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tRNA transport from the nucleus in a eukaryotic cell: Carrier-mediated translocation process

(RNA transport/nuclear transport/*Xenopus laevis* oocyte/$tRNA_i^{Met}$)

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ABSTRACT **The mechanism by which a tRNA molecule is delivered from the nucleus of a cell to the cytoplasm has been studied in the *Xenopus laevis* oocyte utilizing nuclear microinjection and manual microdissection techniques. tRNA nuclear transport in this cell resembles a carrier-mediated translocation process rather than diffusion through a simple pore or channel. tRNA transport is saturable by tRNA, with a maximal rate measured to be about 190×10^7 molecules per min per nucleus (21°C) in the mature oocyte. Competitive inhibition between two different tRNA species can be demonstrated, suggesting that many tRNA species share a common carrier system. tRNA nuclear transport is sharply dependent on temperature, with an optimal rate observed at 31°C. A single G-to-U substitution at position 57 in the vertebrate $tRNA_i^{Met}$ molecule reduces the transport rate of this tRNA by a factor of about 20, implicating this highly conserved region of the tRNA molecule (loop IV) as critical for recognition by the transport mechanism. On morphologic grounds I propose that ribosome-like components surrounding the nuclear pore may function as the tRNA translocation "motor." The tRNA nuclear transport mechanism represents a distinctly eukaryotic process and a site of potential control over cell growth and proliferation.**

Transfer RNA, in common with other cytoplasmic RNA species, is synthesized within the nucleus through a complex series of reactions. Ultimately, the RNA molecule must cross the boundary separating the nucleus from the cytoplasm. By what mechanism does this escape occur? Do RNA molecules, especially tRNAs, the smallest cellular RNA species, simply diffuse across the nuclear envelope once they have been processed or is the transport event more complex? My interest in this problem arose recently in studies of the expression of a cloned human variant $tRNA_i^{Met}$ gene (1–4). By direct microinjection of the cloned gene into the germinal vesicle of the *Xenopus laevis* oocyte, the primary transcript of the variant gene was processed slowly but accurately to a mature variant $tRNA_i^{Met}$ (4). A striking property of this species was its accumulation within the oocyte nucleus in marked contrast to the normal $tRNA_i^{Met}$ species, which was found exclusively in the oocyte cytoplasm (4). The variant $tRNA_i^{Met}$ species, furthermore, appeared to be prevented from free diffusion by the nuclear membrane, and its behavior suggested that a specific transport process between the nucleus and cytoplasm might exist for tRNA species (4). I report here the direct demonstration of a tRNA nuclear transport mechanism in the living *X. laevis* oocyte. This transport system can utilize mature tRNA efficiently as a substrate and discriminates between the normal and variant $tRNA_i^{Met}$ species. I show that tRNA transport from the nucleus can be saturated by tRNA and resembles a carrier-mediated process rather than simple diffusion through a pore or channel. I believe this data to reveal explicitly the functioning of a tRNA nuclear transport mechanism in a living cell.

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METHODS

Intranuclear Injections. *X. laevis* oocytes (stages 5 and 6) were scraped from surgically excised ovaries with a 2-mm platinum loop. Individual oocytes were placed animal-pole-up in single wells of a 96-well microtiter tray with conically shaped wells, each well containing 0.15 ml of OR2 buffer (5) (82.5 mM NaCl/2.5 mM KCl/1 mM $MgCl_2$/1 mM $CaCl_2$/5 mM Hepes·HCl/1 mM sodium phosphate, final pH 7.8). The trays were spun in a Beckman TJ-6 centrifuge at about $1,000 \times g$ for 10 min at 18°C (6). Under optimal conditions, the germinal vesicle is visualized in the animal pole as a distinct target-like structure. Within 5 min of centrifugation, the oocytes were transferred to a Petri dish (60 × 15 mm containing about 20 ml of OR2 buffer), on the bottom of which a nylon grid had been fixed to assure stability of the oocyte during microinjection. The oocytes were oriented animal-pole-up, and 20 nl of a solution was delivered into the visualized germinal vesicle with a beveled glass needle (20- to 25-μm o.d.). The oocytes were maintained in OR2 buffer until further analysis.

Oocyte Dissection. Two dissection techniques were utilized, one using trichloroacetic acid and the other using an aqueous medium described previously (4). In the CCl_3COOH method, oocytes were transferred from OR2 buffer into ice-cold 1% CCl_3COOH and then gently rocked at 4°C for 30 min. tRNA transport ceases at 4°C, and the contents of the oocyte were effectively fixed at this concentration of CCl_3COOH. The oocytes were manually dissected in 0.3 ml of 1% CCl_3COOH on a siliconized microscope slide at room temperature under a standard binocular dissecting microscope with watchmakers' forceps. The oocytes were opened with a single tear in the vegetal pole. The germinal vesicle appeared as a greyish-white ball surrounded by slightly yellow-white cytoplasm and separated from the cytoplasm in much the same way as the pit from a fruit. The nucleus was transferred to a scintillation vial containing 0.1 ml of water. The cytoplasmic contents remaining on the microscope slide were transferred to a separate vial; 15 ml of a scintillant cocktail was added (Aquafluor, New England Nuclear), and the radioactivity present in both nucleus and cytoplasm was determined. For determination of transport rates, at least six oocytes were analyzed for each time point.

The aqueous microdissection technique has been utilized for gel electrophoretic analysis of tRNA species introduced into the oocyte (4). This method requires considerably greater technical skill because the germinal vesicle is a delicate structure easily torn during manual dissection.

tRNA Labeled with ^{32}P at the 5′ End. tRNA labeled with ^{32}P at the 5′ end was prepared by the method of Wurst *et al.*

(7) by utilizing T4 polynucleotide kinase (P-L Biochemicals) and [γ-^{32}P]ATP (New England Nuclear). Labeled tRNA was purified by preparative acrylamide gel electrophoresis (3). tRNA was extracted overnight from the crushed acrylamide gel in a small volume of 2.5 M ammonium acetate and recovered by ethanol precipitation. Specific radioactivities of about 2×10^7 cpm/μg of tRNA were routinely achieved. The samples were resuspended in a small volume of 20 mM Tris·HCl, pH 8.0/88 mM NaCl.

Synthesis of ^{32}P-Labeled tRNA$_i^{Met}$ in the *X. laevis* Oocyte. ^{32}P-Labeled mature variant or normal tRNA$_i^{Met}$ was prepared by injection of the corresponding gene and [α-^{32}P]GTP into the germinal vesicle of the *X. laevis* oocyte as described (4). About 10^3 cpm of labeled tRNA$_i^{Met}$ was recovered per injected oocyte.

RESULTS

tRNA Nuclear Transport Can Be Studied with Mature tRNA. The human variant tRNA$_i^{Met}$ accumulates within the nucleus of the *X. laevis* oocyte after nuclear injection of either the variant gene or the purified primary transcript synthesized *in vitro* (4). The sequence of this variant tRNA is identical to the normal human tRNA$_i^{Met}$ species save for a G-to-U substitution at position 57 and has a mature 3′ C-C-A terminus (4). Of almost 200 prokaryotic and eukaryotic species with sequences determined to date, no other contains a U at this position in the mature sequence, occupied exclusively by a purine (usually G) (8). The defective transport behavior of the variant tRNA$_i^{Met}$ thus appeared to result from a single-base substitution within the highly conserved loop IV region. I wished to determine whether the difference in transport behavior between the normal and variant tRNA$_i^{Met}$ species could be observed after nuclear injection of the mature, processed species, thereby functionally dissecting the transport step from more proximal events in the overall biosynthetic pathway.

^{32}P-Labeled variant tRNA$_i^{Met}$ was synthesized in the intact *X. laevis* oocyte by nuclear injection of the cloned gene (gene 1) and [α-^{32}P]GTP and purified by acrylamide gel electrophoresis from total oocyte RNA (4). The purified tRNA was microinjected into the germinal vesicle of stage 6 *X. laevis* oocytes; the oocytes were maintained at 21°C in OR2 buffer (5) and then were dissected manually at various times thereafter. RNA was extracted from the germinal vesicle and corresponding cytoplasm of individual oocytes, analyzed by electrophoresis in 10% acrylamide gels containing 7 M urea, and autoradiographed as described (3). About 90% of the variant tRNA$_i^{Met}$ remained intranuclear 15 min after injection (Fig. 1*B*, lanes 1 and 2), with between 60–70% persisting in the nucleus some 120 min after injection (Fig. 1*B*, lanes 5 and 6). In contrast, the normal human tRNA$_i^{Met}$ species, prepared from the corresponding gene in the identical fashion, was partitioned into the cytoplasm very rapidly (Fig. 1*A*). To distinguish the rapid escape of the normal species from a simple leak, the ^{32}P-labeled tRNA$_i^{Met}$ species was coinjected into the germinal vesicle with ^{32}P-labeled primary transcript of the variant gene (gene 1). This precursor has a half-life in the oocyte nucleus of about 150 min and, thus, was only marginally processed over the time period of this transport study (unpublished data). In addition, the precursor has been shown previously to be an intranuclear species released into the cytoplasm only on physical disruption of the nuclear envelope and membrane (4). When the two species were coinjected into the oocyte nucleus, the tRNA$_i^{Met}$ species rapidly escaped into the cytoplasm while the primary transcript remained intranuclear. Within 5 min of nuclear injection, about 80% of the normal tRNA$_i^{Met}$ species appeared in the cytoplasm (Fig. 1*A*, lanes 3 and 4). Comparison of the distribution at this time with that observed at 15 min (Fig. 1*A*, lanes 5 and 6) and 30 min (lanes 7 and 8) demonstrates that the full extent of transport occurred within 10 min of injection. (Because the oocytes are not fixed in this method and microdissection of an oocyte takes between 3–5 min, precise measurement of the transport rate by this technique is not possible.)

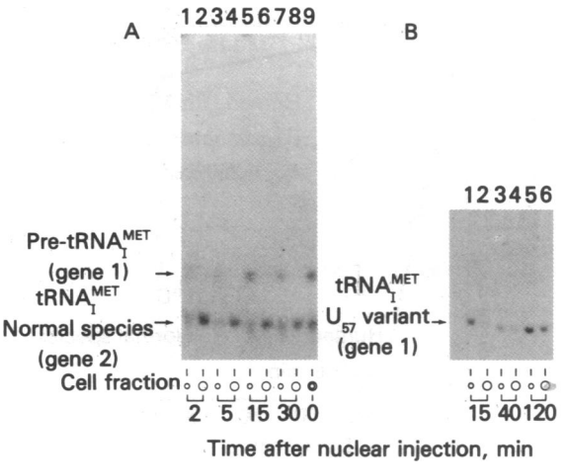

FIG. 1. Intracellular distribution of ^{32}P-labeled normal and variant human tRNA$_i^{Met}$ species in *X. laevis* oocytes after nuclear microinjection. ^{32}P-Labeled tRNA$_i^{Met}$ species were synthesized in intact *X. laevis* oocytes by the injection of [α-^{32}P]GTP and the corresponding gene. ^{32}P-Labeled primary transcript of the variant gene was synthesized in a cell-free transcription system from KB cells and was purified by gel electrophoresis as described (3). Approximately 0.1 ng (200 cpm) of each species in 20 nl of 88 mM NaCl/20 mM Tris·HCl, pH 8.0, was injected into the germinal vesicles of stage 6 *X. laevis* oocytes. The oocytes were maintained at 21°C in OR2 buffer (5) and microdissected in J buffer at indicated times as described (4). RNA was extracted from both cytoplasm and germinal vesicle of individual oocytes, analyzed by electrophoresis in a 10% acrylamide gel containing 7 M urea, and autoradiographed (4). (*A*) Transport of normal tRNA$_i^{Met}$. Normal tRNA$_i^{Met}$ and variant pre-tRNA$_i^{Met}$ (gene 1 primary transcript) were coinjected (volume, 20 nl). Lanes: 9, RNA extracted from the whole oocyte immediately after nuclear microinjection; 1, 3, 5, and 7, species present in the nucleus at indicated times after injection; 2, 4, 6, and 8, species present in the corresponding cytoplasmic fractions. (*B*) Transport of the variant (U57) tRNA$_i^{Met}$. Lanes: 1, 3, and 5, RNA isolated from dissected nuclei; 2, 4, and 6, RNA present in the corresponding cytoplasmic fractions.

The semiquantitative analysis of the tRNA$_i^{Met}$ transport rates was extended by application of a method permitting a quantitative and precise kinetic analysis of intracellular tRNA distribution. ^{32}P-Labeled tRNA species, either synthesized as described above in the intact oocyte or prepared by 5′-labeling of mature tRNA (7), were microinjected into intact stage 6 oocytes. At various times after injection, the oocytes in groups of six were fixed in ice-cold 1% CCl_3COOH. Within 10 min, the germinal vesicle condensed into a dense, whitish rubbery ball, easily dissectable with minimal technical skill from the surrounding cytoplasm. The nucleus was collected, and the tRNA contained in both the cytoplasm and nucleus of an individual oocyte was determined by scintillometry. Electrophoretic analysis of the ^{32}P-labeled tRNA present in each fraction after CCl_3COOH fixation showed that 5′-labeled tRNA remained intact over the time period studied (data not shown). Homogeneous human placental tRNA$_i^{Met}$ (0.2 ng) ^{32}P-labeled at the 5′ end and injected into the germinal vesicle exited with approximately first-order exponential kinetics (Fig. 2). The half-time for transport was calculated to be 8 min. An identical rate was obtained by injection of normal human tRNA$_i^{Met}$ transcribed from the cloned gene in the oocyte (data not shown). In con-

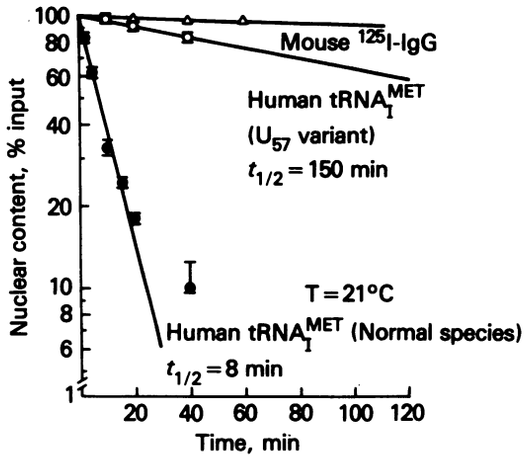

FIG. 2. Kinetics of tRNA$_i^{Met}$ (normal and variant) nuclear transport. ^{32}P-Labeled variant tRNA$_i^{Met}$ was synthesized in intact oocytes as described in Fig. 1. Normal human tRNA$_i^{Met}$ labeled with ^{32}P at the 5′ end was prepared from homogeneous human placental tRNA$_i^{Met}$ (a kind gift of Dr. U. L. Rajbhandary). Each species was injected (volume, 20 nl), and the oocytes were maintained at 21°C in OR2 buffer. At various times after injection, the oocytes in groups of six were fixed in ice-cold 1% CCl_3COOH and manually dissected, and the radioactivity present in the germinal vesicle and cytoplasm of each oocyte was determined separately by liquid scintillation counting. About 4,000 cpm (0.2 ng) of human placental tRNA$_i^{Met}$ and 600 cpm (0.2 ng) of human U57 variant tRNA$_i^{Met}$ were injected into each oocyte. ^{32}P present in the nucleus and cytoplasm of a single oocyte was determined, and the percentage of the total present in the nucleus was calculated. Each point represents the average percentage of the total injected sample remaining in the nucleus for six oocytes, with 1 SD noted: nuclear transport of a normal (●) and U57 variant (○) human tRNA$_i^{Met}$. As control for gross leakage of intranuclear material, a series of oocytes were injected with 20 nl of ^{125}I-labeled mouse IgG (anti-rabbit IgG, affinity purified, 0.1 mCi/ml; 8.4 μCi/μg) and analyzed as above for the tRNA species (△).

trast, the variant tRNA$_i^{Met}$ species (0.2 ng), prepared in the oocyte, was transported into the oocyte cytoplasm with a $t_{1/2}$ of 150 min (Fig. 2), exhibiting a slower rate of escape by almost a factor of 20 than the normal species.

These experiments demonstrate that it is technically possible to measure tRNA nuclear transport within minutes of microinjection of mature tRNA species. Most important, the differences in transport behavior between the normal and variant gene products, seen when the tRNAs are synthesized in the nucleus of the intact oocyte [as shown previously (4)], were also observed upon injection of the processed, mature tRNA species. This observation strongly suggests that mature tRNA introduced into the germinal vesicle utilizes the transport pathway engaged in the biosynthesis of tRNA.

tRNA Nuclear Transport Is Saturable. Differences in the nuclear escape rates measured for the normal and variant human tRNA$_i^{Met}$ species suggested that a selective mechanism is utilized in the transport of tRNA from the nucleus to the cytoplasm. Two simple models for such a process include a tight pore (or channel) and a more complex carrier-mediated translocation mechanism. In the simple pore model, a tRNA molecule would escape from the nucleus by diffusing through a restrictive pore; differences in rate of escape would result from the effects of the base substitution on optimal shape requirements of passage through the hole. In the translocation model, a tRNA molecule would interact with a component capable of translocating the tRNA from one side of the nuclear membrane to the other. Differences in transport rates between the two tRNA$_i^{Met}$ species would result from effects of the base substitution on the affinity of the tRNA molecule for the transmembrane carrier. To distinguish between these two general models we determined the relationship between the rate of tRNA transport and the amount of tRNA introduced into the nucleus. Diffusion-limited processes are generally unsaturable, whereas carrier-mediated events are almost always saturable (9).

Table 1. tRNA$_i^{Met}$ nuclear transport rate as a function of the amount of tRNA$_i^{Met}$ injected per nucleus

Human tRNA$_i^{Met}$ injected, ng	$t_{1/2}$ of nuclear transport, min
0.1	7
0.2	8
0.5	8
1.0	16
2.5	18
5.0	30
10.0	60
40.0	256

Experimental $t_{1/2}$ was determined as described in Fig. 3.

Increasing amounts of 5′-labeled human placental tRNA$_i^{Met}$ were injected into the nuclei of individual oocytes in amounts ranging from 0.1 to 40 ng. The rate of intracellular redistribution was determined by direct radioactivity assay after CCl_3COOH fixation of the oocytes. Up to an input of 0.5 ng, the escape $t_{1/2}$ remained about 7–8 min (Table 1). As the input was increased to about 1 ng per germinal vesicle, the time required for half of the injected tRNA to escape increased from 16 min at 1 ng to 256 min at a 40-ng input of tRNA$_i^{Met}$. In Fig. 3A I have plotted the tRNA$_i^{Met}$ transport rate (pmol/min per nucleus) against the amount of tRNA$_i^{Met}$ injected. A clearly visualized maximal rate was obtained, estimated to be about 3.2×10^{-3} pmol/min per nucleus at 21°C (or about 190×10^7 mol-

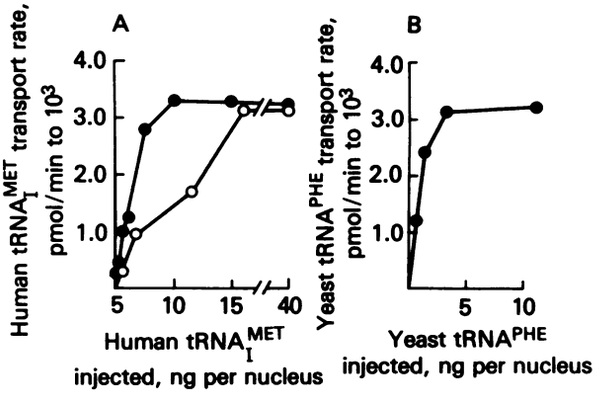

FIG. 3. Rate of tRNA nuclear transport as a function of tRNA injected into the *X. laevis* oocyte nucleus. Human placental tRNA$_i^{Met}$ and yeast tRNA^{Phe} (1,500 pmol phenylalanine incorporated per A_{260} unit; Boehringer Mannheim) were ^{32}P-labeled (specific radioactivity of each was 2×10^7 cpm/μg) at the 5′ end. Increasing amounts of tRNA were injected (volume, 20 nl) into the nucleus of individual oocytes as noted. Oocytes were maintained at 21°C in OR2 buffer for 10 min after injection and fixed in ice-cold 1% CCl_3COOH as described in Fig. 2, and the fraction of the total tRNA injected per oocyte remaining in the nucleus was determined. Rate (and $t_{1/2}$) was calculated assuming first-order exponential kinetics. Each point represents the average value determined from six individual oocytes; the SDs ranged between 5% and 10% of the mean value and have not been included in the figure. (*A*) Nuclear transport of ^{32}P-labeled human tRNA$_i^{Met}$ in the presence or absence of unlabeled yeast tRNA^{Phe}. Various amounts of ^{32}P-labeled tRNA$_i^{Met}$ were injected alone (●) or along with a fixed amount (6.6 ng per nucleus) of unlabeled yeast tRNA^{Phe} (○). (*B*) Nuclear transport of yeast tRNA^{Phe} alone (●).

ecules per min per nucleus). From the data in Fig. 3, an apparent K_m for this transport process can be estimated to occur at about 1.2 ng of $tRNA_i^{Met}$ introduced per nucleus. If we estimate the nuclear volume to be about 2×10^7 liter, the K_m for $tRNA_i^{Met}$ transport would be about 10^{-7} M. Because endogenous tRNA synthesis is low at this stage in oogenesis (10), the concentration of tRNA in the nucleus is represented principally by the injected species.

Yeast $tRNA^{Phe}$ acts as an effective competitive inhibitor of the transport of human $tRNA_i^{Met}$. When increasing amounts of $tRNA_i^{Met}$ were injected in the presence of a fixed input of yeast $tRNA^{Phe}$ (6.6 ng), the amount of $tRNA_i^{Met}$ required to achieve a half-maximal transport rate was shifted from about 1.2 ng to about 6 ng (Fig. 3A). The maximal rate achieved was approximately the same as that observed in the absence of added yeast $tRNA^{Phe}$. From the data presented, a K_i for the yeast $tRNA^{Phe}$ species can be estimated at about 1.7 ng per nucleus, almost identical to the K_m of the human $tRNA_i^{Met}$ species.

The transport rate of yeast $tRNA^{Phe}$ was measured directly as a function of the amount injected into the oocyte nucleus. Both the K_m (about 1.5 ng per nucleus) and the maximal transport rate (3.1×10^{-3} pmol/min per nucleus) were almost identical to those kinetic parameters measured for the human $tRNA_i^{Met}$ species (Fig. 3B).

Although $tRNA_i^{Met}$ was transported rapidly from the nucleus to the cytoplasm of the oocyte, transport in the other direction could not be detected. Thus, injection of ^{32}P-labeled $tRNA_i^{Met}$ into the cytoplasm of the oocyte resulted in no significant accumulation of this species in the nucleus after 6 hr (data not shown). The absence of back-transport of unfractionated total HeLa cell tRNA into the oocyte nucleus has been reported recently (11).

These experiments demonstrate unequivocally that the transport of mature tRNA from the nucleus operates through a saturable process. In principle, saturation could result from a mechanism in which (*i*) tRNA was forced through a tight pore or channel that admitted only a limited number of molecules at one time and (*ii*) the entry or passage through the channel was sufficiently slow to become rate limiting (12). To explain the 20-fold difference in transport rates between the variant and normal $tRNA_i^{Met}$ species, one must postulate that such a restrictive pore could discriminate between the shapes or molecular volumes of these two molecules. From x-ray crystallographic studies, it appears that the purine normally occupying position 57 does not play a major role in maintaining tRNA tertiary structure, suggesting that the G-to-U substitution in the variant should have only a small effect on the overall shape of the molecule (13). A pore or channel with this degree of selectivity would operate through a mechanism requiring highly specific interactions with the transported tRNA molecule and would resemble a transmembrane carrier more than a simple hole-limiting free diffusion.

The ability of yeast $tRNA^{Phe}$ to compete with the transport of human $tRNA_i^{Met}$ demonstrates a rate-limiting component in the transport pathway is nonspecific with respect to tRNA sequence and suggests that all tRNAs share a common carrier system.

It is interesting to compare the experimentally determined maximal tRNA nuclear transport rate of 190×10^7 molecules per min per nucleus with an estimate of the tRNA transport rates utilized in the oocyte during its development. Over the course of the 3-month period of oogenesis between stages 2 and 6, the *X. laevis* oocyte accumulates about 5×10^{12} molecules of tRNA (10), requiring an average transport rate of about 4×10^7 molecules per min per nucleus, or about 2% of the maximal capacity measured for the mature oocyte nucleus.

Temperature Dependence of tRNA Nuclear Transport. The temperature dependence of tRNA transport was determined by measuring the kinetics of $tRNA_i^{Met}$ escape after nuclear injection. Oocytes were injected with human $tRNA_i^{Met}$ labeled with ^{32}P at the 5′ end at 21°C and, within 10 sec of injection, were transferred to OR2 buffer maintained at various temperatures. Oocytes were removed at subsequent times, fixed in ice-cold 1% CCl_3COOH, and manually dissected into nuclear and cytoplasmic fractions, and the intracellular distribution of ^{32}P-labeled tRNA was determined. $tRNA_i^{Met}$ nuclear transport exhibited a maximal rate at 31°C decreasing about 50% at temperatures 10°C above or below the optimum temperature (Fig. 4). At 12°C transport was reduced by 90%. At 4°C virtually no $tRNA_i^{Met}$ transport could be detected. The linearity of the plotted data, extrapolating to the origin at all temperatures analyzed, demonstrates that the transport mechanism responds rapidly to shifts in temperature from 21°C.

The inhibition of tRNA nuclear transport at 4°C was reversible. Recovery of tRNA nuclear transport on warming of the oocyte to 21°C was not instantaneous but exhibited a measurable lag (5-min lag after chilling at 4°C for 1 hr), with the rate achieved being approximately the same as that of the unchilled oocyte (data not shown).

The temperature dependence of $tRNA_i^{Met}$ transport in the living oocyte (4.5-fold increase from 12°C to 21°C; 2.5-fold increase from 21°C to 31°C) is considerably greater than that generally observed for simple diffusional transport processes but is consistent with a carrier-mediated translocation mechanism (9). The temperature dependence of tRNA nuclear transport is similar in magnitude to that observed for such processes as protein uptake from solution by the oocyte (5) and total protein synthesis in the intact cell (14). Although similarity in temperature dependence may be coincidental or may be secondary to major changes in the internal structure of the oocyte, it also may reflect utilization by several systems of similar energy-dependent processes.

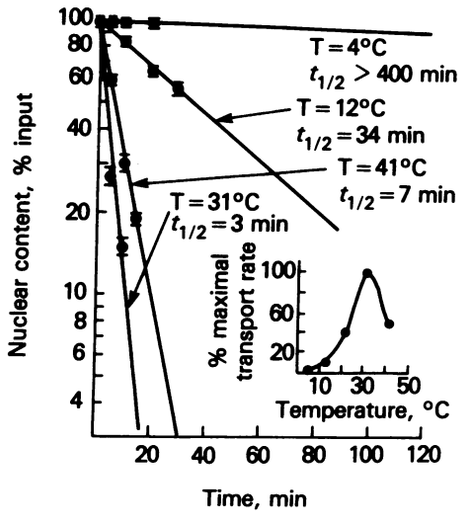

FIG. 4. Temperature dependence of human $tRNA_i^{Met}$ nuclear transport rate. Human placental $tRNA_i^{Met}$ ^{32}P-labeled at the 5′ end (4,000 cpm; 0.2 ng) was injected into the germinal vesicles of individual oocytes at an ambient room temperature of 21°C, and the oocytes immediately were transferred to OR2 buffer at the indicated temperatures. Oocytes in groups of six were fixed in ice-cold 1% CCl_3COOH at the times noted and microdissected; the radioactivity was assayed as described in Fig. 2. $t_{1/2}$ values are calculated from the slopes of the best-fit first-order exponential plot of the data.

IMPLICATIONS

I have demonstrated that tRNA is transported across the nuclear membrane of the *X. laevis* oocyte by a saturable process. This shows that transport rather than simple diffusion underlies the passage of tRNA between the nucleus and cytoplasm of a living eukaryotic cell. The mechanism can transport species with sequences as diverse as that of yeast $tRNA^{Phe}$, vertebrate $tRNA_i^{Met}$, and even prokaryotic initiator $tRNA_f^{Met}$ (data not shown) with almost identical kinetic parameters. Presumably, the sequence or shape requirements, or both, of the tRNA nuclear transport mechanism are evolutionarily primitive, shared by both prokaryotic and eukaryotic tRNA species. The effect of the single G-to-U substitution at position 57 in the vertebrate $tRNA_i^{Met}$ molecule clearly implicates this highly conserved region of the molecule as critical for recognition by the transport mechanism.

The physical basis of the transport mechanism remains to be defined. The nuclear envelope is comprised of a vesicle-like double membrane and numerous pore complexes, organized together in a mosaic-like array (15). In principle, a tRNA molecule could escape from the nucleus either by crossing the double membrane barrier or by traversing the nuclear pore complex. The pore has been implicated as the route by which ribosomal particles emerge from the nucleus after assembly (16). It is conceivable that tRNA might exit the nucleus through the pore by associating with an outgoing ribosomal particle. I view this mechanism as unlikely, however, primarily because tRNA is synthesized most actively in the oocyte prior to the onset of ribosomal synthesis and yet nevertheless accumulates within the cytoplasm of the oocyte (17).

A transport model I favor is one in which the pore itself actively translocates tRNA between the cellular compartments. Recent high resolution electron microscopic studies of the pore complex of the mature *X. laevis* oocyte nuclear membrane reveal the presence of eight ribosomes (or "ribosome-like" particles) organized in radial array around the cytoplasmic face of the pore (15). These "structural" ribosomes represent very attractive candidates for the basic "motor" of the tRNA transport mechanism, both because of their presence in great numbers on the nuclear membrane and because the ribosome is an organelle specifically designed to translocate tRNA. Thus, in this model, tRNA transport could be accomplished by the binding of tRNA to an entry site on the pore-associated ribosome, followed by translocation of the molecule to an acceptor site on the ribosome, with subsequent release of the tRNA into the cytoplasm in an overall series of topological events analogous to events that occur in translation.

The existence of a tRNA transport mechanism leads me to ask whether tRNA transport is (or could be) coupled to the transport of other RNA species, such as mRNA, from the nucleus. This mechanism would provide a simple explanation for the observation that certain cells in which a predominant protein is synthesized, such as the reticulocyte, appear to contain amino acid acceptor species in the same relative proportion as the amino acid composition of the major protein (18, 19). Coupling of tRNA and mRNA transport could be accomplished by the ribosome present at the pore complex in a process envisioned as a "threading out" of mature mRNA simultaneous with the codon-dependent translocation of cognate tRNAs from the nucleus to the cytoplasm, in a process resembling ribosome-dependent mRNA translocation.

The tRNA nuclear transport mechanism represents a biological process with great potential for the regulation of cell growth and proliferation. A detailed analysis of the workings of this distinctly eukaryotic mechanism and its functional relationship to other intracellular processes should be studied further.

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