Import of Nuclear Deoxyribonucleic Acid Coded Lysine-Accepting Transfer Ribonucleic Acid (Anticodon C-U-U) into Yeast Mitochondria†

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ABSTRACT: We have previously shown that *Saccharomyces cerevisiae* mitochondria contain a full set of mitochondrial DNA-coded isoaccepting tRNAs. We report here the presence of two lysine tRNA isoacceptors in purified yeast mitochondria, only one of which ($tRNA_2^{Lys}$) is of mitochondrial origin. The other mitochondria-associated lysine tRNA ($tRNA_1^{Lys}$) does not hybridize with mitochondrial DNA (mtDNA) and elutes with one of the cytoplasmic lysine tRNA isoacceptors in the RPC-5 column chromatographic system. This tRNA was purified by two-dimensional polyacrylamide gel electrophoresis of in vivo ^{32}P-labeled total mitochondrial tRNA. Sequence analysis showed that it corresponds to cytoplasmic $tRNA^{Lys}$ (anticodon C-U-U), whose primary structure has already been determined by Smith and coworkers [Smith, C. J., Teh, H. S., Ley, A., & D'Obrenan, P. (1973) *J. Biol. Chem. 248*, 4475–4485]. $tRNA_1^{Lys}$ is present in tRNA preparations extracted from RNase-treated mitochondria as well as from submitochondrial fractions, such as inner-membrane or mitosol preparations. It is absent in the mitochondrial outer-membrane preparation. These results show that the presence of $tRNA_1^{Lys}$ in mitochondria is not a result of cytoplasmic contamination and suggest strongly that it is imported from the cytoplasm. This cytoplasmic tRNA seems not to be involved in the transfer of lysine into mtDNA-coded polypeptide chains since (1) it is not acylated by the mitochondrial aminoacyl-tRNA ligase preparation and (2) mtDNA-coded mitochondrial $tRNA_2^{Lys}$ recognizes both lysine codons. It is postulated that the presence of $tRNA_1^{Lys}$ in yeast mitochondria results from active transport involving a specific receptor (other than mitochondrial lysyl-tRNA ligase). This tRNA may have a regulatory role, and its function could give an important insight into the relationship between the mitochondrial and the nuclear–cytoplasmic systems.

Mitochondria contain their own protein synthesizing system, whose components are of dual origin, mitochondrial, or nuclear. In yeast, mitochondrial tRNAs corresponding to the 20 amino acids are specified by the mitochondrial genome (Martin, N. C., et al., 1977; Martin, R. P., et al., 1977). The genes for 22 tRNA species have been mapped by hybridization to the mtDNA of a series of "petite" deletion mutants (Wesolowski & Fukuhara, 1979). tRNA isoacceptors have been detected by fractionation of total mitochondrial tRNA using RPC-5 column chromatography and two-dimensional polyacrylamide gel electrophoresis (Martin & Rabinowitz, 1978; Martin, R. P., et al., 1977; R. P. Martin, J. M. Schneller, A. P. Sibler, A. J. C. Stahl, and G. Dirheimer, unpublished experiments). The fractionated isoacceptors corresponding to methionine (Martin et al., 1976a; Martin, N. C., et al., 1977; Wesolowski & Fukuhara, 1979) and to threonine (Macino & Tzagoloff, 1979; Wesolowski & Fukuhara, 1979) have been shown to be transcription products of distinct mitochondrial tRNA genes. The number of mitochondrial tRNA cistrons being at least 30 (Martin, R. P., et al., 1977), isoacceptor tRNAs specific for some other amino acids may also derive from distinct mitochondrial tRNA genes. Despite a somewhat low number of mitochondrial tRNA cistrons to ensure proper response to all codons [for a discussion, see Martin, R. P., et al. (1977)], one needs not to postulate a massive import of nDNA-coded tRNAs to supplement mtDNA-coded mitochondrial tRNAs in yeast mitochondrial protein biosynthesis, as has been suggested to be the case in *Tetrahymena pyriformis* mitochondria by Chiu et al. (1975). Indeed, in a previous work we have reported that all but one yeast mitochondrial tRNA species fractionated by two-dimensional polyacrylamide gel electrophoresis are mtDNA-coded (Martin, R. P., et al., 1977). We suggested that the tRNA species which did not hybridize to mtDNA in our experiments was one of the two lysine tRNAs present in purified mitochondria preparations. The present report deals with the detailed study of yeast mitochondria associated lysine tRNA isoacceptors, especially the isoacceptor which does not hybridize to mtDNA.

Materials and Methods

Culture and Mitochondria Isolation. Yeast strains were a generous gift from Dr. H. Fukuhara. Wild-type *Saccharomyces cerevisiae* IL8-8C and the related mtDNA-less mutant IL8-8C/H71 were grown as previously reported (Schneller et al., 1976). Preparation of mitochondria from yeast protoplasts (Faye et al., 1974) and further purification on a sucrose gradient (Accoceberry & Stahl, 1972) were as described.

RNase Treatment of Mitochondria. Mitochondria purified from 100 g of yeast cells (wet weight) were suspended in 0.5 M Sorbitol and 10 mM Tris-HCl buffer (pH 7.4) containing 0.25 mg of pancreatic RNase (Worthington), 1000 units of T_1 RNase (Sankyo), and 0.5 mg of snake venom phosphodiesterase (Worthington). The mixture was incubated for 20 min at 23 °C, and mitochondrial RNA was directly phenol-extracted.

Lysis of Mitochondria. Preparation of Submitochondrial Fractions. Mitochondria obtained by differential centrifugation and several washes were lysed in phosphate buffer (pH 8) in the presence of 1 mM ATP and 20 mM $MgCl_2$ (Cassady & Wagner, 1971), and the submitochondrial fractions were separated on a discontinuous sucrose gradient as described by Accoceberry & Stahl (1972). Mitochondrial outer-membrane and inner-membrane fractions were recovered separately,

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diluted with 0.5 M Sorbitol and sedimented at 20000*g* for 30 min. RNA was phenol-extracted from both fractions. The supernatant of the discontinuous sucrose gradient was sedimented at 105000*g* for 2 h, and the resulting supernatant, called the "mitosol" fraction, was phenol-treated for RNA extraction.

Preparation of tRNA and Acylation Enzymes. tRNA Acylation. Preparation of unlabeled mitochondrial tRNA has been described by Schneller et al. (1975a). Isolation of in vivo ^{32}P-labeled mitochondrial tRNA has been reported previously (Martin, R. P., et al., 1977).

Preparation procedures of total-cell and mitochondrial aminoacyl-tRNA ligases (crude extracts) and tRNA aminoacylation have been described elsewhere (Accoceberry et al., 1973; Schneller et al., 1975b, 1976). Cytoplasmic tRNA was acylated by total-cell enzyme or postmitochondrial enzyme at pH 7.4 and mitochondrial tRNA was acylated by mitochondrial enzyme at pH 8 by using [^{14}C]lysine (sp act. 160 mCi/mmol; CEA Saclay) or [^{3}H]lysine (sp act. 23 Ci/mmol; Amersham/Searle).

tRNA Fractionation. Reversed-phase column chromatography on RPC-5 of lysyl-tRNAs was as previously described (Accoceberry et al., 1973).

Two-dimensional polyacrylamide gel electrophoresis of uncharged mitochondrial tRNA was according to Fradin et al. (1975), with minor modifications. The fractionated tRNAs were revealed by staining with methylene blue (0.2% in 0.2 M sodium acetate, pH 4.8). tRNAs were eluted by grinding the gel slices in 1 mL of extraction buffer (20 mM sodium acetate, pH 4.5, 0.3 M NaCl, and 10 mM $MgCl_2$) and by shaking for 3 h with 1 volume of buffer-saturated phenol. The aqueous phases were filtered on 10-mL Sephadex G-25 columns, eluted with water, and lyophilized prior to aminoacylation. Extraction from the gel and recovery of ^{32}P-labeled tRNAs^{Lys} were as described earlier (Martin et al., 1978).

tRNA–DNA Hybridization. DNA-loaded filters were a generous gift from Dr. H. Fukuhara. Hybridization experiments of [^{3}H]lysyl-tRNAs with mtDNA IL8-8C or nuclear DNA IL8-8C/H71 were performed as previously reported (Schneller et al., 1975c).

Ribosome Binding Tests. The trinucleotide A-A-G was prepared from a T_1 RNase hydrolysate of yeast tRNA^{Thr}, and the A-A-A triplet was purchased from Boehringer-Mannheim. Washed *Escherichia coli* ribosomes were gifts from Dr. J. Ramiasa. Binding experiments were performed following the procedure of Nirenberg & Leder (1964) in the presence of trinucleotide codons or alkali-treated poly(A,G) (gift from Dr. J. Weissenbach) in the conditions described in the legend to Table III. After incubation the samples were diluted with 3 mL of ice-cold 50 mM Tris–acetic acid (pH 7.2), 50 mM NH_4Cl, and 20 mM magnesium acetate and filtered on Sartorius SM 11406 nitrocellulose membranes, and the filters were washed with 10 mL of the same ice-cold buffer. After drying, we determined the radioactivity on the membranes in Omnifluor–toluene (4:1000 w/v).

tRNA Sequencing. Standard procedures (Barrell, 1971) were used for enzymic digestions, fingerprinting of in vivo ^{32}P-labeled tRNA$_1^{Lys}$, and also for the sequence analyses of the resulting oligonucleotides. Nucleotide compositions of the different oligonucleotides after hydrolysis with a mixture of T_1 and T_2 ribonucleases were determined as previously reported (Martin et al., 1978).

Results

Cochromatography of Mitochondrial and Cytoplasmic Lysyl-tRNAs. Two major isoacceptors are revealed in mitochondrial tRNA acylated with lysine by a total-cell ligase preparation, using the RPC-5 column chromatographic system (Figure 1). The mitochondrial tRNA$_1^{Lys}$ peak coincides with one of the cytoplasmic lysine isoacceptors, whereas mitochondrial tRNA$_2^{Lys}$ elutes distinctly from cytoplasmic tRNAs^{Lys}. Only the mitochondrial tRNA$_2^{Lys}$ peak is detected when mitochondrial tRNA is acylated with lysine by the mitochondrial ligase preparation (result not shown). This is confirmed by acylation to the plateau of the two lysine isoacceptors fractionated by preparative RPC-5 column chromatography of mitochondrial tRNA. Table I shows that mitochondrial tRNA$_1^{Lys}$ is not significantly acylated by the mitochondrial enzyme.

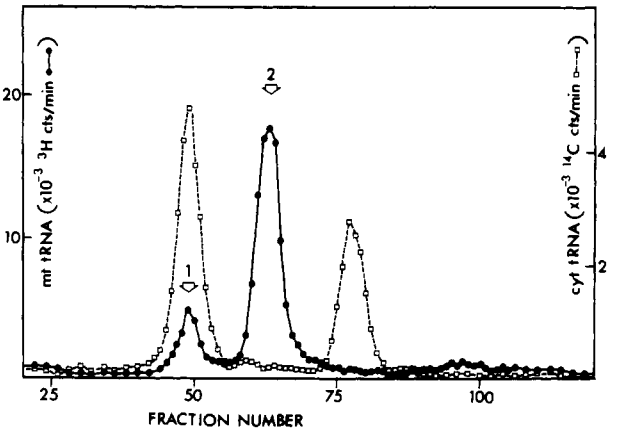

FIGURE 1: Chromatography in the RPC-5 system of cytoplasmic [^{14}C]lysyl-tRNA (□) and mitochondrial [^{3}H]lysyl-tRNA (●) on a 0.5 × 90 cm column with a linear gradient (400 mL) from 0.35 to 0.65 M NaCl in 10 mM sodium acetate buffer, pH 4.5, containing 10 mM $MgCl_2$. Fractions of 2.8 mL were collected at a flow rate of 0.7 mL min^{-1}.

Table I: Extent of Aminoacylation of tRNA$_1^{Lys}$ and tRNA$_2^{Lys}$ Carried Out with an Excess of Mitochondrial or Total-Cell Ligase Preparations

ligase prepn	[^{14}C]lysyl-tRNA formed[a]	
	tRNA$_1^{Lys}$	tRNA$_2^{Lys}$
mitochondrial ligase	2.4	200.0
total-cell ligase	218.0	184.0

[a] Results are expressed in pmol per A_{260} unit tRNA.

Table II: Transcriptional Origins of tRNA^{Lys} Isoacceptors Isolated from Mitochondria[a]

[^{3}H]lysyl-tRNA input	mtDNA ρ^+IL8-8C (cpm)[b]	nDNA ρ^0IL8-8C/H71 (cpm)[b]	blank (cpm)
Lys 1: 8500 cpm	2	21	27
Lys 2: 9200 cpm	98	1	31
cytoplasmic tRNA: 12000 cpm (unfractionated)	−2	17	34

[a] [^{3}H]Lysyl-tRNAs were recovered after RPC-5 chromatography and hybridized with 10 μg of mtDNA or 20 μg of nuclear DNA. [b] Counts per minute above blank values.

Hybridization of Mitochondrial Lysyl-tRNAs. Hybridization of [^{3}H]lysyl-tRNAs (isolated peaks) with mtDNA shows that mitochondrial tRNA$_2^{Lys}$ hybridizes to saturation, whereas no significant hybridization with mtDNA was observed for the tRNA$_1^{Lys}$ isoacceptor. Table II summarizes the values of the different hybridization plateaus obtained. A very low level of hybridization to nuclear DNA is obtained with tRNA$_1^{Lys}$ or with cytoplasmic lysyl-tRNA (unfractionated).

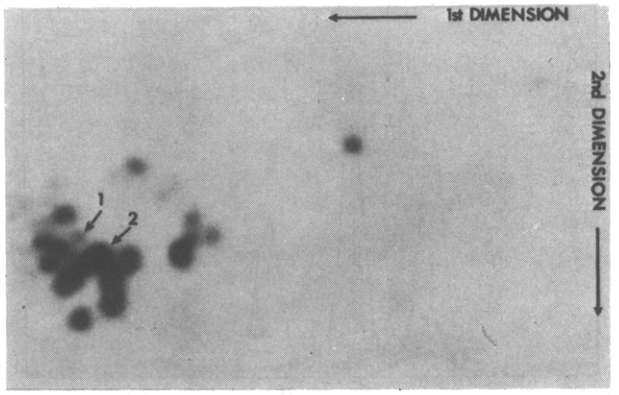

FIGURE 2: Two-dimensional polyacrylamide gel electrophoretic pattern of in vivo ^{32}P-labeled mitochondrial tRNA. Arrows indicate the positions of $tRNA_1^{Lys}$ (1) and $tRNA_2^{Lys}$ (2) [compare with Figures 3 and 4 in Martin, R. P., et al. (1977)].

Table III: Binding Specificities of $tRNA_1^{Lys}$ and $tRNA_2^{Lys}$ to *E. coli* Ribosomes in Response to Lysine Codons[a]

trinucleoside diphosphate or poly-nucleotide	[^{14}C]lysyl-tRNA bound[b]			
	$tRNA_1^{Lys}$	$tRNA_2^{Lys}$	mt tRNA[c]	cyt tRNA[d]
A-A-A	0.10	0.37	0.28	0.68
A-A-G	1.45	0.41	0.61	1.24
poly(A,G) (1:1.5)	3.48	1.52	1.51	2.51
none	(0.29)	(0.21)	(0.47)	(0.89)

[a] Incubation mixture: 50 μL of 50 mM Tris–acetic acid (pH 7.2), 50 mM NH_4Cl, and 20 mM magnesium acetate containing 1.3 A_{260} units of ribosomes; 0.4 A_{260} unit of A-A-A or 0.5 A_{260} unit of A-A-G or poly(A,G) (5 nmol of phosphate); 15.2 pmol of $tRNA_1^{Lys}$ or 12.8 pmol of $tRNA_2^{Lys}$ or 19.4 pmol of bulk mitochondrial tRNA or 38 pmol bound per assay above background (none). [c] mt tRNA = mitochondrial tRNA (unfractionated). [d] cyt tRNA = cytoplasmic tRNA (unfractionated).

Analysis by two-dimensional polyacrylamide gel electrophoresis of the $tRNA_1^{Lys}$ fraction (isolated peak from preparative RPC-5 column chromatography, acylated with lysine and coelectrophoresed with total mitochondrial tRNA) demonstrates that this tRNA maps in the position of spot X tRNA [see Figure 3 in Martin, R. P., et al. (1977) and compare with Figure 2 in this paper]. This tRNA was shown to be the only tRNA species present in highly purified yeast mitochondria which did not hybridize with mtDNA.

Coding Properties of the Two tRNAs^{Lys} Associated with Yeast Mitochondria. $tRNA_1^{Lys}$ and $tRNA_2^{Lys}$ have been tested for their abilities to bind *E. coli* ribosomes in response to the trinucleoside diphosphate codons of lysine. The results, summarized in Table III, show that the coding pattern for the two mitochondria-associated lysine isoacceptors is different. $tRNA_1^{Lys}$ shows binding to A-A-G, but little or no response to A-A-A, whereas $tRNA_2^{Lys}$ exhibits weaker but significant response to both the codons A-A-G and A-A-A. The binding data of $tRNA_1^{Lys}$, $tRNA_2^{Lys}$, or unfractionated mitochondrial lysyl-tRNAs in response to poly(A,G) are in agreement with these results.

Sequence Analysis of $tRNA_1^{Lys}$. $tRNA_1^{Lys}$ was isolated by two-dimensional polyacrylamide gel electrophoresis of in vivo ^{32}P-labeled mitochondrial tRNA (Figure 2).

Figure 3 shows the two-dimensional electrophoretic separation of exhaustive pancreatic RNase (a) and T_1 RNase (b) digestion products. The fingerprints are identical with those obtained for cytoplasmic $tRNA_1^{Lys}$ from haploid yeast (strain α S288C) by Smith et al. (1973). Nucleotide compositions,

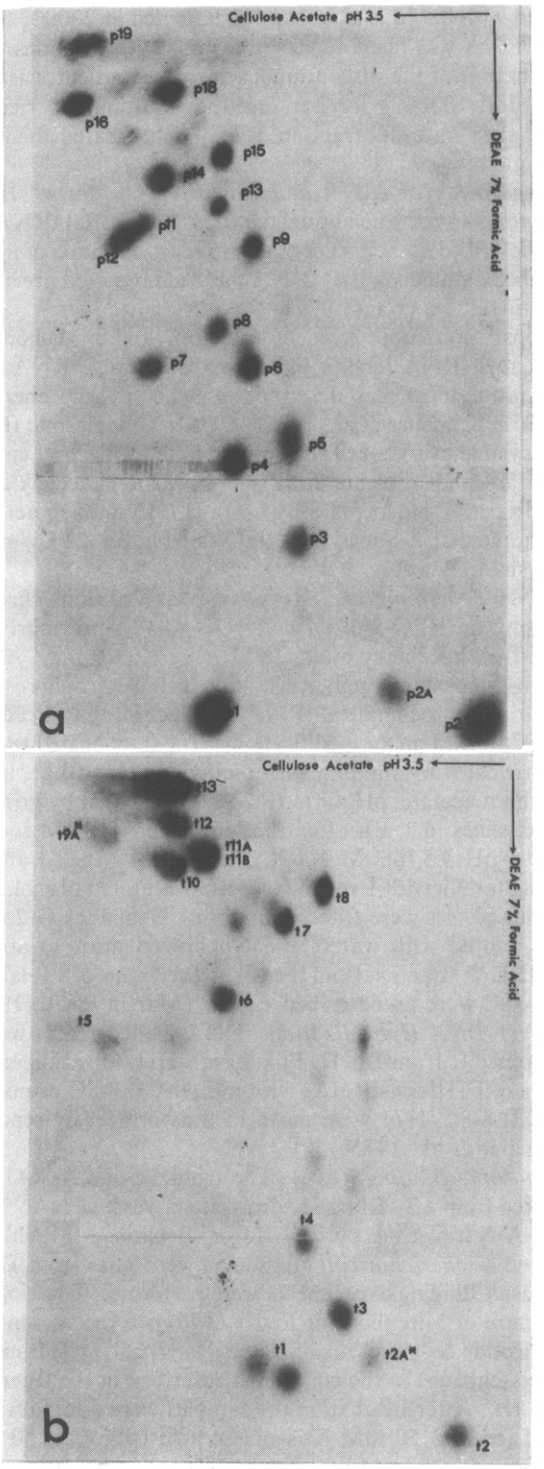

FIGURE 3: Autoradiograms of the pancreatic RNase (a) and T_1 RNase (b) fingerprints of in vivo ^{32}P-labeled $tRNA_1^{Lys}$. The numbering of the spots is the same as that used by Smith et al. (1973). (*) These oligonucleotides are absent on the fingerprints published by these authors. Oligonucleotides p10 and t9 present in Smith et al. (1973) fingerprints are absent.

molar yields, and partial nucleotide sequences of the pancreatic and T_1 RNases resulting oligonucleotides listed in Table IV are consistent with the previously determined sequences. These results show unambiguously that $tRNA_1^{Lys}$ associated with yeast mitochondria corresponds to the nuclear DNA-coded $tRNA^{Lys}$ (anticodon C-U-U), whose cloverleaf structure is shown in Figure 4.

Presence of Cytoplasmic $tRNA_1^{Lys}$ inside Yeast Mitochondria. To find out whether cytoplasmic $tRNA_1^{Lys}$ is found

Table IV: Oligonucleotides Produced by Complete Digestion of tRNA$_1^{Lys}$ with Pancreatic and T_1 RNases

pancreatic RNase end products				T_1 RNase end products			
		molar ratio[b]				molar ratio[b]	
spot no.	sequence[a]	obsd	exptd	spot no.	sequence[a]	obsd	exptd
p1	{Up, ψp}	9.30	{8, 1}	t1	{Gp, m^{2}Gp}	5.25 0.65	6 1
p2	Cp	10.25	11	t2	C-U-C-C-(A_{OH})	0.70	1
p2A	A-Cp	1.05	1	t2A	C-m$_2^2$G>	0.30[e]	–
p3	G-Cp	1.65	2				
p4	A-Up	2.10	2	t3	C-Gp	2.10	2
p5	{A-G-Cp, G-A-Cp}	1.50	{1, 1}	t4	A-Gp	1.05	1
				t5	pGp	0.75	1
p6	A-A-Dp	1.00	1	t6	D-A-Gp	0.90	1
p7	m$_2^2$G-ψp	0.75	1	t7	C-A-A-D-C-Gp	0.75	1
p8	m^{2}G-m^{2}G-Cp	0.65	1	t8	C-C-C-C-C-U-A-C-A-Gp	0.70	1
p9	G-m^{1}A-G-Cp	0.40[c]	–	t9	U-U-Gp	nf[d]	–
p10	G-m^{2}G-Cp	nf[d]	–	t9A	U-U-m^{2}Gp	0.85	1
p11	G-Up	1.20	1	t10	T-ψ-C-Gp	1.05	1
p12	pG-Cp	0.85	1	t11A	C-C-U-U-Gp	1.80	{1, 1}
p13	G-A-G-Cp	0.70	1	t11B	m^{7}G-U-U-A-Gp		
p14	t^{6}A-A-ψp	0.85	1	t12	C-m$_2^2$G-ψ-A-U-Gp	0.75	1
p15	A-A-G-m^{7}G-Up	0.65	1	t13	A-C-U-C-U-U-t^{6}A-A-ψ-C-A-U-A-A-Gp	1.05	1
p16	G-G-Dp	1.10	1				
p18	A-G-G-G-Cp	0.75	1				
p19	A-G-G-G-G-Tp	0.65	1				

[a] The base compositions of the oligonucleotides were determined and complementary digestions were done with T_1 RNase (oligonucleotides p5, p9, p13, p15, p18, and p19), pancreatic RNase (oligonucleotides t6–t8 and t11–t13, and U_2 RNase (oligonucleotides t7, t8, and t11–t13). Oligonucleotide sequences were deduced by comparison with the results published by Smith et al. (1973). [b] Obsd, observed; exptd, expected. [c] Results from partial methylation of the adenylic residue of oligonucleotide p13. [d] nf, not found. [e] Results from incomplete splitting of oligonucleotide t12.

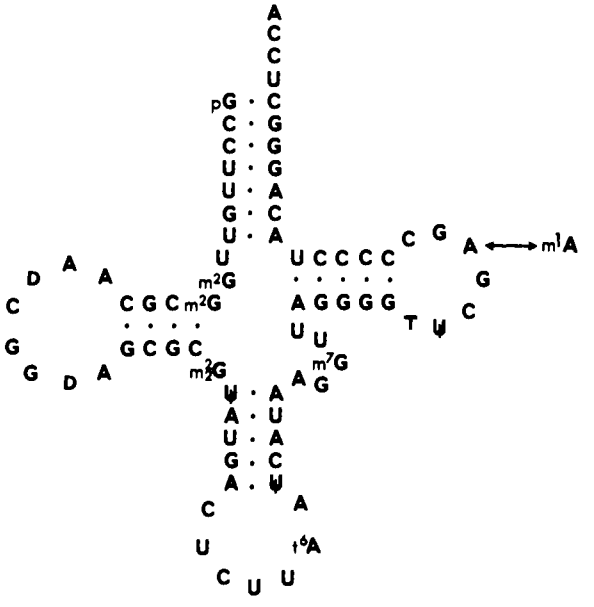

FIGURE 4: Cloverleaf structure of cytoplasmic tRNA$_1^{Lys}$ present in yeast mitochondria.

in purified mitochondria preparations because of a special adherence to the outer membrane or whether it is in fact specifically imported into the organelle, we extracted mitochondrial tRNA from RNase-treated mitochondria (see Materials and Methods). After fractionation by two-dimensional polyacrylamide gel electrophoresis, the tRNA^{Lys} spots (for their location, see Figure 2) were tested for lysine acceptor activity. Table V shows that the amount of tRNA$_1^{Lys}$ in RNase-treated mitochondria is comparable to that found in nontreated mitochondria. Furthermore, this tRNA is also found in tRNA extracted from the mitosol fraction as well as from the inner-membrane fraction (Table V). Analysis of [^{3}H]lysyl-tRNA by RPC-5 column chromatography showed invariably the presence of the tRNA$_1^{Lys}$ peak besides the

Table V: Presence of Cytoplasmic tRNA$_1^{Lys}$ in Mitochondrial tRNA Extracted from Mitochondria after RNase Treatment and from Submitochondrial Fractions

	[^{3}H]lysyl-tRNA formed[a]	
tRNA source	tRNA$_1^{Lys}$ [b]	tRNA$_2^{Lys}$ [b]
nontreated mitochondria (control)	4600	24 500
RNase-treated mitochondria	3950	23 300
mitosol fraction	4800	26 700
inner-membrane fraction	4700	26 100
none	(1100)	(900)

[a] Results are expressed in counts per minute above background (none) and correspond to the amounts of tRNA$_1^{Lys}$ and tRNA$_2^{Lys}$ recovered after two-dimensional polyacrylamide gel fractionation of 120 μg each time. Acylation of tRNA$_1^{Lys}$ was by whole-cell enzyme at pH 7.4, and acylation of tRNA$_2^{Lys}$ was by mitochondrial enzyme at pH 8.0. [b] See Figure 2.

mtDNA-coded mitochondrial tRNA$_2^{Lys}$ peak in the tRNA preparations from the different mitochondrial fractions (results not shown). Furthermore, no RNA could be detected in the outer-membrane fraction. These results strongly suggest that cytoplasmic tRNA$_1^{Lys}$ is in fact imported into yeast mitochondria.

Discussion

S. cerevisiae mitochondria contain two lysine tRNAs, only one (tRNA$_2^{Lys}$) of which hybridizes with mtDNA. tRNA$_1^{Lys}$ comigrates with one of the cytoplasmic tRNAs^{Lys} but does not result from cytoplasmic tRNA contamination for the following reasons. (1) RPC-5 column chromatography of several mitochondrial aminoacyl-tRNAs showed no other cytoplasmic tRNAs in our mitochondrial tRNA preparations (Schneller et al., 1975a,c; Martin et al., 1976a; R. P. Martin, J. M. Schneller, A. P. Sibler, A. J. C. Stahl, and G. Dirheimer, unpublished experiments). (2) Two-dimensional polyacrylamide gel electrophoresis of total mitochondrial tRNA showed

that all but 5–6 mitochondrial tRNAs migrated distinctly from their cytoplasmic counterparts. Furthermore, the gel pattern obtained with mitochondrial tRNAs eluted from mitochondrial DNA–tRNA hybrids was identical with the one obtained with nonhybridized mitochondrial tRNAs, except for one spot (Martin, R. P., et al., 1977). In this paper we show that $tRNA_1^{Lys}$ maps in this spot (Figure 2). (3) This tRNA is present in RNase-treated mitochondria as well as in submitochondrial preparations such as mitosol or inner-membrane fractions. The ratio of $tRNA_1^{Lys}$ vs. $tRNA_2^{Lys}$ is the same as that in mitochondrial tRNA extracted from untreated mitochondria. However, $tRNA_1^{Lys}$ is absent in the outer-membrane preparation. This excludes the possibility that this tRNA may be protected against RNase degradation by tight binding to some structure present on the outer side of the mitochondria and indicates that it is present inside the organelle. The presence of mitochondrial tRNAs in the inner-membrane fraction is consistent with the general belief that mitochondrial ribosomes are tightly bound to the inner-membrane of yeast mitochondria.

The fractionation pattern of cytoplasmic and mitochondrial lysyl-tRNAs obtained by RPC-5 column chromatography in the present experiments is quite different from the elution profiles published by Martin & Rabinowitz (1978). The chromatographic mobilities of both the cytoplasmic and mitochondrial $tRNA^{Lys}$ species show very different patterns. In addition, only the mtDNA-coded mitochondrial $tRNA^{Lys}$ was detected by these authors because they analyzed mitochondrial tRNA acylated by lysine with use of a mitochondrial ligase preparation. The difference in acylation of $tRNA_1^{Lys}$ using mitochondrial or total-cell ligases shows that mitochondrial and cytoplasmic lysyl-tRNA ligases must be distinct enzymes. As has been shown for several other yeast aminoacyl-tRNA ligases (Schneller et al., 1976), cytoplasmic lysyl-tRNA ligase is not found in the mitochondria. This in turn implies that $tRNA_1^{Lys}$ could not be acylated inside the organelle.

T_1 and pancreatic RNase fingerprints of $tRNA_1^{Lys}$ and analyses of the resulting oligonucleotides were sufficient to show that $tRNA_1^{Lys}$ is identical with a cytoplasmic lysine tRNA, i.e., $tRNA^{Lys}$ (anticodon C-U-U) whose primary structure was determined by Smith et al. (1973). The only difference from the previously published sequence is that the guanosine residue in position 9 was found to be fully modified to m^2G in our $tRNA_1^{Lys}$. The G + C content of this tRNA (55%) is much higher than those of the mtDNA-coded mitochondrial tRNA species (Martin, R. P., et al., 1977), and it contains rare nucleosides such as m^7G and m^1A which are absent in yeast mitochondrial tRNAs (Martin et al., 1976b).

The coding pattern of $tRNA_1^{Lys}$ is in agreement with the previously published results (Smith et al.,1973) indicating that this tRNA recognizes only the A-A-G lysine codon. But $tRNA_1^{Lys}$ translates both A-A-A and A-A-G codons (Mitra, 1978). As shown for other tRNAs, negative results in ribosome binding experiments with one codon do not exclude the possibility of translation of this codon (Weissenbach et al., 1977). On the other hand, mtDNA-coded $tRNA_2^{Lys}$ recognizes, although weakly, both A-A-G and A-A-A lysine codons. This weaker binding is perhaps due to the fact that the experiments were done by using *E. coli* ribosomes. Macino & Tzagoloff (1979), for yeast mitochondrial $tRNA_1^{Thr}$, and Chiu et al. (1975), for *Tetrahymena* mitochondrial $tRNA^{Phe}$, observed very little or no binding in the presence of the appropriate codons using *E. coli* ribosomes. It could be that optimal triplet-codon binding of organellar tRNAs can only be observed by using homologous ribosome preparations. Yeast mitochondrial $tRNA^{Phe}$ (Martin et al., 1978), as well as other mitochondrial tRNAs from yeast (our unpublished experiments) or from *Neurospora crassa* (Heckman et al., 1979), shows original structural features, which may hinder proper recognition in heterologous systems. In any case, taking into account the coding properties of mitochondrial $tRNA_2^{Lys}$, $tRNA_1^{Lys}$ does not seem to be necessary for the transfer of lysine into mtDNA-coded polypeptide chains, unless it plays a role in promoting efficient mitochondrial protein biosynthesis. However, since cytoplasmic lysyl-tRNA ligase is absent in mitochondria, the participation of lysyl-$tRNA_1^{Lys}$ in mitochondrial protein synthesis would require import of this tRNA in an acylated state and a high exchange rate between uncharged $tRNA_1^{Lys}$ from the mitochondria and charged $tRNA_1^{Lys}$ from the cytoplasm. Suyama & Hamada (1976) proposed a model for the transport of nDNA-coded tRNAs into *Tetrahymena* mitochondria involving their cognate aminoacyl-tRNA ligases. This mechanism is ruled out for the import of $tRNA_1^{Lys}$ unless mitochondrial lysyl-tRNA ligase binds this cytoplasmic tRNA without acylating it. Several authors showed the possibility of a passive entry of ribonucleic acids into isolated mitochondria from various sources (Swanson, 1971; Grivell & Metz, 1973; Dimitriadis & Georgatsos, 1974). We suggest, however, since $tRNA_1^{Lys}$ is the only cytoplasmic tRNA found in yeast mitochondria, that an active transport mechanism using a specific receptor could be involved. This receptor would be highly specific for cytoplasmic $tRNA_1^{Lys}$ whereas lysyl-tRNA synthetase interacts easily with both cytoplasmic lysine-tRNA isoacceptors. An alternative hypothesis could be entry of $tRNA_1^{Lys}$ as a complex with some protein whose functioning would be promoted by this tRNA.

tRNAs are known to have other functions in addition to the transfer of amino acids into polypeptide chains. On one hand, a $tRNA^{Lys}$ isoacceptor from mouse cells seems to be implicated in regulation of cell division (Raba et al., 1979). On the other hand, host-cell $tRNA^{Trp}$ and $tRNA^{Pro}$ have been found to serve as primers for the initiation of animal virus RNA-directed DNA synthesis [for a review, see Waters & Mullins (1977)]. By analogy, one could imagine that yeast nDNA-coded $tRNA_1^{Lys}$ could participate in conjunction with a nDNA-coded protein in mtDNA replication or transcription. The following are alternative hypotheses: One hypothesis is a primer function for replication of yeast viruses inside mitochondria. Though viruses have been demonstrated in yeast (Oliver et al., 1977), viruslike particles have only been postulated in *N. crassa* mitochondria (Küntzel et al., 1974). Another hypothesis is a regulation function for coordinate division of cell and mitochondria. The function of cytoplasmic $tRNA_1^{Lys}$ in yeast mitochondria deserves further investigation.

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Amino Acid Sequence of the Ribosomal Protein L21 of *Escherichia coli*†

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ABSTRACT: The primary structure of protein L21 from the 50S subunit of *Escherichia coli* ribosomes has been completely determined by sequencing the peptides obtained by digestion of L21 with trypsin before and after modification of the arginine residues with 1,2-cyclohexanedione, *Staphylococcus aureus* protease, thermolysin, and pepsin. Automated Edman degradation using a liquid-phase sequenator was carried out on the intact protein as well as on a fragment arising from cleavage with cyanogen bromide. Protein L21 consists of a single polypeptide chain of 103 amino acids of molecular weight 11 565. An estimation of the secondary structure of protein L21 and a comparison with other *E. coli* ribosomal protein sequences are presented.

Protein L21 is part of the large subunit of *Escherichia coli* ribosomes. The protein L21 does not bind directly to ribosomal RNA but is bound to the 23S RNA in the presence of L20 (Roth and Nierhaus, unpublished experiments). The protein is located in the neighborhood of protein L17 as established by cross-linking experiments (Kenny & Traut, 1979). It belongs to a group of six proteins present in a ribonucleoprotein core remaining after controlled trypsin and RNase digestion of the 50S subunit (Kühlbrandt & Garrett, 1978).

In reconstitution experiments it was found that the 50S particle without protein L21 has full activity in the poly(U)-dependent poly(Phe) synthesis (Spillman et al., 1977). It was also found that protein L21 is not essential for the peptidyltransferase activity (Schulze and Nierhaus, unpublished experiments).

Using a mutant with an altered protein L21 (Dabbs, 1978), it was recently possible to map the gene of this protein at 68 min of the *E. coli* chromosome (Kitakawa et al., 1979; Takata, 1978).

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