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Mitochondrial Transfer RNAs in Yeast: Identification of Isoaccepting Transfer RNAs†

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ABSTRACT: To delineate the total number of tRNAs encoded by yeast mitochondrial DNA (mtDNA), we have examined mitochondrial tRNA preparations for the presence of heterogenic isoaccepting tRNAs. Analyses of ^{3}H-labeled aminoacylated mitochondrial tRNAs by reversed-phase column chromatography (RPC-5) coupled with hybridization to mtDNA detected only one major mitochondrially coded tRNA for alanine, arginine, aspartic acid, glycine, histidine, isoleucine, leucine, lysine, proline, serine, and tryptophan. Some of these profiles also contained one or more minor peaks that may represent small amounts of heterogenic isoacceptors, but their low concentrations prevented their characterization. Cysteinyl-, methionyl-, phenylalanyl-, threonyl-, tyrosyl-, and valyl-tRNAs separated into multiple species upon RPC-5 chromatography. Two cysteinyl-, two methionyl-, two phenylalanyl-, two threonyl-, four tyrosyl-, and two valyl-tRNA species hybridized to mtDNA. The hybridization of the phenylalanyl- and the valyl-tRNA species was not additive, indicating that their sequences are similar, if not identical, and suggesting that they may be transcribed from the same genes. The two methionyl-, and two threonyl-, and probably the two cysteinyl-tRNAs are transcribed from separate genes, since their hybridizations to mtDNA are additive. That the methionyl-tRNAs are transcribed from separate genes was further confirmed through deletion mapping experiments which showed that the genes coding for these tRNAs are at different locations on the mtDNA. The transcriptional relationship of the four tyrosyl-tRNAs was not established. There is at least a cistron coding for tRNAs corresponding to each of the common amino acids except asparagine and several amino acids (methionine, threonine, and cysteine) are accepted by more than one transcriptionally distinct tRNA. Thus, a minimum of 22 tRNA cistrons have been identified.

The mitochondrion contains its own protein-synthetic system, which is distinct from that found in the cytoplasm and presumably serves to translate mitochondrially coded components of the respiratory system (for review, see Locker & Rabinowitz, 1978). The mitochondrial protein-synthetic system is of dual origin, since most, if not all, of the ribosomal proteins are encoded by nuclear genes whereas rRNA is specified by the organelle genome. Although it has been established that mtDNA codes for some tRNAs, it is not yet known whether the mitochondrial protein-synthetic system is autonomous with respect to its tRNA complement or whether nuclear-coded tRNAs must be imported to supply a complete set of tRNAs for organelle protein synthesis, as has been suggested to be the case for *Tetrahymena* by Chiu et al. (1975).

Estimates of the number of tRNA cistrons in mtDNA have varied with experimental procedure. Early estimates based on saturation-hybridization experiments with bulk labeled 4S RNA indicated the presence of 12–15 tRNA cistrons in HeLa cells and *Xenopus* (Aloni & Attardi, 1971; Dawid & Chase, 1972) to 20–25 in yeast (Reijnders & Borst, 1972; Schneller et al., 1975a,b). Though these estimates raised the possibility that mtDNA may not code for tRNAs for all the 20 common amino acids, more recent experiments using hybridization of [^{3}H]aminoacyl-tRNAs have demonstrated the presence of mt-coded tRNAs in HeLa mitochondria corresponding to 16 amino acids (Lynch & Attardi, 1976) and in yeast corresponding to 19 amino acids (Martin et al., 1977). Since the

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[^{3}H]aminoacyl-tRNA preparations may consist of a mixture of isoaccepting species, the actual number of tRNA cistrons may be greater than the number of amino acids for which tRNAs have been identified. Indeed, Lynch & Attardi (1976) have demonstrated the presence of two seryl-tRNA cistrons in HeLa mtDNA, bringing estimates of HeLa mitochondrial tRNA cistrons to a minimum of 17.

If all the sense codons are to be recognized during mitochondrial protein synthesis, then many amino acids must have more than one corresponding tRNA. In addition, redundant tRNAs which respond to the same codon, but are structurally distinct, have been identified in other systems and may well occur in mitochondria. In order to establish the total number of mitochondrially encoded tRNAs, we have analyzed yeast mitochondrial tRNAs acylated with radioactive amino acids by reversed-phase column chromatography and tRNA–mtDNA hybridization. In most cases, we have cochromatographed the mitochondrial tRNAs with differentially labeled cytoplasmic tRNAs isolated from a strain containing no mtDNA. We have identified mitochondrial tRNAs that chromatograph differently from corresponding cytoplasmic tRNAs, and we have ascertained their mitochondrial origin by hybridization studies. Some aminoacyl-tRNAs yielded more than one peak of radioactivity distinct from their cytoplasmic counterparts. In several cases, these peaks showed additive hybridization to mtDNA, indicating that they are transcribed from separate genes. Furthermore, we were able to demonstrate, by using the petite mutant deletion mapping technique (Fukuhara et al., 1976), that the isoaccepting methionyl-tRNAs hybridize to separate regions of the genome.

Methods

Isolation of Mitochondrial DNA and Nuclear DNA. Mitochondria were prepared from protoplasts, treated with pancreatic DNase and washed and lysed as described by Casey et al. (1972). Following lysis, the suspension was deproteinized with chloroform/methanol and the DNA purified on hydroxylapatite columns (Casey et al., 1972). The mtDNA obtained was free of detectable nuclear DNA contamination when analyzed by isopycnic CsCl centrifugation in the analytical ultracentrifuge.

An mtDNA-less petite strain, IL8-8C/HF-71 (Casey et al., 1974), was broken in a Braun homogenizer and nuclear DNA was isolated from the 2000*g* pellet by the procedure used for mtDNA.

Isolation and Acylation of tRNA. Mitochondria were obtained from glusalase-treated cells and were washed four times in 0.8 M sorbitol, 0.1% bovine serum albumin, according to the procedure of Casey et al. (1972). Mitochondrial tRNAs were isolated and acylated with mitochondrial synthetase as previously described (Martin et al., 1977). Cytoplasmic tRNA was isolated from an mtDNA-less strain (IL8-8C/HF-71) (Casey et al., 1974); cells were broken in a Braun glass bead mill. For acylation of cytoplasmic tRNAs, we used whole-cell synthetase preparations in reaction mixtures containing ATP and $MgCl_2$ concentrations known to be optimal for their mitochondrial counterparts (Martin et al., 1977).

Reversed-Phase Column Chromatography. For analysis of tRNA, reversed-phase column chromatography on RPC-5 (Miles Laboratories) was used, essentially as described by Pearson et al. (1971). Acylated tRNAs were loaded on a 0.63 × 24 cm column equilibrated with 10 mM ammonium acetate (pH 4.6), 10 mM $MgCl_2$, 0.2 mM dithiothreitol, and NaCl at a concentration corresponding to the starting concentration of the salt gradient. The tRNAs were eluted with a 200-mL linear salt gradient (0.3–0.7 M for alanyl-tRNAs; 0.4–0.7 M arginyl-, aspartyl-, cysteinyl-, glutamyl-, histidyl-, isoleucyl-, leucyl-, methionyl-, prolyl-, threonyl-, and valyl-tRNAs; 0.4–0.8 M for phenylalanyl-, seryl-, glycyl-, and tryptophanyl-tRNAs; and 0.6–0.9 M for tyrosyl-tRNAs). For preparative work, aliquots of each fraction were counted; those fractions containing aminoacyl-tRNAs were pooled, and the tRNA was collected by ethanol precipitation or absorbed onto a 1-cm DEAE-cellulose column and eluted with 0.8 M NaCl.

TABLE I: Transcriptional Origins of Mitochondrial tRNAs Comigrating with Cytoplasmic tRNAs.[a]

tRNA	Input counts	Blank (cpm)	mtDNA (cpm)	cpm hybridized
Asp I	4040	24	29	5
Cys II	5160	76	78	2
Lys	2450	26	178	152
Met I	2270	13	12	−1
Met IV	3140	15	17	2
Phe III	3390	20	21	1
Ser I	4700	15	14	−1
Tyr II	6310	41	171	130
Val I	9260	83	260	177

[a] tRNAs were recovered after RPC-5 chromatography and hybridized to mtDNA as described in Methods.

Hybridization was carried out essentially as described by Martin et al. (1976a). Filters containing 20 μg of wild type or petite DNA were incubated in the presence of aminoacyl-tRNAs in a reaction mixture containing 50 mM ammonium acetate, 2 × SSC (SSC is 0.15 M NaCl, 0.015 M sodium citrate), and 33% formamide. The reaction volume used was 0.1 mL per filter. Hybridization proceeded for 4 h at 33 °C. Filters were washed in 2 × SSC, treated with T1 ribonuclease (5 μg/mL, 30 min, 37 °C), washed again in 2 × SSC, and incubated for 50 min at 33 °C in 33% formamide, 2 × SSC, and 50 mM ammonium acetate. Following a final wash, the filters were dried and counted in toluene-based scintillation fluid.

Results

The results of RPC-5 chromatography of mitochondrial and cytoplasmic tRNAs acylated in vitro with 18 different labeled amino acids are shown in Figures 1 and 2. The cytoplasmic tRNA was isolated from a strain containing no mtDNA (Casey et al., 1974) and thus contained no mitochondrially specified tRNAs. Nine mitochondrial aminoacyl-tRNA preparations (alanine, arginine, glycine, histidine, isoleucine, leucine, lysine, proline, and tryptophan) showed only one major peak by RPC-5 chromatography (Figure 1). In the case of alanine, arginine,[1] glycine, histidine, isoleucine, leucine, and tryptophan, this peak migrated differently than did the nuclear-coded tRNAs for that amino acid. Because we were unable to acylate cytoplasmic tRNA preparations with proline, we could not compare the mobility of the mitochondrial and cytoplasmic species; hybridization experiments with the prolyl-tRNA, however, establish its mitochondrial origin. The mitochondrial lysyl-tRNA cochromatographed with a cytoplasmic tRNA, but its mitochondrial origin was ascertained by hybridization (Table I). Thus, comigration of tRNA isolated from mitochondria with a cytoplasmic tRNA does not necessarily indicate a nuclear origin. Several of these mito-

[1] The size of the reproduction makes it difficult to discern that the major arginine mt tRNA peak consistently eluted one fraction earlier than the second arginine cytoplasmic tRNA.

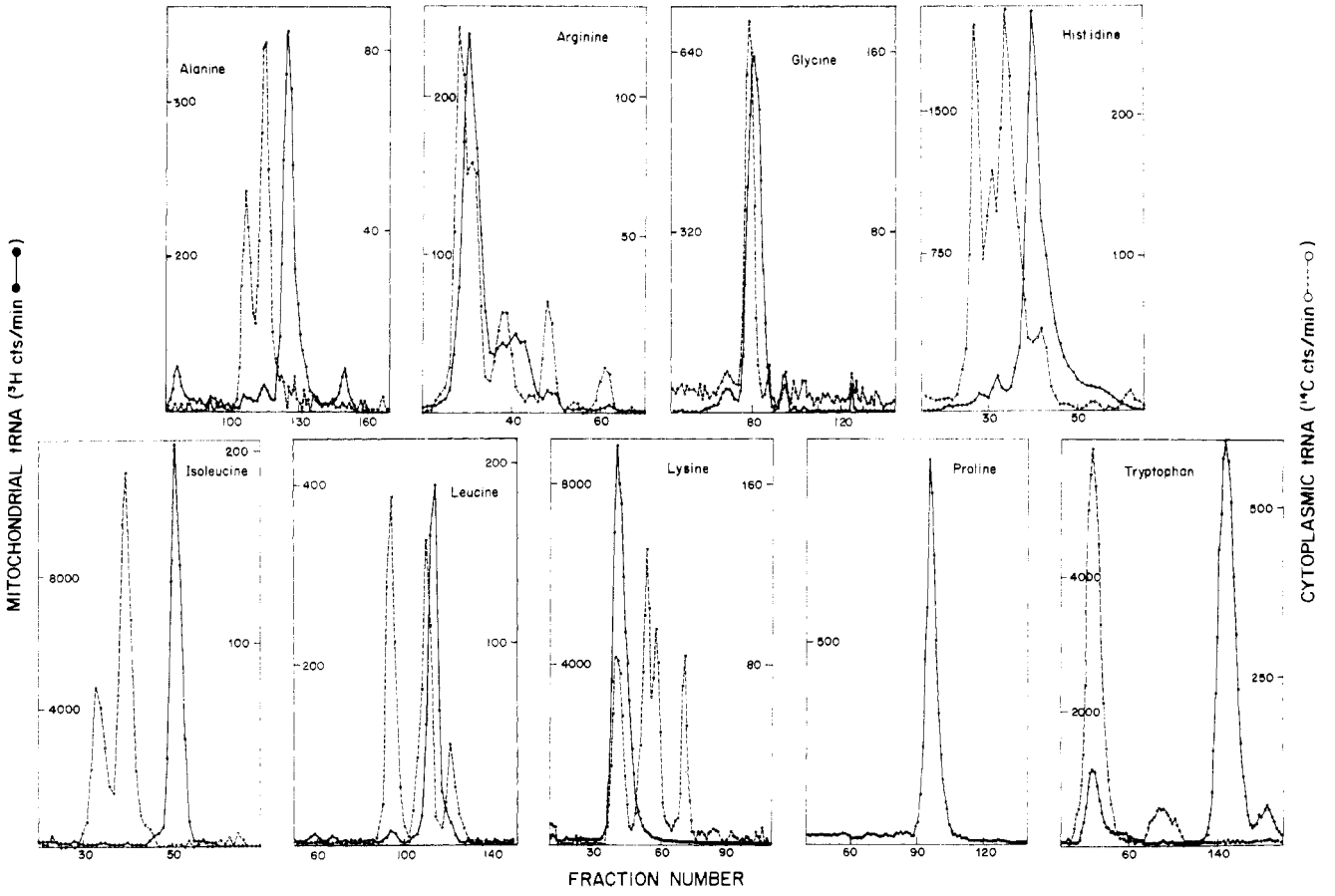

FIGURE 1: RPC-5 chromatographs of [^{14}C]cyt (○ - - ○) and [^{3}H]mt (●—●) tRNAs containing only one major aminoacyl-tRNA derived from mitochondrial tRNA preparations.

chondrial tRNA preparations (alanine, arginine, glycine, and tryptophan) showed one or more minor peaks (Figure 1), some of which did not cochromatograph with cytoplasmic tRNA. Because of difficulties in obtaining enough material to do hybridization studies, we have not yet demonstrated that these species are encoded by mtDNA.

Other mitochondrial tRNA preparations (Figure 2) (aspartic acid, serine, and valine) showed one peak that migrated with a mobility distinct from nuclear-coded tRNA and a second peak that migrated with a nuclear-coded tRNA. Several other profiles (cysteine,[2] methionine, phenylalanine, and tyrosine; Figure 2) contained more than one peak with a mobility that differed from nuclear-coded tRNA, but they also had peaks that migrated with a cytoplasmic species. In order to determine whether those peaks which comigrated with nuclear-coded tRNAs were due to the presence of cytoplasmic tRNAs in our mitochondrial preparations or whether they represented comigrational mitochondrially coded species, we performed hybridization experiments. Table I shows that the peaks that comigrate with a cytoplasmic species in the aspartyl-, seryl-, cysteinyl-, methionyl-, and phenylalanyl-tRNA profiles do not hybridize to mtDNA. Furthermore, the levels of cytoplasmic tRNAs varied in different mitochondrial tRNA preparations, suggesting that they may result from nonspecific contamination of our mitochondria pellets with nuclear RNA. The valyl and tyrosyl species which comigrate with a cytoplasmic component do hybridize to mtDNA (Table I). That these species are encoded by mtDNA is further confirmed by the fact that their hybridization was completed with cold mitochondrial tRNA, but not cold cytoplasmic tRNA (data not shown).

Finally, both the glutamyl and the threonyl profile showed two mitochondrial species which migrated distinct from cytoplasmic species (Figure 2). As we reported previously, the two tRNAs acylated with glutamic acid are transcribed from different genes (Martin et al., 1976a). The first peak is a glutamyl-tRNA, whereas the second appears to be an intermediate in the formation of a glutaminyl-tRNA (Martin et al., 1977).

All of the remaining tRNAs which showed evidence of multiple mitochondrially coded species (i.e., cysteinyl-, methionyl-, phenylalanyl-, threonyl-, tyrosyl-, and valyl-tRNAs) (Figure 2) were subjected to hybridization analysis in our effort to determine whether they also might be transcribed from separate genes. Methionyl peaks II and III were isolated from preparative RPC-5 columns, purified by rechromatography, and used in hybridization experiments to determine their transcriptional relationship. At saturation the sum of the Met II + Met III hybridization was 80% of the amount expected from their individual hybridization. This suggested to us that one cycle of rechromatography was not enough to eliminate all cross contamination of the two peaks so they were chromatographed a second time before deletion mapping studies were carried out. We have previously mapped mitochondrial tRNA cistrons by hybridization of [^{3}H]aminoacyl-tRNAs to mtDNA isolated from a series of petite deletion mutants. By correlating the presence or absence of hybridization of tRNAs with the presence or absence of antibiotic

[2] ^{35}S-labeled, cytoplasmic cysteinyl-tRNA and ^{35}S-labeled, mitochondrial cysteinyl-tRNA were run separately and together in sequential runs to determine their relative mobilities. Cytoplasmic cysteinyl-tRNA cochromatographed with peak II (Figure 2).

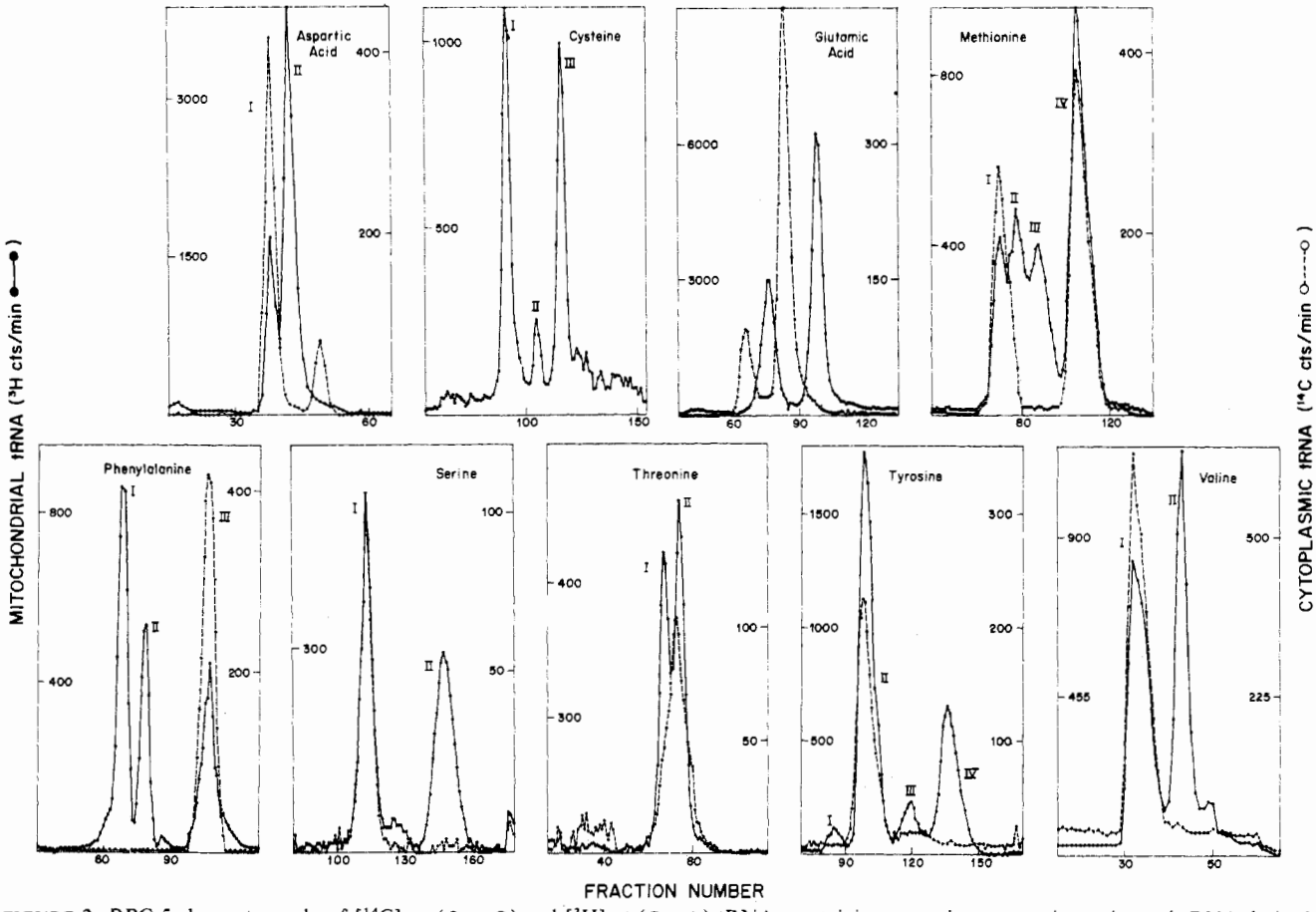

FIGURE 2: RPC-5 chromatographs of [^{14}C]cyt (○ - - ○) and [^{3}H]mt (●—●) tRNAs containing more than one major aminoacyl-tRNA derived from mitochondrial tRNA preparations. The bands are sequentially numbered with Roman numerals.

TABLE II: Hybridization of Met-tRNA^{II} and Met-tRNA^{III} to Petite mtDNA Relative to Grande mtDNA.[a]

Strain	mt loci					Ratio of hybridization rel to grande M441-78 mtDNA	
						Met-tRNA^{II}	Met-tRNA^{III}
	C	E	O_I	O_{II}	P		
MH41-7B (grande)	+	+	+	+	+	1	1
CEP 2	+	+	−	−	+	4.5	5.5
CEP 3	+	+	−	−	+	0	0
CEP 4	+	+	−	−	+	2.5	0.13
CEP 5	+	+	−	−	+	0	0.35
C 1	+	−	−	−	−	0.02	NT
D41	+	−	−	−	−	0	NT
R53	+	+	−	−	−	0	0.02
C42	−	−	−	−	−	0	0
O_IP_2	−	−	+	+	+	0	1.2
O_IO_{II-1}	−	−	+	+	−	NT	0.04
O_IP	−	−	+	−	+	NT	0.31
P 1	−	−	−	−	+	0.02	1.54
P 2	−	−	−	−	+	NT	0.36
P 22	−	−	−	−	+	NT	1.5
EO_I	−	+	+	−	−	0	0

[a] Petite strains and their use in deletion mapping have been described (Fukuhara et al., 1976; Martin et al., 1977).

resistance markers chloramphenicol (C^R), erythromycin (E^R), paromomycin (P^R), and oligomycin I and II (O_I^R, O_{II}^R), we were able to order the tRNA genes with respect to the markers and to each other. The pattern of hybridization observed with unfractionated methionyl-tRNA suggested that there might be two methionyl-tRNA genes (Martin et al., 1977).

As can be seen in Table II and Figure 3, some petites (i.e., CEP2) retained the cistrons for both methionyl-tRNAs. Other petites (i.e., CEP4) lost the gene coding for methionyl tRNA^{II}, and still others (i.e., CEP5, O_I, P_2, P_{22}) retained the gene coding for methionyl-tRNA^{III}, but not the gene coding for methionyl-tRNA^{II}. By comparing the hybridization of the methionyl-tRNAs with that observed for prolyl-, valyl-, and isoleucyl-tRNAs (Martin et al., 1977), we were able to order the methionyl-tRNA cistrons with respect to these other tRNA genes.

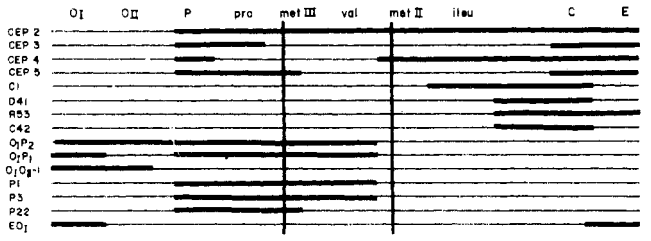

FIGURE 3: Deletion diagram. From the data of Table II, methionyl-tRNA genes were placed relative to antibiotic resistance markers arranged linearly in the order described by Martin et al. (1977). Hybridization greater than 0.2 relative to the hybridization observed with grande DNA is considered positive and is represented by heavy lines.

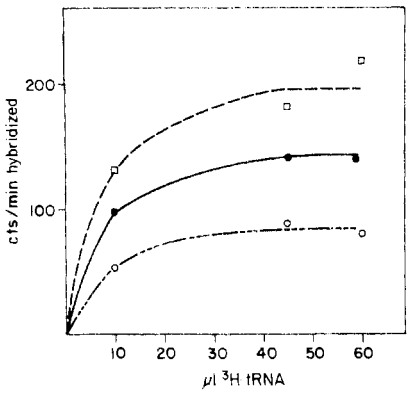

FIGURE 4: Hybridization of [^{3}H]threonyl-tRNAs to mtDNA. Increasing amounts of threonyl-tRNA^{I} (47 cpm/μL) (●—●) and threonyl tRNA^{II} (82 cpm/μL) (○- - - - -○) were hybridized individually or together (□ - - □). Aminoacyl-tRNAs (expressed as μL of tRNA per filter) were incubated with blank and mtDNA containing filters. Reaction volumes were 0.1 mL per filter.

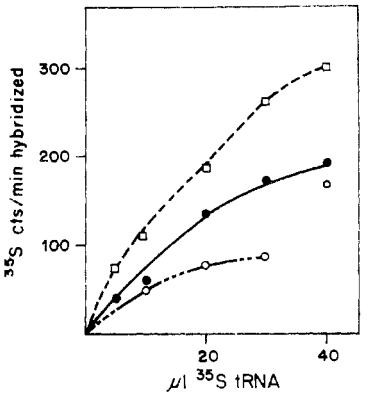

FIGURE 5: Hybridization of [^{35}S]cysteinyl-tRNAs to mtDNA. Increasing amounts of cysteinyl-tRNA^{I} (57 cpm/μL) (●- - - -●) and tRNA^{III} (112 cpm/μL) (●—●) were hybridized separately or together (□ - - □), as described in Figure 4.

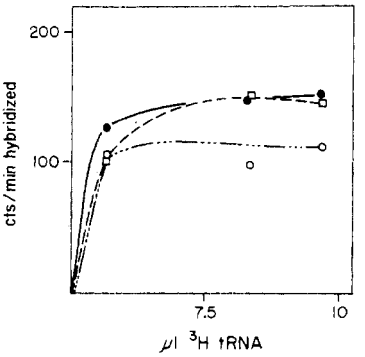

FIGURE 6: Hybridization of [^{3}H]phenylalanyl-tRNAs to mtDNA. Increasing amounts of phenylalanyl-tRNA^{I} (115 cpm/μL) (●—●) and tRNA^{II} (126 cpm/μL) (○- - - - -○) were hybridized separately or together (□ - - □), as described in Figure 4.

Threonyl-tRNA peaks I and II were isolated from preparative RPC-5 columns, rechromatographed twice, and then used in hybridization experiments. As shown in Figure 4, additive hybridization was observed. Mapping studies with the separated peaks have not been done.

Cysteinyl-tRNA proved difficult to analyze definitively. Repeated analyses of numerous samples of cysteinyl-tRNA yielded two different chromatographic profiles. One observed profile is shown in Figure 2; the other was characterized by a reduced or absent peak I, coupled with the appearance of a peak eluting in the high-salt wash (1.5 M NaCl) routinely used after the gradient elution. Although the tRNA recovered from peak I (Figure 2) or from the high-salt wash clearly hybridized to mtDNA, the apparent chromatographic shift could not be correlated with cell growth, sample preparation, or chromatographic technique. Cysteinyl-tRNA represented the only case in this study for which such chromatographic variation was observed. In three separate experiments, tRNA recovered from peak I appeared to display additive hybridization with the tRNA in peak III. Hybridizations in these experiments approached saturation (Figure 5), but complete saturation was not reached. Therefore, although the results suggest the presence of two cysteinyl-tRNA genes, further analyses will be necessary before the behavior of mitochondrial cysteinyl-tRNAs becomes completely clear.

Phenylalanyl- and valyl-tRNAs did not show additive hybridization of chromatographically separated peaks. Phenylalanyl-tRNA peaks I and II were isolated from preparative RPC-5 columns and used in hybridization experiments. As can be seen in Figure 6, both peaks hybridized to mtDNA. The sum of their hybridization was not additive; therefore, their sequences must be very similar, if not identical. We also obtained nonadditive hybridization for valyl peaks I and II (data not shown) similar to results obtained previously by Schneller et al. (1975b). These authors also reported the presence of a small additional valyl peak which hybridized to mtDNA; we have not observed this peak.

Finally, all four peaks seen in the tyrosyl-tRNA profiles hybridized to mtDNA (Table III). Saturation plateaus were not attained. It proved to be technically impossible to increase yields of [^{3}H]tyrosyl tRNA to levels sufficient for the carrying out of hybridization experiments for each peak. Therefore, we have not determined the transcriptional relationship of the four tyrosyl-tRNA peaks.

Discussion

Over the past few years, estimates of the number of tRNAs encoded by mitochondrial DNA have risen steadily. It now appears likely that mtDNA in both lower (Martin et al., 1977) and higher (Lynch & Attardi, 1976) eukaryotes will prove to code for at least one tRNA that accepts each of the common amino acids. We initiated the studies reported here to evaluate the possibility that mtDNA might code for more than one tRNA for some amino acids.

As an internal control, we chromatographed cytoplasmic tRNAs from a strain containing no mtDNA, so that peaks differing chromatographically from cytoplasmic tRNA are likely to be of mitochondrial origin. Transfer RNAs isolated from mitochondria that migrate with nuclear-coded tRNAs but do not hybridize to mitochondrial DNA were observed. Because the levels of these tRNAs varied in different tRNA

preparations, we consider it probable that their presence is due to nonspecific contamination of our mitochondria with cytoplasmic constituents. When the same tRNA preparation was used in acylation reactions with different amino acids, sometimes both nuclear-coded and mitochondrial-coded tRNAs were acylated whereas, in other cases, amino acids were only found ligated to mitochondrial species. This is probably related to the variable specificities of mitochondrial aminoacyl synthetases, some may acylate only mitochondrial tRNA, and others act in a heterologous fashion with cytoplasmic tRNAs.

For the determination of the genetic origin of mitochondrial tRNAs, hybridization analysis was necessary because several factors, other than differences in primary base sequence, can alter the chromatographic behavior of tRNAs. Posttranscriptional modification of bases (Huang & Mann, 1974; White et al., 1975), differential cross-linking between bases (Egan et al., 1973), and conformational changes (Ishide et al., 1971) all result in sufficient structural difference to allow chromatographic separation of tRNAs having an otherwise identical primary base sequence.

The wobble hypothesis predicts that multiple species of tRNAs are necessary to recognize all the codons for alanine, arginine, glycine, leucine, proline, serine, threonine, and valine. A consideration of our data and those of others shows that this prediction has not yet been met. Minor peaks were observed here for alanine, arginine, and glycine tRNAs; these may represent tRNAs encoded by additional genes, but further analyses must be done before this can be determined. We observed only one species of leucyl-tRNA, as has also been reported by Schneller et al. (1975b). We are unaware of any other studies of mitochondrial prolyl-tRNA and, again, we observed only one species of this tRNA. Baldacci et al. (1976) reported the presence of two mitochondrially coded isoaccepting seryl-tRNAs in yeast and observed that the amount of one of them is greatly affected by growth conditions. We found two species of threonyl-tRNA, transcribed from separate genes, in yeast mitochondrial tRNA. Finally, both we and Schneller et al. (1975b) observed multiple species of mitochondrial valyl-tRNAs, but there is no evidence that these tRNAs differ in base sequence.

The finding by Baldacci et al. (1976) that the appearance of at least one tRNA depends on growth conditions raises the possibility that more tRNAs may be detected if growth conditions are varied. It is also possible that more than one tRNA elutes in the same position with RPC-5 chromatography so that, for example, if leucyl-tRNAs were analyzed by other methods, multiple species might be detected. An alternate possibility, suggested by Chiu et al. (1975), is that mitochondria do not code for some essential tRNAs, but that nuclear-coded tRNAs are transported into mitochondria for use in mitochondrial protein synthesis. Such imported tRNAs have yet to be demonstrated conclusively.

The separation of two mitochondrial methionyl-tRNAs by RPC-5 chromatography and the fact that they hybridize to different regions of the mitochondrial genome conclusively demonstrate the presence of two methionyl-tRNA cistrons in yeast mitochondrial DNA. Some years ago, Halbreich & Rabinowitz (1971) demonstrated that mitochondrial $tRNA^{fMet}$ was encoded by mtDNA in yeast. The methionyl mitochondrial tRNA labeled Met III (Figure 2) corresponds to the initiator tRNA (Martin et al., 1976b).

Compared with the information available about mitochondrial tRNA isoacceptors in yeast, little is known for other organisms. Wallace & Freeman (1974) showed that methionyl- and formylmethionyl-tRNA from mouse liver mitochondria could each be separated into a major and minor species by RPC-5 chromatography. Chiu et al. (1974) demonstrated the presence of three leucyl-tRNA species from *Tetrahymena* mitochondria. That HeLa mitochondrial DNA codes for at least two seryl-tRNAs has been shown by the hybridization of [^{3}H]seryl-tRNA to both the light and heavy strands of that genome (Lynch & Attardi, 1976).

TABLE III: Hybridization of Tyrosyl tRNA Species from RPC-5 Columns.[a]

tRNA	Input counts	Blank (cpm)	mtDNA (cpm)	cpm hybridized to mtDNA
Tyr I	143	18	30	12
	286	16	41	25
	572	20	63	43
Tyr II	2450	22	61	39
	4910	30	91	61
	9820	60	214	154
Tyr III	396	17	59	42
	792	21	92	71
	1580	27	167	140
Tyr IV	1870	25	160	135
	3740	41	333	292
	7480	69	516	447

[a] tRNAs were recovered after RPC-5 chromatography and hybridized to mtDNA as described in Methods.

It is clear that mitochondrial DNA from both lower and higher eukaryotes codes for some isoacceptor, organelle-specific tRNAs. Some of the species separable by RPC-5 chromatography may differ in sequence but be similar enough that they cannot be differentiated by hybridization. Further analyses of mitochondrial tRNAs in yeast as well as in higher organisms will be necessary to determine whether mitochondrial protein synthesis is autonomous with respect to organelle-coded tRNAs and to determine the exact number of tRNA cistrons in mtDNA. At present, it is established that yeast mtDNA codes for 22 tRNA species. Each amino acid except asparagine has been shown to have a corresponding mt-coded tRNA, and methionine, threonine, and cysteine each appear to have two corresponding tRNAs. Several other species must be identified before it can be stated definitely that mitochondrial protein synthesis can depend exclusively on organelle-encoded tRNAs.

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Mitochondrial Phospholipase A_2 Activity and Mitochondrial Aging†

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ABSTRACT: The changes in mitochondrial phospholipid metabolism and energy-linked functions have been followed as coupled mitochondria are allowed to age in isotonic sucrose at 18 °C. Analysis of the aging process has provided an approach for studying the structure-function relationships within the mitochondrion without adding external agents to perturb the membrane structure. The initial event observed in this process of deterioration is a loss of respiratory control which is paralleled by diminishing levels of ATP. As ATP levels decline, so do the rates of reacylation of monoacylglycerophosphorylethanolamine and fatty acid oxidation. In most cases the previously inactive phospholipase A_2 (EC 3.1.1.4, phosphatide 2-acyl-hydrolase) begins rapid hydrolysis of membrane phosphatidylethanolamine as ATP levels approach zero. The final energy-linked phenomenon observed to decline is the anilinonaphthalenesulfonic acid fluorescence response. Evidence is presented which suggests strongly that the activity of the mitochondrial phospholipase A_2 on endogenous phospholipids is suppressed in tightly coupled mitochondria. This suppression is temporally linked to ATP levels in the mitochondrion. Furthermore, this study demonstrates that mitochondria which are only slightly damaged have the potential to effect membrane repair through reacylation of monoacyl phospholipids.

In the past few years the process of mitochondrial aging has received considerable attention (Waite et al., 1969a; Scarpa & Lindsay, 1972; Cheah et al. 1973; Siliprandi et al., 1973; Jurkowitz et al., 1974; Ozelkok et al., 1974; Yamaguchi & Satomura, 1974). Analysis of this process provides an approach for studying the structure-function relationships within the mitochondrion without adding external agents to disrupt the membrane. A number of the above studies have suggested that the endogenous phospholipase A_2 may be involved in the deterioration of mitochondrial energy-linked functions. Waite et al. (1969a) have shown a direct relationship between endogenous phospholipase A_2 activity and irreversible mitochondrial swelling, a change generally associated with progressive loss in energy-linked functions (Jurkowitz et al., 1974). Moreover, Scarpa & Lindsay (1972) have presented evidence suggesting that the phospholipase is partially responsible for the loss in respiratory control observed during the aging process.

In the present study we have investigated more completely the participation of the endogenous phospholipase A_2 in the mitochondrial aging process. In order to determine the contribution of this enzyme in the loss of energy-linked properties we have measured simultaneously the changes in RCR,[1] ATP levels, the energy-linked Ans fluorescence response, and phospholipase A_2 activity as mitochondria were aged at 18 °C. We have also measured the kinetics of loss of monoacyl phospholipid reacylation during the aging process. Moreover, this

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[1] Abbreviations used: RCR, respiratory control ratio; Ans, 1-anilino-8-naphthalenesulfonic acid; diacyl-GPE, diacylglycerophosphorylethanolamine; monoacyl-GPE, monoacylglycerophosphorylethanolamine; FFA, free fatty acid; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; diacyl-GPC, diacylglycerophosphorylcholine.

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