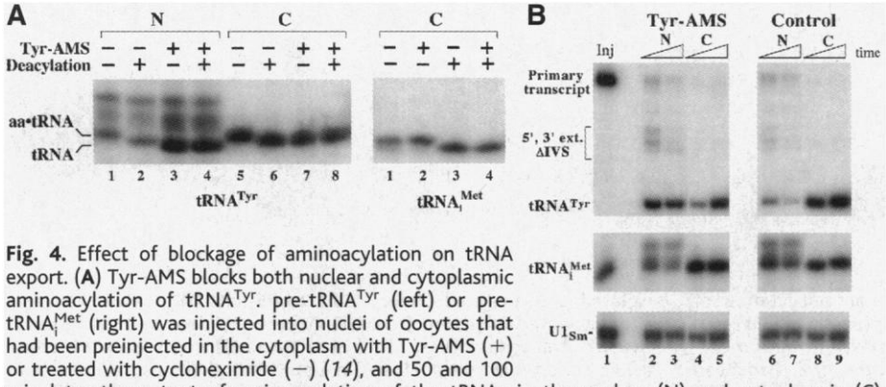

Fig. 4. Effect of blockage of aminoacylation on tRNA export. (**A**) Tyr-AMS blocks both nuclear and cytoplasmic aminoacylation of $tRNA^{Tyr}$. pre-$tRNA^{Tyr}$ (left) or pre-$tRNA_i^{Met}$ (right) was injected into nuclei of oocytes that had been preinjected in the cytoplasm with Tyr-AMS (+) or treated with cycloheximide (−) (*14*), and 50 and 100 min later the extent of aminoacylation of the tRNAs in the nuclear (N) and cytoplasmic (C) fractions were assayed. Differences in gel mobilities of the nuclear nonacylated tRNAs may represent differences in modifications. (**B**) Tyr-AMS specifically inhibits export of $tRNA^{Tyr}$. Oocytes were injected as in (A), and RNA export was monitored with time. The intracellular distributions of $tRNA^{Tyr}$, $tRNA_i^{Met}$, and $U1_{Sm^-}$ at 50 min (lanes 2, 4, 6, and 8) and 100 min (lanes 3, 5, 7, and 9) after RNA injection are shown; the injected RNAs are shown in lane 1 (Inj).

References and Notes

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14. Templates for in vitro synthesis of primary transcripts of $tRNA^{Tyr}$ were made from pxt62 DNA (*13*) by polymerase chain reaction (PCR) with primers 5′GGGAATTCATTTAGGTGACACTATAGAACCGGCCTTCGATAGC and 5′-GGCAAGCTTAAAGCGTCCTTCGAGCCGGAAT(g, c, a)G(a, g)ACCAGC; lowercase letters represent sequence variants used to generate mutants (T55C, G57T/C) within loop III of mature $tRNA^{Tyr}$. Template for the precursor of $tRNA_i^{Met}$ lacking the 3′ CCA end (*23*) was used in PCR to make template for the precursor containing the mature 3′ end with primers 5′-GTGAATTCTAATACGACTCACTATAGGG and 5′-CTCTGGATCCTGGTAGCAGAGGATGGTTTCGAT followed by digestion with Mva I. Unlabeled yeast $tRNA^{Phe}$ was from Sigma. Transcription, purification, injection, and isolation of the RNAs from oocytes were as described (*32*). Transfer RNA transcripts were coinjected with $U1_{sm}$ and U3 RNAs, serving as controls for export and nuclear injection and dissection, respectively, and low and high amounts of $tRNA^{Tyr}$ primary transcripts (Fig. 1) were 10 to 20 and 80 to 100 fmol per oocyte, respectively. For DNA injections, low and high amounts of the *X. laevis* $tRNA^{Tyr}$ gene were 0.125 and 1.0 ng per oocyte, respectively, of pxt62 plasmid DNA (*13*); for in vivo labeling, [α-^{32}P]GTP was used at 0.5 μCi/oocyte. For depletion of nuclear RanGTP, 20 to 30 ng of RanT24N [an inhibitor of the guanine-nucleotide exchange factor for Ran, RCC1 (*9*, *19*)] or RanGAP was preinjected into the nucleus; comparable results were obtained with RanT24N or RanGAP (*18*). For standard RNA analyses under neutral conditions, electrophoresis was in 8% (30:0.8) polyacrylamide, 7 M urea, 0.5× TEB (45 mM tris-borate, 1.15 mM EDTA, pH 8.3) gels. For analyses under acid conditions, RNAs were isolated at pH 5.0 and on occasion deacylated at pH 9.0, as described (*24*), and electrophoresis was in 6.5% (19:1) polyacrylamide, 8 M urea, 0.1 M NaOAc (pH 5.0) gels. Periodate oxidation of RNAs was as described by E. Lund and J. E. Dahlberg [*Science* **255**, 327, (1992)]. For in vivo aminoacylation of $tRNA_i^{Met}$ (Fig. 3C), oocytes were injected with ^{35}S-methionine (0.2 μCi per oocyte), and comigration of the ^{35}S-label and met-$tRNA_i^{Met}$ was determined by RNA staining before autoradiography of the dried gels (*18*). For blockage of aminoacylation or protein synthesis, Tyr-AMS (*27*) was injected to final concentrations of 150 to 300 μM or cycloheximide was added to 200 μg/ml of medium; inhibition of protein synthesis was monitored by labeling with ^{35}S-methionine (50 μCi/ml of medium).
15. Precursors with an intron or extra sequences at the ends were differentiated by RNase T1 fingerprinting (*32*), with reference to published sequences (*13*).
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21. Human pre-$tRNA_i^{Met}$ lacking an intron but with a comparable mutation at position 57 also is processed and exported slowly [M. Zasloff, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 6439 (1983); J. A. Tobian, L. Drinkard, M. Zasloff, *Cell* **43**, 415 (1985); C. Traboni, G. Ciliberto, R. Cortese, *ibid.* **36**, 179 (1984); (*23*)]. Mutations at position 55 were not tested previously, because they alter the B-box of the tRNA promoter, which is required for producing tRNAs in vivo from injected genes.
22. Mutated tRNAs or end-immature processing intermediates either may not be recognized by the export machinery or may be actively retained by nuclear proteins that bind to such molecules [C. J. Yoo and S. Wolin, *Cell* **89**, 393 (1997); E. Bertrand, F. Houser-Scott, A. Kendall, R. H. Singer, D. R. Engelke, *Genes Dev.* **12**, 2463 (1998)]. At least some form of retention appears likely because wild-type and mutant pre-tRNAs containing 5′ m^7G caps were not exported efficiently (*18*). These molecules are recognized as having some tRNA character, because they undergo base modifications soon after synthesis (*16*, *18*).
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25. When aminoacylated, both forms of $tRNA^{Tyr}$ migrated during electrophoresis as broad bands (denoted by the brackets in Fig. 3D, lanes 3 and 5) that could be resolved into doublets (lane 5). Deacylation of each form produced a species with a single electrophoretic mobility (lanes 4 and 6), indicating that alternative conformers may form upon aminoacylation; however, aminoacylated $tRNA_i^{Met}$ migrated as a single band (Fig. 3A), showing that formation of the doublet is not general to all tRNAs.
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28. If tRNAs remain aminoacylated during translocation through the nuclear pore complex, they could be passed directly into the translational machinery in the cytoplasm, in agreement with the "channeling" hypothesis for recycling of tRNAs [R. Stapulionis and M. P. Deutscher, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 7158 (1995)].
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Defective LPS Signaling in C3H/HeJ and C57BL/10ScCr Mice: Mutations in *Tlr4* Gene

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Mutations of the gene *Lps* selectively impede lipopolysaccharide (LPS) signal transduction in C3H/HeJ and C57BL/10ScCr mice, rendering them resistant to endotoxin yet highly susceptible to Gram-negative infection. The codominant Lps^d allele of C3H/HeJ mice was shown to correspond to a missense mutation in the third exon of the Toll-like receptor-4 gene (*Tlr4*), predicted to replace proline with histidine at position 712 of the polypeptide chain. C57BL/10ScCr mice are homozygous for a null mutation of *Tlr4*. Thus, the mammalian Tlr4 protein has been adapted primarily to subserve the recognition of LPS and presumably transduces the LPS signal across the plasma membrane. Destructive mutations of *Tlr4* predispose to the development of Gram-negative sepsis, leaving most aspects of immune function intact.

Conservative estimates hold that in the United States alone, 20,000 people die each year as a result of septic shock brought on by Gram-negative infection (*1*). The lethal effect of a Gram-negative infection is linked, in part, to the biological effects of bacterial lipopolysaccharide (endotoxin), which is produced by all Gram-negative organisms. A powerful activator of host mononuclear cells, LPS prompts the synthesis and release of tumor necrosis factor (TNF) and other toxic cytokines that ultimately lead to shock in