Merrick, W. C., and Anderson, W. F. (1975), *J. Biol. Chem. 250,* 1197–1206.
Ochoa, S., and Mazumder, R. (1974), *Enzymes, 3rd Ed., 10,* 1–51.
Ranu, R. S., and Wool, I. G. (1976), *J. Biol. Chem. 251,* 1926–1935.
Sadnik, I., Herrera, F., McCuiston, J., Thompson, H. A., and Moldave, K. (1975), *Biochemistry 14,* 5328–5335.
Safer, B., Adams, S. L., Anderson, W. F., and Merrick, W. C. (1975a), *J. Biol. Chem. 250,* 9076–9082.
Safer, B., Anderson, W. F., and Merrick, W. C. (1975b), *J. Biol. Chem. 250,* 9067–9075.
Schreier, M. H., and Staehelin, T. (1973), *Nature (London) 242,* 35–38.
Schroer, R. A., and Moldave, K. (1973), *Arch. Biochem. Biophys. 154,* 422–430.
Siegel, L. M., and Monty, K. J. (1966), *Biochim. Biophys. Acta 112,* 346–362.
Siler, J., and Moldave, K. (1969), *Biochim. Biophys. Acta 195,* 123–129.
Skogerson, K., and Moldave, K. (1967), *Biochem. Biophys. Res. Commun. 27,* 568–572.
Skogerson, L., and Moldave, K. (1968), *Arch. Biochem. Biophys. 125,* 497–505.
Smith, K. E., and Henshaw, E. C. (1975), *Biochemistry 14,* 1060–1067.
Smith, A. E., and Marcker, K. A. (1970), *Nature (London) 226,* 607–610.
Staehelin, T., Trachsel, H., Erni, B., Boschetti, A., and Schreier, M. H. (1975), *Fed. Eur. Biochem. Soc. Meet. [Proc.] 10,* 309–323.
Thompson, H. A., Sadnik, I., and Moldave, K. (1977), *Biochemistry 16,* 2221–2230.
Traugh, J. A., Tahara, S. M., Sharp, S. B., Safer, B., and Merrick, W. C. (1976), *Nature (London) 263,* 163–165.
Treadwell, B. V., and Robinson, W. G. (1975), *Biochem. Biophys. Res. Commun. 65,* 176–183.
Treadwell, B. V., and Robinson, W. G. (1976), *Fed. Proc., Fed. Am. Soc. Exp. Biol. 35,* 1515.
Weber, K., and Osborne, M. (1969), *J. Biol. Chem. 244,* 4406–4412.
Weissbach, H., and Ochoa, S. (1976), *Annu. Rev. Biochem. 45,* 191–216.
Zasloff, M., and Ochoa, S. (1971), *Proc. Natl. Acad. Sci. U.S.A. 68,* 3059–3063.
Zasloff, M., and Ochoa, S. (1973), *J. Mol. Biol. 73,* 65–76.

Yeast Mitochondrial DNA Specifies tRNA for 19 Amino Acids. Deletion Mapping of the tRNA Genes†

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ABSTRACT: We have previously identified 14 aminoacyl-tRNAs that are specified by yeast mitochondrial DNA (mtDNA). We now report four more amino acids (Arg, Cys, Trp, Thr) that acylate tRNAs which hybridize with mtDNA. Furthermore one of the two mitochondrial tRNAs that we had earlier demonstrated to be directly charged with glutamic acid responds to glutamine but not to glutamic acid codons. Thus Gln-tRNA^{Gln} appears to be formed by transamidation of a missense intermediate Glu-tRNA^{Gln}. This brings to 19 the number of amino acids which have corresponding tRNAs specified by mtDNA. Only tRNA^{Asn} has not yet been shown to be a mtDNA transcript. We have also mapped the genes for the newly identified mitochondrial tRNAs, as well as several others that were previously identified but unmapped, by hybridization to the mtDNA of a series of petite deletion mutants. We now have ordered 20 mitochondrial tRNA genes (including two methionyl-tRNAs) with respect to the antibiotic resistance markers chloramphenicol (C^R), erythromycin (E^R), paromomycin (P^R), and oligomycin I and II (O^R_I, O^R_{II}). Eighteen tRNA genes map between the C and E resistance markers. Only the serinyl-tRNA and glutamyl-tRNA genes are localized near the O_I and O_{II} resistance markers.

Mitochondrial assembly depends upon the coordination of nuclear and mitochondrial genetic and protein-synthetic systems. The respiratory elements responsible for electron transport and coupled phosphorylation, i.e., cytochrome oxidase, coenzyme Q–cytochrome *c* reductase, and oligomycin-sensitive ATPase, are oligomeric complexes consisting of as many as 9 peptides. In each complex, some peptides are synthesized on cytoplasmic and others on mitochondrial ribosomes (Schatz and Mason, 1974). The mitochondrial ribosome itself has a dual origin; most of its components are synthesized extramitochondrially and imported into the organelle. The only presently established transcription products of mitochondrial DNA are tRNAs, the two subunits of mitochondrial rRNA, and 9–10 presumptive mRNA species which probably code for three peptides of cytochrome oxidase, one of cytochrome *b*, four of the oligomycin-sensitive ATPase, and probably one ribosomal protein (Locker and Rabinowitz, 1977). It is not yet known whether the tRNA complement of mitochondria has a dual origin, but the possibility of the import of cytoplasmic tRNAs into the mitochondria has been raised (Chiu et al., 1975).

This possibility was suggested by RNA–mtDNA hybrid-

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ization experiments with labeled total 4S RNA, in which only 12–15 tRNA cistrons were noted in HeLa cells and *Xenopus* (Aloni and Attardi, 1971; Dawid, 1972). Similar studies in yeast indicated 20–25 tRNA cistrons (Reijnders and Borst, 1972; Schneller et al., 1975a,b); this number is lower than required for response to all codons as sense codons. More recently, electron-microscopic examination of mitochondrial tRNA/mtDNA hybrids has indicated the presence of 19 tRNA genes in HeLa mtDNA (Angerer et al., 1976). and at least 15 genes in *Xenopus* mtDNA (Dawid et al., 1976). Although these estimates are somewhat higher than those obtained from hybridization of bulk-labeled tRNA, they still indicate that mtDNA codes for a limited number of tRNAs.

Our laboratory has sought for some time to identify and characterize tRNAs specified by yeast mtDNA by using hybridization with tRNAs aminoacylated with individual labeled amino acids of high specific activity. We previously identified 14 aminoacyl-tRNAs specified by yeast mtDNA and suggested that tRNAs for all common amino acids were probably mitochondrial gene products (Casey et al., 1969, 1972, 1974a,b; Halbreich and Rabinowitz, 1971; Cohen et al., 1972). We now report that four more amino acids are accepted by tRNAs which are gene products of mtDNA. Furthermore, we present evidence for the presence of a mitochondrially coded tRNA that responds to glutamine codons. This work brings to 19 the number of amino acids which have corresponding tRNAs coded for by mtDNA. We have also analyzed mitochondrial tRNAs by reverse-phase column chromatography and detected several isoaccepting mitochondrial tRNA species (Martin and Rabinowtiz, 1976). Taken together, the results suggest the likelihood that the mitochondria of yeast will prove to be autonomous with respect to tRNAs.

We have also used hybridization of mitochondrial tRNA with the mtDNA of a number of petite deletion mutants to examine the arrangement of tRNA genes in yeast mtDNA. We previously reported on the ordering of 12 tRNA cistrons with respect to 5 antibiotic resistance markers (Fukuhara et al., 1976) [chloramphenicol (C), erythromycin (E), oligomycin I (O_I), oligomycin II (O_{II}), and paromomycin (P)] by use of a large series of petite mutants that retain different segments of the grande genome (Casey et al., 1974a,b). We have extended the study to include the newly identified tRNAs as well as several previously identified, but unmapped tRNAs. We now have a deletion map of 20 tRNA genes, including one isoacceptor tRNA.

Materials and Methods

(a) *Strains and Cultures.* A haploid, respiratory-sufficient (grande) strain of *Saccharomyces cerevisiae,* MH41-7B, was used for the isolation of mitochondrial tRNA, mtDNA, and mitochondrial aminoacyl synthetases. Strain MH41-7B was constructed by Bolotin-Fukuhara and Fukuhara (1976) and has been characterized with respect to 5 mitochondrial alleles. For a complete genotype of this strain, as well as for information on the cytoplasmic petite strains used in these mapping studies, the reader is referred to Fukuhara et al. (1976).

(b) *Preparation of DNA and RNA.* Mitochondria were prepared from protoplasts as described previously (Casey et al., 1972). For the isolation of mtDNA, the mitochondria were treated with pancreatic DNase, washed, and lysed, and the DNA was isolated as described by Casey et al. (1974a,b). A mtDNA-less petite strain, IL8-8C/HF71, was broken in a Braun homogenizer and the nuclear DNA isolated from the 2000*g* pellet by the procedure used for mtDNA.

Mitochondrial tRNA was isolated from mitochondria washed four times in 0.8 M sorbitol, 0.1% bovine serum albumin, following the procedure of Casey et al. (1972). Cytoplasmic tRNA was prepared from the 100 000*g* supernatant of IL8-8C/HF71 cell homogenates; this strain contains no mtDNA.

(c) *Isolation of Acylation Enzymes and Acylation of tRNAs.* Mitochondrial aminoacyl synthetases were prepared from washed mitochondria and tRNA acylated as described previously (Casey et al., 1974a,b). Pilot experiments for the determination of optimal $MgCl_2$ (range tested, 5–20 mM) and ATP (range tested, 3–10 mM) concentrations were done during a search for previously undetected mitochondrial tRNAs. The tRNA was acylated with arginine (New England Nuclear, sp act. 28.7 Ci/mM) or cysteine (New England Nuclear, sp act. 63.7 Ci/mM) in a reaction mixture containing 100 mM Tris,[1] pH 7.5, 5 mM $MgCl_2$, 3 mM ATP, 0.2 mM dithiothreitol (DTT). The reaction mixture for tryptophan (New England Nuclear, sp act. 10.7 Ci/mM) was 100 mM Tris, pH 7.5, 15 mM $MgCl_2$, 6 mM ATP, and 0.2 mM DTT; that for threonine (New England Nuclear, sp act. 2.0 Ci/mM) was 100 mM Tris, pH 7.5, 15 mM $MgCl_2$, 3 mM ATP, and 0.2 mM DTT. Characteristic specific activities of these tRNA preparations (counts min^{-1} (μg of RNA)$^{-1}$) were 2.2×10^3 arginyl-tRNA, 3.6×10^3 cysteinyl-tRNA, 6.3×10^3 tryptophanyl-tRNA, and 9.9×10^2 threonyl-tRNA. Other tRNAs were acylated as described previously (Halbreich and Rabinowitz, 1971; Casey et al., 1974b), by use of either mitochondrial synthetase or *E. coli* synthetase prepared in the same way.

(d) *Hybridization of tRNAs to DNA.* Nitrocellulose filters (Selectron, Schleicher and Schuell) were loaded with 20 μg of grande mtDNA, 20 μg of various petite mtDNAs, or 100 μg of nuclear DNA. Hybridizations were carried out as described previously (Martin et al., 1976).

(e) *Binding of Aminoacyl-tRNA to Ribosomes in Response to Polynucleotides.* The methods for the binding of tRNAs to ribosomes in response to polynucleotides were essentially those described by Nirenberg and Leder (1964). Reaction mixtures contained 40 mM Tris, pH 7.5, 80 mM NH_4Cl, 0.2 mM GTP, 25 mM $MgCl_2$, 0.5 mM β-mercaptoethanol, 1.2 A_{260} units of washed *E. coli* MRE 600 ribosomes, and 0.5 A_{260} polynucleotides (Miles). Following incubation for 15 min at 37 °C, the ribosomes were collected on nitrocellulose filters, and the counts bound were determined following the solubilization of the filters in Triton X-100 scintillation fluid.

Results

(a) *Hybridization of Arginyl-, Cysteinyl-, Tryptophanyl-, and Threonyl-tRNA to Grande mtDNA.* Hybridizations of mitochondrial arginyl-, cysteinyl-, tryptophanyl-, and threonyl-tRNAs are shown in Figure 1. In all cases, saturation plateaus were reached when these tRNAs were hybridized to filters containing 20 μg of grande mtDNA. No hybridization above background was observed with filters containing nuclear DNA.

Further evidence demonstrating that these tRNAs are mitochondrial in origin comes from hybridization–competition experiments. Figures 2A and 2B show representative competition curves which result when radioactive tRNA at saturating levels is competed by cold tRNA. Unlabeled mitochondrial tRNA reduced the hybridization to expected levels, whereas unlabeled cytoplasmic tRNA, isolated from a mtDNA-less petite, did not affect hybridization.

[1] Abbreviations used: Tris, tris(hydroxymethyl)aminomethane; DTT, dithiothreitol.

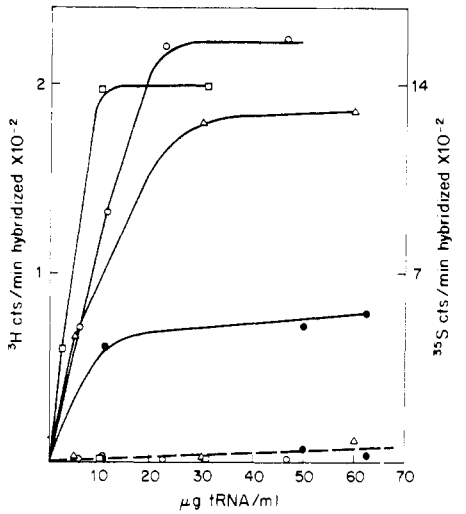

FIGURE 1: Hybridization of arginyl-, tryptophanyl-, threonyl-, and cysteinyl-tRNAs to yeast mtDNA. Increasing amounts of labeled aminoacyl-tRNAs were hybridized to filters containing 20 μg of mtDNA. (○—○) Arg; (□—□) Cys; (△—△) Trp; (●—●) Thr. Same symbols with interrupted lines indicate hybridization with 100 μg of nuclear DNA.

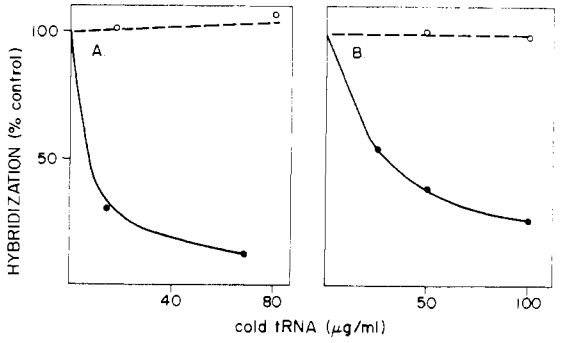

FIGURE 2: Hybridization-competition of (A) arginyl- and (B) threonyl-[^{3}H]tRNAs with cold mitochondrial tRNA or cold cytoplasmic tRNA. Saturating amounts of the labeled tRNAs were hybridized with 20 μg of mtDNA in the presence of increasing amounts of cold mitochondrial tRNA (●-●) or cold cytoplasmic tRNA (○ - - - ○).

(b) *Identification of a Glutamine-tRNA.* Efforts to acylate mitochondrial tRNA with radioactive glutamine have consistently yielded negative results. In examining the codon responses of two mitochondrial tRNAs which are acylated with glutamic acid (Martin et al., 1976), we found that one (previously designated as tRNA^{Glu}II) responded to oligonucleotides which contain the CAA codon specifying glutamine; the other responded, as expected, to oligonucleotides containing the GAA and GAG codons specifying glutamic acid (Table I).

To ascertain that the tRNA responding to glutamine codons was directly acylated with glutamic acid, we did aminoacylation experiments in the presence of unlabeled amino acids. As can be seen in Table II, the addition of unlabeled glutamic acid decreased incorporation of [^{3}H]glutamic acid into tRNA, whereas unlabeled glutamine had no effect. In contrast, unlabeled glutamine reduced the formation of cytoplasmic glutamine tRNA, as expected. From these results, we conclude that we are unable to detect direct acylation of mitochondrial tRNA with glutamine because glutamine-tRNA is synthesized by a pathway in which a missense aminoacyl-tRNA (Glu-tRNA^{Gln}) is formed and then converted to Gln-tRNA^{Gln} by an as yet unidentified transamidase. Such a reaction has been shown to occur in several gram-positive bacteria (Wilcox and Nirenberg, 1968).

TABLE I: Codon Recognition of Yeast Glu-tRNAs. Increase in Aminoacyl-tRNA Bound to Ribosomes in Response to Oligonucleotides.[a]

Oligonucleotides	tRNAs (cpm)		
	Cytoplasmic	mt GluI	mt GluII
None	63	38	32
U	96	28	40
GA	610	352	40
CA	109	38	227

[a] GA contains GAA and GAG glutamic acid codons. CA contains CAA glutamine codon. Binding assays were carried out as described in Materials and Methods and follows the procedure of Nirenberg and Leder (1964).

TABLE II: Effect of Unlabeled Glutamic Acid and Glutamine on the Acceptance of Radioactively Labeled Amino Acid by Mitochondrial and Cytoplasmic tRNAs.[a]

tRNA	Unlabeled amino acid	cpm/min	% control act.
Mitochondrial acylated with 0.032 μg of [^{3}H]Glu	No addition	4792	100
	0.064 μg of Glu	3191	67
	0.124 μg of Glu	1695	35
	0.064 μg of Gln	4992	103
	2.92 μg of Gln	4840	101
Cytoplasmic acylated with 0.62 μg of [^{14}C]Gln	No addition	2432	100
	1.4 μg of Gln	1762	72
	2.9 μg of Gln	874	36
	5.8 μg of Gln	690	28

[a] Acylation reactions were carried out as described in Materials and Methods.

(c) *Mapping of mt tRNA Genes.* Seven tRNAs which we had not previously mapped were initially localized by hybridization with mtDNA from petites CEP-2, O_1P-2, and EO_1. Genetic analyses and restriction enzyme analysis of these DNAs show that these three petites together appear to contain all the sequences present in the grande genome (A. Lewin, personal communication). Hybridization of these DNAs with the acylated transfer RNAs defined the general localization of each tRNA cistron (Table III), which was then refined by hybridization to additional selected petite strains (Table IV).

Cysteinyl- and threonyl-tRNAs show significant hybridization to petites which retain the C marker (Figure 3), analogous to the results obtained previously for histidyl-tRNA (Fukuhara et al., 1976). We have been able to order these tRNAs with respect to each other and to the C marker (i.e., C, Thr, Cys, His) because petite C7 retained only the Thr gene, CEP-5 retained Thr and Cys, but not His, and other C petites such as C4 and C6 retained all three genes. We tentatively place Thr, Cys, and His to the right of C toward P (Figure 3), but some or all of these genes could well be to the left of C.

Glycinyl- and arginyl-tRNAs show an identical pattern of hybridization to petite mtDNAs. The pattern is similar to that which we previously observed for phenylalanyl-, alanyl-, tyrosyl-, and aspartyl-tRNAs (Fukuhara et al., 1976). All of

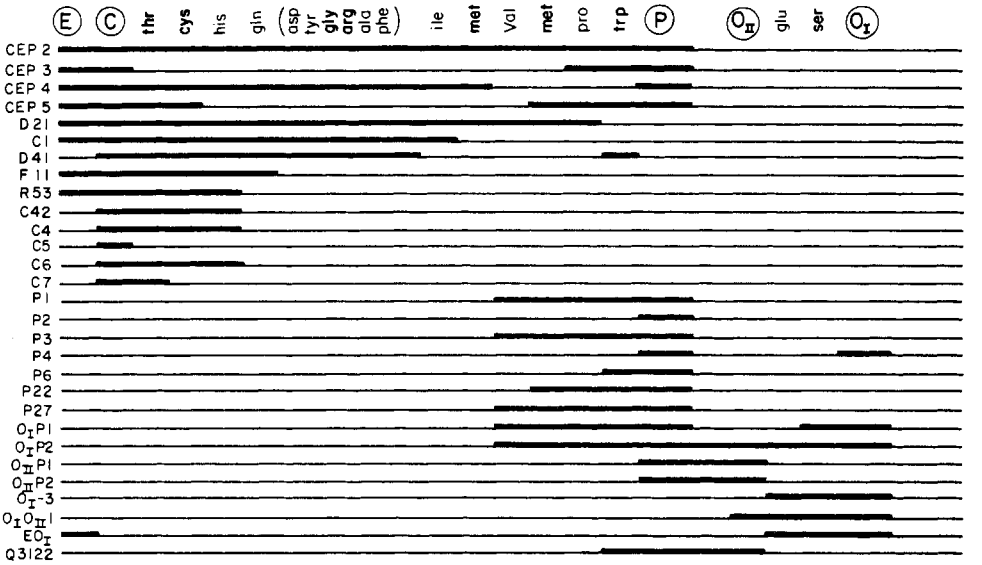

FIGURE 3: Deletion diagram. From the data of Table IV, tRNA genes and antibiotic resistance markers were arranged linearly in the order described by Martin and Rabinowitz (1976). Hybridization greater than 0.2 is represented by heavy lines. Petites not tested with individual tRNAs are listed in Table IV. Some previously mapped tRNAs (Fukuhara et al., 1976) are included for clarity. Bold lettering indicates new data.

TABLE III: Relative Hybridization of [^{3}H]Aminoacyl-tRNAs with Petite mtDNAs.[a]

Strain	Aminoacyl-tRNA						
	Trp	Met	Arg	Gly	Cys	Thr	Ser
CEP-2	0.53	4.22	0.63	0.73	0.89	2.00	0.00
O_IP-2	0.72	0.20	0.01	0.07	0.05	0.12	6.17
EO_I	0.00	0.04	0.03	0.00	0.04	0.00	1.03

[a] Filter hybridizations were carried out as described in the Materials and Methods. The data are expressed as the ratio of hybridization Petite:grande mtDNA.

TABLE IV: Hybridization of [^{3}H]Aminoacyl-tRNAs with Petite mtDNA Relative to the Hybridization with Grande mtDNA.[a]

Strain	Trp	Met	Arg	Gly	Cys	Thr	Ser
CEP-2	0.53	4.22	0.63	0.73	0.89	2.0	0
CEP-3		0.03					
CEP-4		0.36	2.07				
CEP-5	1.54	0.54	0	0.02	0.72	0.44	0.15
D21	0.02		0.69			2.90	
Cl		0.02			0.35		
D41	1.3	0.05	2.05	4.65	0.77	1.82	
F11	0	0.07	0.03	0	1.4		0
R53	0	0.02	0.01	0.02	0.70	2.32	0
C42	0.03	0	0	0.02	2.62	3.61	
C4				0	4.18		
C5					0	0	
C6	0	0	0	0	5.6	3.27	
C7	0				0.10	1.28	
P1	0.60	0.90	0		0.06	0.21	
P2	0.05					0.04	
P3	0.89	0.36	0.02	0		0.00	0.00
P4	0.02	0.06					
P6	5.1						
P22	0.94	1.5	0.01			0.03	
P27	0.93						
O_IP-1		0.41					0.91
O_IP-2	0.72	0.20	0.01	0.07	0.05	0.12	6.17
O_{II}P-1	0.01						0.07
O_{II}P-2	0.01						0.05
O_I-3	0				0.00	0.03	3.71
O_IO_{II}-1	0.02	0.04	0.04		0.04	0.09	0.22
EO_I	0	0.04	0.03	0	0.04	0	1.03
Q3122	1.13	0.06				0.14	0.09

[a] Data are expressed as the ratio of hybridization with petite mtDNA to hybridization with grande mtDNA. Hybridizations not done are indicated by no entry.

these tRNAs map to a region of the mtDNA between the C and P markers. All petites so far tested hybridized either with all six of these tRNAs or with none; therefore these six tRNA genes cannot yet be further ordered with respect to each other. As a group, they can be placed on the map between glutaminyl- and isoleucyl-tRNAs (Fukuhara et al., 1976).

Methionyl-tRNA maps to a region of the genome between the Ile-tRNA gene and the P marker. It is placed to the right of the Ile-tRNA gene because petite CEP-4 retains both Ile-tRNA and Met-tRNA genes while petite Cl has lost the Met-tRNA gene. Analogously, Met-tRNA genes can be placed to the left of the Pro-tRNA gene because petite CEP-3 retains the Pro-tRNA gene but not the Met-tRNA gene, whereas petites CEP-5 and P22 retain both (Figure 3). An inconsistency in the mapping arises when the position of the Val-tRNA gene (Figure 4) is taken into consideration. Hybridization to petites previously shown to have both Pro- and Val-tRNA genes (P1, P3, O_IP-1, O_IP-2) as well as those having only the Pro-tRNA genes (CEP-5, P22) suggested the presence of a Met-tRNA gene between the Pro- and Val-tRNA genes. However, the fact that Met-tRNA also hybridized to a petite containing the Ile-tRNA cistron but not Pro- and Val-tRNA cistrons (CEP-4) suggested that there was a second Met-tRNA gene between the Val- and Ile-tRNA genes. Preliminary results confirming the presence of two distinct mitochondrial methionyl-tRNAs and their mapping to petites have been presented (Martin and Rabinowitz, 1976).

Tryptophanyl-tRNA hybridizes to petite DNAs that retain the P resistance marker. When the pattern of hybridization is compared with other tRNAs demonstrating a strong linkage to the P marker [Pro-, Val-tRNA (Fukuhara et al., 1976), and Met-tRNA], it is evident that some P-containing petites retain all of these genes, others retain the Trp-, Pro-, and Met-tRNA genes, still others the Trp- and Pro-tRNA genes, whereas some retain the Trp-tRNA gene alone. The Trp-tRNA gene thus appears to be more closely linked to P than is Pro and must be

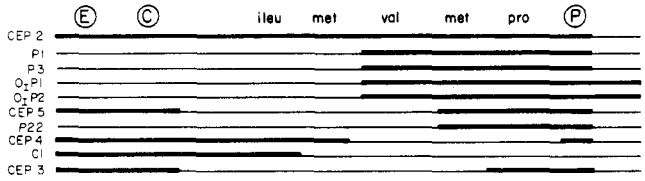

FIGURE 4: Partial deletion diagram of Met-tRNA region. See text for discussion.

located either between P and Pro or close to P, but on the side opposite the Pro-tRNA gene. Petite D41 showed hybridization with Trp-tRNA. The most likely explanation for this result is that D41, known to be multiply deleted (Fukuhara et al., 1976), has a deletion between Trp and other tRNA genes closer to C.

Serine-tRNA is the second example of a tRNA gene located distant from the CEP region of the yeast mitochondrial genome. Serinyl-tRNA and glutamyl-tRNA show a similar pattern of hybridization with petite DNAs, except that serinyl-tRNA hybridizes to O_1P-1, whereas glutamyl-tRNA does not. Because the petites in this region of the genome are extensively rearranged and contain double deletions, it is not possible at this time to localize these tRNAs further.

Discussion

One of the major aims in this study was to determine whether yeast mtDNA codes for tRNAs corresponding to each of the common amino acids. Our laboratory had previously used [^{3}H]aminoacyl-tRNA/DNA hybridization (Casey et al., 1969, 1972, 1974; Halbreich and Rabinowitz, 1971; Cohen et al., 1972) to identify mitochondrially coded tRNAs. The identification of tryptophanyl-, cysteinyl-, arginyl-, and threonyl-tRNAs brings to 18 the total number of tRNAs detected by this method.

We have previously reported the presence of two distinct mitochondrial tRNAs acylated with glutamic acid and shown that they were transcribed from different sequences in widely separated regions of the genome (Martin et al., 1976). In examining the codon responses of these two tRNAs, we found that, as expected, one of them responded to glutamic acid codons. An unexpected observation, however, was that the other responded to glutamine codons in ribosome-binding experiments. These results are analogous to observations by Wilcox et al. in *B. megaterium, B. subtilis,* and several other gram-positive bacteria in which glutamic acid is accepted by a $tRNA^{Gln}$ to form a missense Glu-$tRNA^{Gln}$ and is then enzymatically converted to Gln-$tRNA^{Gln}$ by a specific aminotransferase (Wilcox, 1969a,b; Wilcox and Nirenberg, 1968). Experiments for the identification of a similar enzyme activity in yeast mitochondrial extracts are currently under way.

We have been unable to identify an asparaginyl-tRNA. The presence of an unusual pathway for the formation of glutaminyl-tRNA in mitochondria suggested that a similar mechanism might be involved in asparaginyl-tRNA formation. However, mitochondrial tRNA acylated with aspartic acid did not respond to asparagine codons in ribosome-binding studies (N. Martin, unpublished observation). Because we have now identified tRNAs corresponding to 19 of the common amino acids, we believe it likely that tRNAs for all 20 of the common amino acids are present, and that technical difficulties such as instability or inactivity of the appropriate aminoacyl-tRNA ligase, coupled with the low specific activity of the radioactive asparagine, have prevented detection of this last tRNA. It should be noted, however, that asparaginyl-tRNA was one of those not found by Lynch and Attardi (1976) in their study of HeLa mitochondrial tRNAs. All of the other tRNAs not found in the HeLa cell study, namely, histidinyl, prolinyl, and glutaminyl, were observed in yeast mitochondrial preparations.

The fact that yeast mitochondrially coded tRNAs will accept most of, and probably all, the common amino acids suggests that the mitochondrial protein-synthetic system is autonomous with respect to tRNAs, but it does not prove conclusively that this is the case. To provide flexibility in mitochondrial protein synthesis, more than one tRNA for some amino acids would be necessary if all synonym codons were to be recognized as sense codons. In the hybridization experiments reported here, no attempt was made to determine whether some of the aminoacyl-tRNAs consist of mixed populations of isoacceptor tRNAs. Mapping studies presented in this paper suggested that two methionyl-tRNA genes were present in yeast mtDNA. This has been confirmed by the separation, by means of RPC-5 chromatography, of two distinct species of Met-tRNAs which have different sequences and map to different cistrons (Martin and Rabinowitz, manuscript in preparation). Studies of other mitochondrial aminoacyl-tRNAs by RPC-5 chromatography in our laboratory (Martin and Rabinowitz, 1976), as well as in several others (Schneller et al., 1975a,b; Martin et al., 1976; Baldacci et al., 1976), indicate that several isoacceptor tRNAs are present in yeast mitochondria. Further identification and characterization of isoacceptor tRNAs should establish the number of tRNAs coded for by yeast mtDNA and allow an evaluation as to whether mitochondrially coded tRNAs provide a full complement of tRNAs for use in mitochondrial protein synthesis in yeast.

Yeast mtDNA is considerably larger than mtDNA of higher animals; therefore, the possibility exists that it codes for more gene products, including tRNAs, than the mtDNA of higher eukaryotes. Although it has recently been reported that mitochondrial tRNA in locusts consists of at least 27 species (Feldman and Kleinow, 1976), estimates closer to 20 have been made for the mitochondrial tRNA of HeLa cells and *Xenopus* (Aloni and Attardi, 1971; Dawid, 1972; Angerer et al., 1976; Dawid et al., 1976; Lynch and Attardi, 1976). It may be that further analysis of tRNAs from mitochondria of higher organisms will lead to the identification of additional tRNAs. Alternatively, the apparently rigid system implied by this limited number of tRNAs might be circumvented by the import of nuclear-coded tRNAs into the mitochondria (Chiu et al., 1975).

The tRNA cistrons in yeast mtDNA are concentrated in the region of the genome between the C and P antibiotic resistance markers. That tRNA cistrons are lost in various combinations in various petites suggests that not all are closely clustered. The only genes which may consist of a tight cluster of tRNA cistrons are those that have not yet been separated by the deletion mapping technique. These would be composed of phenylalanyl-, alanyl-, tyrosyl-, aspartyl-, glycinyl-, and arginyl-tRNA genes. Mapping of these tRNA genes by electron microscopy or the isolation of multicistronic tRNA precursor molecules would be the definitive methods of determining whether these genes exist as a tight cluster on the mitochondrial genome, as has been found for some tRNA cistrons in phage (Siedman et al., 1975) and bacterial (Ghysen and Celis, 1974; Ilgan et al., 1976) genomes. Electron-microscopic mapping of tRNA–DNA hybrids in HeLa cells and *Xenopus* mtDNA has shown that the tRNA cistrons are widely spaced, and, further, that tRNAs are transcribed from both strands (Angerer et al., 1976; Dawid et al., 1976). Until it becomes technically feasible to separate DNA strands of yeast mtDNA, it is not possible to determine whether the same is true in this organism.

The availability of the petite mutation, antibiotic resistance

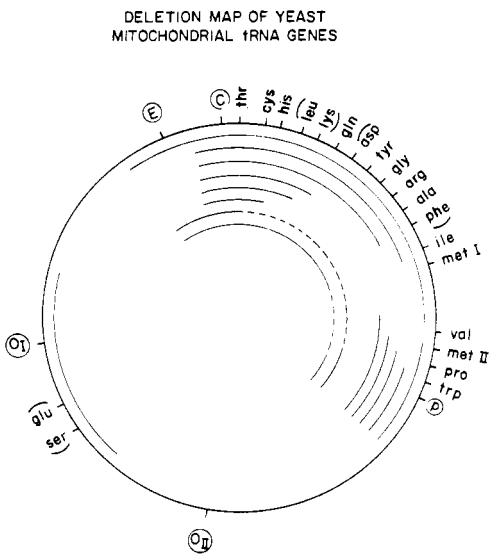

FIGURE 5: Deletion map of yeast mitochondrial tRNA genes.

markers, and a number of aminoacyl-tRNAs has enabled us to construct a tentative map of yeast mitochondrial tRNA cistrons (Figure 5). As pointed out in the Results section, a final ordering of tRNA genes has not always been possible because some of the tRNA cistrons have not been separated from each other in the generation of petites. Also, it has not been possible to determine physical distances between the antibiotic markers and the tRNA genes, or between the tRNA genes themselves. Another limiting factor in this type of mapping is that some of the petite mtDNAs contain multiple deletions. The uncertainties that remain in the deletion map may become clear through the mapping of tRNAs to restriction fragments. Such studies are currently under way in our laboratory. The evidence to date suggests that the pattern of tRNA cistrons obtained from deletion mapping is very similar to that found by restriction mapping and thus attests to the overall accuracy of the former technique.

References

Aloni, Y., and Attardi, G. (1971), *J. Mol. Biol. 55,* 271.

Angerer, L., Davidson, N., Murphy, W., Lynch, D., and Attardi, G. (1976), *Cell 9,* 81.

Baldacci, G., Falcone, C., Frontali, L., Macino, G., and Palleschi, C. (1976), in Genetics and Biogenesis of Chloroplasts and Mitochondria, Bücher, T., Neupert, W., Sebald, W., and Werner, S., Ed., Amsterdam, North-Holland Publishing Co., p 759.

Bolotin-Fukuhara, M., and Fukuhara, H. (1976), *Proc. Natl. Acad. Sci. U.S.A. 73,* 4608.

Casey, J., Cohen, M., Rabinowitz, M., Fukuhara, H., and Getz, G. S. (1972), *J. Mol. Biol. 63,* 431.

Casey, J., Fukuhara, H., Getz, G. S., and Rabinowitz, M. (1969), *J. Cell Biol. 43,* 18A.

Casey, J., Gordon, P., and Rabinowitz, M. (1974a), *Biochemistry 13,* 1059.

Casey, J., Hsu, H. J., Rabinowitz, M., Getz, G. S., and Fukuhara, H. (1974b), *J. Mol. Biol. 88,* 717.

Chiu, N., Chiu, A., and Suyama, Y. (1975), *J. Mol. Biol. 99,* 37.

Cohen, M., Casey, J., Rabinowitz, M., and Getz, G. S. (1972), *J. Mol. Biol. 63,* 441.

Dawid, I. (1972), *J. Mol. Biol. 63,* 201.

Dawid, I. B., Klukas, C., Ohi, S., Ramirez, J. L., and Upholt, W. B. (1976), in The Genetic Function of Mitochondrial DNA, Saccone, C., and Kroon, A. M., Ed., Amsterdam, North-Holland Publishing Co., p 3.

Feldman, H., and Kleinow, W. (1976), *FEBS Lett. 69,* 300.

Fukuhara, H., Bolotin-Fukuhara, M., Hsu, H. J., and Rabinowitz, M. (1976), *Mol. Gen. Genet. 145,* 7.

Ghysen, A., and Celis, J. E. (1974), *Nature (London) 249,* 418.

Halbreich, A., and Rabinowitz, M. (1971), *Proc. Natl. Acad. Sci. U.S.A. 68,* 294.

Ilgan, C., Kirk, L., and Carbon, J. (1976), *J. Biol. Chem. 251,* 922.

Locker, J., and Rabinowitz, M. (1977), in Methods in Enzymology—Biomembranes Part D Bioenergetics, Section VII, Fleischer, S., Ed., New York, N.Y., Academic Press (in press).

Lynch, D., and Attardi, G. (1976), *J. Mol. Biol. 102,* 125.

Martin, N. C., and Rabinowitz, M. (1976), in Genetics and Biogenesis of Chloroplasts and Mitochondria, Bücher, T., Neupert, W., Sebald, W., and Werner, S., Ed., Amsterdam, North-Holland Publishing Co., p 749.

Martin, N. C., Rabinowitz, M., and Fukuhara, H. (1976), *J. Mol. Biol. 101,* 285.

Martin, R., Schneller, J. M., Stahl, A. C., and Dirheimer, G. (1976), in Genetics and Biogenesis of Chloroplasts and Mitochondria, Bücher, T., Neupert, W., Sebald, W., and Werner, S., Ed., Amsterdam, North-Holland Publishing Co., p 755.

Nirenberg, M., and Leder, P. (1964), *Science 145,* 1399.

Reijnders, L., and Borst, P. (1972), *Biochem. Biophys. Res. Commun. 46,* 126.

Schatz, G., and Mason, T. L. (1974), *Annu. Rev. Biochem. 43,* 51.

Schneller, J. M., Faye, G., Kujawa, C., and Stahl, A. J. C., (1975a), *Nucleic Acids Res. 2,* 821.

Schneller, J. M., Stahl, A., and Fukuhara, H. (1975b), *Biochimie 57,* 1051.

Siedman, J. G., Barrel, B. G., and McClain, W. H. (1975), *J. Mol. Biol. 99,* 733.

Wilcox, M. (1969a), *Cold Spring Harbor Symp. Quant. Biol. 34,* 521.

Wilcox, M. (1969b), *Eur. J. Biochem. 11,* 405.

Wilcox, M., and Nirenberg, M. (1968), *Proc. Natl. Acad. Sci. U.S.A. 61,* 229.