Biochemistry

© Copyright 1975 by the American Chemical Society Volume 14, Number 10 May 20, 1975

Sequence Homology of the Mitochondrial Leucyl-tRNA Cistron in Different Organisms[†]

Smilja Jakovcic, James Casey,[‡] and Murray Rabinowitz*

ABSTRACT: Sequence divergence of the mitochondrial leucyl-tRNA cistron in several eukaryotes has been examined by RNA-DNA hybridization. Rat mitochondrial leucyl-tRNA was hybridized with rat, mouse, guinea pig, monkey, chicken, and yeast mitochondrial DNAs (mtDNA) immobilized on filters. Hybridization was carried out in 50% formamide (T_m −12°) or in 20% formamide (T_m −21°). Melting profiles of the hybrids were obtained for evaluation of the extent of base sequence mismatching. Under the more stringent hybridization conditions (50% formamide, T_m −12°), only mouse and guinea pig mtDNAs hybridized with rat mitochondrial leucyl-tRNA. The T_m's of the heterohybrids were depressed by 2 and 9°, respectively. Under less stringent hybridization conditions (T_m −21°), monkey mtDNA also hybridized, and the T_m was depressed by about 15°. Chicken and yeast mtDNAs did not form specific hybrids with rat mitochondrial leucyl-tRNA under these hybridization conditions. Mitochondrial leucyl-tRNA sequences in different eukaryotes appear to be conserved to a less extent than cytoplasmic rRNA, 5S RNA, or hemoglobin mRNA sequences.

Although the gene products of mitochondrial DNA (mtDNA) have not been completely defined, it has been shown that mitochondrial ribosomal RNA (Rifkin et al., 1967; Aloni and Attardi, 1971; Reijnders et al., 1972) and several mitochondrial tRNAs are coded for by mtDNA (Dawid, 1969; Dawid and Chase, 1972; Nass and Buck, 1969, 1970; Aloni and Attardi, 1971; Halbreich and Rabinowitz, 1971; Casey et al., 1972; Cohen and Rabinowitz, 1972; Wu et al., 1972). Furthermore, in yeast (Mason and Schatz, 1973) and *Neurospora* (Sebald et al., 1972), some components of cytochrome oxidase, cytochrome *b* (Weiss, 1972), and oligomycin-sensitive ATPase (Tzagaloff and Meagher, 1972) are synthesized by mitochondria, but absolute proof that they are specified by mtDNA is not yet available.

Hybridization experiments (Aloni and Attardi, 1971) and electron microscopic mapping of mitochondrial tRNA cistrons using ferritin-labeled tRNAs (Wu et al., 1972) indicate that HeLa cell mtDNA, and presumably mtDNA of other higher organisms, contain only 12 tRNA cistrons. If this is the case, either the proteins synthesized by mitochondria are deficient in some amino acids (Wu et al., 1972) or cytoplasmic tRNAs are transported into and function within mitochondria. In yeast, on the other hand, a full complement of tRNAs appears to be specified by mtDNA. All 14 tRNA species tested so far hybridize with mtDNA (Casey et al., 1974), and the hybridization level of total mitochondrial tRNA indicates that mtDNA contains more than 20 tRNA cistrons (Reijnders and Borst, 1972).

Since the circular mtDNAs of higher organisms uniformly have similar molecular sizes (about 5 μ), and since their gene products are closely related functionally, it was of interest to determine the extent to which specific mtDNA base sequences are conserved during evolution. A remarkable conservation of cytoplasmic ribosomal RNA sequences in organisms that are far removed from one another has been demonstrated (Sinclair and Brown, 1971; Bendich and McCarthy, 1970). In contrast to the conservation of rRNA sequences, which are presumably essential for ribosomal function, there was no homology between ribosomal "spacer" sequences even in closely related organisms (Brown et al., 1972). Thus, spacer sequences, which presumably have no informational content, evolve rapidly.

In this paper, we have examined the sequence homology of mitochondrial leucyl-tRNA cistrons in the rat, mouse, guinea pig, monkey, chicken, and in yeast. We have used the tRNA-DNA hybridization system first employed by Weiss et al. (1968), in which tRNA is acylated with tritiat-

---

[†] From the Departments of Medicine and Biochemistry, University of Chicago, and The Franklin McLean Memorial Research Institute,[§] Chicago, Illinois 60637. *Received December 18, 1974.* This work was supported in part by Grants HL04442, HL09172, and HL05673 from the National Institutes of Health, U.S. Public Health Service, and the Louis Block Fund of the University of Chicago.

[‡] Present address: Department of Chemistry, California Institute of Technology, Pasadena, California.

[§] Operated by the University of Chicago for the U.S. Atomic Energy Commission.

ed amino acids of high specific activity, and hybridization is carried out at low pH and at a low temperature in 50% formamide to minimize deacylation. The same system has subsequently been used by Nass and Buck (1969, 1970) to define some tRNA transcripts of rat liver mtDNA, and by Casey et al. (1969, 1972, 1974) and Cohen et al. (1972) to study mitochondrial tRNAs in grande and petite yeast.

The information obtained in this study will be compared in the following paper (Jakovcic et al., 1975) to the sequence homology of the total mtDNA estimated by DNA-DNA hybridization. In this way, it may be determined whether mitochondrial tRNA sequences are conserved to a greater extent than total mtDNA sequences.

Methods

Preparation of Closed Circular mtDNA. Mitochondria were prepared from livers of decapitated adult female rats, guinea pigs, mice, and chickens, and from the liver of an anesthetized rhesus monkey as previously described (Jakovcic et al., 1971). The mitochondrial pellets were lysed in 0.15 M NaCl, 0.01 M EDTA, and 1% sodium dodecyl sulfate for 30 min at 23°. The DNA was then extracted once with phenol saturated with 0.22 M sodium phosphate buffer (pH 7.4) at 4°. The aqueous layer was separated, extracted with ether, and passed through a 10 cm × 2.5 cm hydroxylapatite column (Bernardi, 1971) which had been equilibrated in 0.1 M sodium phosphate buffer (pH 6.8). The column was washed with 0.2 M sodium phosphate buffer (pH 6.8) until $A_{260\text{nm}}$ reached zero, and the DNA was eluted with 0.4 M sodium phosphate buffer (pH 6.8). Closed circular DNA was then isolated from the pooled samples by the procedure of Hudson et al. (1969). Propidium iodide was added to a final concentration of 300 μg/ml and the density adjusted to 1.58 g/cm^3 with CsCl. The samples were centrifuged at 40,000 rpm in a SW50 Beckman rotor for 60 hr. The centrifuge tubes were punctured, and 40-50 fractions were collected. The fluorescence intensities were measured with an Aminco-Bowman spectrofluorometer with excitation-emission wavelengths of 548 and 590 nm, respectively. Samples containing covalently closed DNA were pooled and the propidium iodide was removed by passage through a 3 cm × 0.5 cm Dowex 50 column (Radloff et al., 1967). The DNA was stored at −20°.

Preparation of Rat Liver Mitochondrial tRNA. Mitochondria were prepared from livers of decapitated adult female rats as previously described (Jakovcic et al., 1971). The mitochondrial pellets were suspended in the solution containing 0.02 M Tris-HCl buffer (pH 7.5), 0.15 M NaCl, 2% sodium dodecyl sulfate, and 0.1% Macaloid (w/v) for 15 min at room temperature. The tRNA was then extracted by the method of Casey et al. (1972). The suspension was deproteinized four times with equal volumes of phenol-cresol (Kirby, 1965), followed by four ether extractions of the aqueous phase. Ether was removed by bubbling N_2 through the solution. The RNA was precipitated with 2 volumes of absolute ethanol at −20° and collected by centrifugation. The tRNA was deacylated in 1.0 M Tris buffer (pH 8.8) by incubation for 1 hr at 37°. The RNA was then precipitated with 0.1 volume of 20% potassium acetate and 2 volumes of absolute EtOH at −20°, and the precipitate was collected by centrifugation. The precipitate was dissolved in 0.1 × standard sodium citrate, SSC (SSC is 0.015 M sodium citrate-0.15 M NaCl), and rRNA was precipitated by addition of an equal volume of 4 M LiCl (Avital and Elson, 1969). The tRNA was dialyzed against 0.001 M magnesium acetate overnight at 4° and again precipitated with 0.1 volume of 20% potassium acetate and 2 volumes of absolute ethanol at −20°. The tRNA was finally dissolved in 0.01 M Tris buffer (pH 7.8) and stored at −20°.

Preparation of Mitochondrial Aminoacyl-tRNA Synthetases. Aminoacyl-tRNA synthetases were prepared from rat liver mitochondria by the method of Buck and Nass (1968). The mitochondrial pellets were suspended in 0.1 M Tris (pH 7.8), 0.01 M KCl, 0.01 M $MgCl_2$, and 0.006 M mercaptoethanol. The suspensions were sonicated with a Branson sonifier at 4° for eight 15-sec periods and cooled for 45 sec between pulses. The suspension was then homogenized with five strokes in a glass Potter-Elvejhem homogenizer. The solution was centrifuged at 100,000g for 1 hr, and the supernatant was passed through a Sephadex G-100 column (2.5 cm × 10 cm) equilibrated with the buffer used to suspend the mitochondria. The protein eluting in the void volume was collected and stored at −20° in 25% glycerol.

Aminoacylation of tRNA. Mitochondrial tRNA was acylated with [^{3}H]leucine (40 Ci/mmol; New England Nuclear Corp., Boston, Mass.) in a reaction mixture of the following composition: 0.1 M Tris-HCl (pH 7.8), 0.01 M $MgCl_2$, 0.01 M KCl, 0.001 M ATP, 1×10^{-4} M CTP, 0.006 M 2-mercaptoethanol, 1 mCi of [^{3}H]leucine, 750 μg of tRNA, and 14 mg of mitochondrial aminoacyl synthetase protein in a final volume of 3 ml. After incubation at 37° for 30 min, 1 volume of 0.4 M sodium acetate, (pH 5.2), 0.4 M NaCl, and 0.01 MgCl was added, and the solution was extracted at 4° with 1 volume of phenol-cresol mixture (Kirby, 1965). The phenol-cresol phase was reextracted with an equal volume of 0.05 M ammonium acetate (pH 5.2). The combined aqueous phase was extracted with ether and the ether removed by bubbling N_2 through the solution.

A tenth volume of 25% diethyl pyrocarbonate (Abadom and Elson, 1970) was added and the tRNA was loaded at 4° on a Sephadex G-100 column (2.5 cm × 100 cm) that was equilibrated in 0.05 M ammonium acetate buffer (pH 5.2) (Weiss et al., 1968). The eluted tRNA was precipitated with 2.5 volumes of ethanol, resuspended in 0.05 M ammonium acetate (pH 5.2) and stored at −80°.

Hybridization Procedures. Hybridization was carried out in formamide at low temperature by procedures modified from those of Weiss et al. (1968), which are similar to those subsequently used by Nass and Buck, (1969) and by Casey et al. (1972). The mtDNA was denatured according to the procedure of Nass and Buck (1969). Solutions of DNA were first dialyzed for 2 hr against 1:100 SSC and sheared by five passages through a 25-gauge needle of a disposable tuberculin syringe. Next, the DNA solution was heated to 100° for 10 min and rapidly cooled on ice. It was then denatured again in 0.1 M NaOH for 10 min. After neutralization to pH 7, the SSC concentration was adjusted to 6 ×, and the DNA was slowly applied to membrane filters (Schleicher and Schuell Selectron 25 mm). The filters were air-dried at room temperature overnight and then dried in a vacuum oven at 88° for 2 hr. All the DNA was retained on the filters, as indicated by quantitative DNA determinations according to the method of Gillespie and Gillespie (1971).

Hybridizations were carried out in 20 or 50% formamide, 2 × SSC, 0.05 M ammonium acetate buffer, and varying concentrations of [^{3}H]leucyl-tRNA. The final pH of the mixture was adjusted to 5.0 (pH meter reading). Annealing was carried out for 17 hr at 33°. The filters were washed in

1 l. of 2 × SSC (pH 5.2) for 13 min, treated with 25 units/ml T1 RNase in 2 × SSC (pH 5.2) at 37° for 30 min, and washed again in 1 l. of 2 × SSC. The filters were then incubated at 38° for 1 hr in the same solution used for hybridization, except without [^{3}H]leucyl-tRNA, to lower the nonspecific binding (Gillespie and Gillespie, 1971). The filters were again washed in 1 l. of 2 × SSC for 15 min and then dried and counted in 15 ml of a toluene-based scintillation mixture (0.5% 2,5-diphenyloxazole and 0.05% 1,4-bis[2-(5-phenyloxazolyl)]benzene), using a Packard liquid scintillation spectrometer.

Thermal Stability Measurements. Melting curves were obtained from mitochondrial [^{3}H]leucyl-tRNA-mtDNA hybrids bound to filters following the procedure of Gordon and Rabinowitz (1973). Filters which had been counted in the scintillation counter were washed four times in 30 ml of toluene and air-dried. The filters were then incubated in successive vials with 0.6 ml of 2 × SSC and 20 or 50% formamide. Temperature increments were 5°, and each temperature was maintained for 5 min; 0.4 ml of H_2O and 15 ml of Triton scintillation fluid were added to each vial for counting. Each liter of Triton fluid contained 5.0 g of 2,5-diphenyloxazole, 0.1 g of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene, and 250 ml of Triton X-100 (Emulsion Engineering, Inc., Elk Grove Village, Ill.). The counts released from the hybrid at successive temperatures were used to construct the melting curves.

Results

Hybridization of Rat Liver Mitochondria [^{3}H]leucyl-tRNA with mtDNAs Under Restrictive Conditions (50% Formamide ≃ T_m −12°). The mtDNAs used were quite pure, since all except yeast mtDNA were isolated from mitochondria as covalently closed circular molecules by use of propidium iodide-CsCl gradients (Hudson et al., 1969). The purity of yeast and chicken mtDNA was further documented by their characteristic buoyant densities when they were analyzed by CsCl isopyknic gradient centrifugation in the analytic ultracentrifuge.

The hybridization of rat liver mitochondrial [^{3}H]leucyl-tRNA to filters charged with 10 µg of rat, mouse, guinea pig, monkey, and chicken liver mtDNA is shown in Figure 1. Saturation of rat mtDNA appears to be reached with the leucyl-tRNA concentrations used in the experiment. The hybridization levels with mouse and guinea pig mtDNA were about 55 and 30%, respectively, of the level obtained in the homologous rat tRNA-rat mtDNA hybridization (Figure 1). Binding to chicken mtDNA was only 2% of that with rat mtDNA, and binding to monkey and yeast mtDNA was not significantly greater than that obtained with *Escherichia coli* DNA or with blank filters containing no DNA. Thus there seems to be considerable sequence homology of the leucyl mitochondrial tRNA cistron in rat, mouse, and guinea pig, but little or no cross-hybridization in organisms that are less closely related.

The thermal stability of the leucyl-tRNA-mtDNA hybrids was examined to evaluate further the homology of the mitochondrial leucyl-tRNA cistron in the rat, mouse, and guinea pig. Composite melting curves representing data from two to four separate determinations are shown in Figure 2A. The T_m of the homologous rat tRNA-rat mtDNA hybrid was 45°, the T_m of the rat tRNA-mouse mtDNA hybrid was 43°, and that for the rat tRNA-guinea pig mtDNA was 36°. The tRNA-chicken mtDNA hybrids released radioactivity over a broad temperature range, with no evidence for specific melting. The modest depression in T_m (2°) of the rat tRNA-mouse mtDNA hybrid, and the greater decrease (9°) for the rat tRNA-guinea pig mtDNA hybrid, reflect an increasing extent of base sequence mismatching in these heterohybrids.

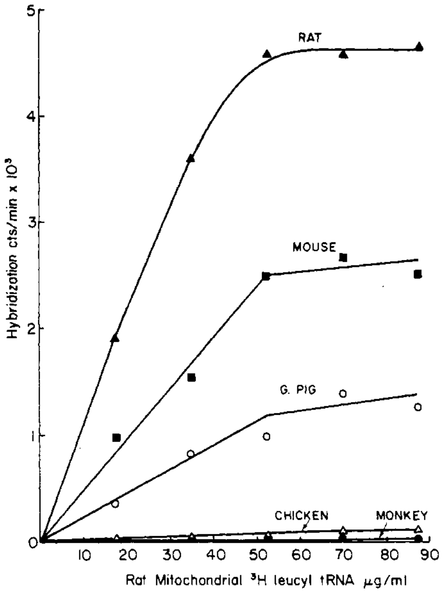

FIGURE 1: Hybridization of rat mitochondrial leucyl-tRNA with mtDNA of various eukaryotes. Filters containing 10 µg of rat (▲), mouse (■), guinea pig (○), monkey (●), chicken (△), and yeast (not shown) mtDNAs were hybridized with increasing concentrations of rat mitochondrial [^{3}H]leucyl-tRNA of specific radioactivity 3.6 × 10^3 cpm/µg of RNA, as described in Methods. Blank filters containing no DNA, or *E. coli* DNA, bound 13-65 cpm, which were subtracted from the counts bound to the other filters. Yeast mtDNA bound 2-30 cpm above blank values. The eight filters were incubated together in a reaction mixture having a final volume of 0.7 ml.

Hybridization Under Less Stringent Conditions (20% Formamide ≃ T_m −21°). Since the T_m of the homologous rat tRNA-rat mtDNA hybrid was only about 12° above the temperature used for annealing, mitochondrial leucyl tRNA cistrons that deviate in base sequence by more than about 15% from that in the rat would not be expected to form a stable hybrid (assuming that there is a 1° drop in melting temperature for each 1.4% difference in base sequence [Laird et al., 1969]). Therefore, we examined the hybridization levels under less stringent annealing conditions. The formamide concentration in the hybridization reaction was lowered from 50 to 20%, which would be expected to raise the T_m by 18 to 21° (Blüthmann et al., 1973; McConaughy et al., 1969). In fact, the effect of formamide on the T_m of the tRNA-mtDNA hybrids was less than previously reported for other hybridization systems. The T_m of the rat leucyl tRNA-rat mtDNA homohybrid in 20% formamide was increased by only about 9° to approximately 54° (Figure 2B). Hybridization in 20% formamide at 33° therefore represents annealing at about 21° below the T_m of the hybrid.

Hybridization results under these conditions are shown in Table I. The absolute hybridization levels with rat, mouse, and guinea pig mtDNA at high tRNA input are 50-90% of those obtained in 50% formamide, but the order of hybridization remains the same, i.e., rat > mouse > guinea pig. The most striking change is greatly enhanced hybridization with monkey mtDNA, raising it to a level similar to that for guinea pigs. Hybridization to chicken and yeast mtDNAs was negligible under these conditions, too.

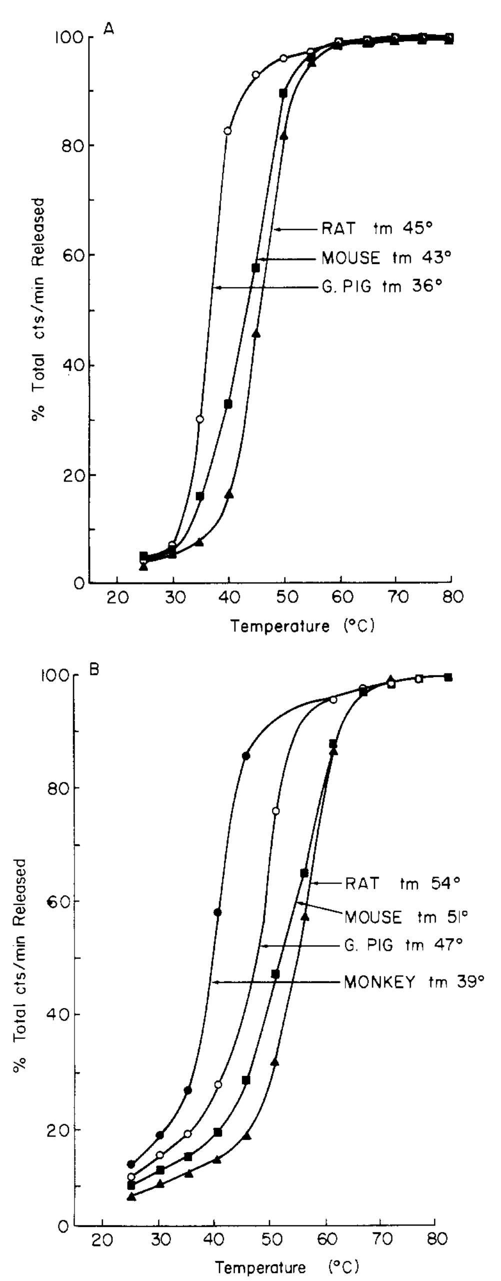

FIGURE 2: Melting profiles of rat mitochondrial leucyl-tRNA-mtDNA hybrids. (A) Hybrids formed and melted in 50% formamide. (B) Hybrids formed and melted in 20% formamide. Filters containing [^{3}H]leucyl-tRNA-mtDNA hybrids were incubated in 2 × SSC, and 20 or 50% formamide, and the radioactivity released at 5° temperature increments was measured as described in Methods. The filters contained 1.2-4.8 × 10^3 cpm in (A), and 0.89-2.4 × 10^3 cpm in (B). Rat tRNA-rat mtDNA hybrid (▲), rat tRNA-mouse mtDNA hybrid (■), rat tRNA-guinea pig mtDNA hybrid (○), and rat tRNA-monkey mtDNA hybrid (●) are shown. The chick mtDNA maximally bound 136 cpm above blank values and showed a broad, nonspecific melting profile.

Table I: Hybridization of Rat Mitochondrial Leucyl-tRNA of Different Organisms in 50 and 20% Formamide.[a]

	Hybridization (cpm)	
mtDNA	50% Formamide	20% Formamide
Rat	4660	2346
Mouse	2313	1710
Guinea pig	1272	1116
Monkey	8	890
Chicken	117	136
Yeast	17	47
E. coli	0	0
None	65[b]	131[b]

[a] Filters charged with 10 μg of DNA were hybridized to apparent saturation (see Figure 1) with 87.5 μg/ml of rat mitochondrial [^{3}H]-leucyl-tRNA in a final reaction volume of 0.7 ml, as described in the legend of Figure 1. [b] Blank values that were subtracted from the other data.

Melting curves of the [^{3}H]leucyl-tRNA-mtDNA hybrids formed in 20% formamide at 33° and melted in 20% formamide are shown in Figure 2B. All of the hybrids displayed a higher fraction of radioactivity released at low temperatures than that observed when hybridization was carried out at 50% formamide. The T_m of the heterohybrids between rat tRNA and mouse and guinea pig mtDNA hybrids were again slightly depressed, below that of the homohybrid, i.e., 3 and 7°, and the T_m of the tRNA-monkey mtDNA hybrid was depressed by 15°. Hybrids with chicken mtDNA gave broad melting curves, probably due to nonspecific binding.

Hybridization carried out in the absence of formamide, or in 10% formamide, resulted in greatly increased nonspecific binding to filters and depressed hybridization levels that prevented a clear-cut interpretation of these data.

Discussion

The results presented in this paper indicate a fairly close homology among mitochondrial leucyl-tRNA cistrons of rat, mouse, and guinea pig. Under stringent hybridization conditions (T_m −12°), substantial hybridization of rat leucyl-tRNA with mouse and guinea pig mtDNAs was observed. At input levels of tRNA that gave apparent saturation, hybridization to mouse and guinea pig mtDNA was respectively about 55 and 30% of that in the homologous rat tRNA-rat mtDNA reaction. In comparison with the homohybrids, melting curves showed a 2° depression in T_m for the rat tRNA-mouse mtDNA heterohybrid and a 9° depression for the rat tRNA-guinea pig mtDNA heterohybrid. Thus, there is approximately a 3-13% base sequence mismatching in these hybrids (Laird et al., 1969). Under the same stringent hybridization conditions (T_m −12°), rat mitochondrial leucyl-tRNA did not hybridize significantly with monkey, chicken, or yeast mtDNAs or with *E. coli* DNA. Differences in base sequence therefore must be greater than about 15% (Laird et al., 1969), the maximal degree of mismatching that would be compatible with detectable hybridization under the conditions selected.

When annealing conditions were relaxed (T_m −21°) by hybridizing in 20% formamide solution, substantial hybridization was also seen between rat leucyl-tRNA and monkey mtDNA. The T_m of the rat leucyl-tRNA-monkey mtDNA hybrid was 15° below that of the homohybrid. Again there was no significant hybridization with chicken or yeast mtDNAs, or with *E. coli* DNA. Differences in sequence homology between rat mitochondrial leucyl-tRNA and the leucyl-tRNA cistrons in these DNAs must therefore be greater than about 30%. Hybridization conditions that were even less stringent led to high nonspecific binding to filters and prevented us from evaluating greater degrees of sequence mismatching.

It should be noted that the leucyl-tRNA used in our hybridization system is labeled exclusively in the aminoacyl group. Hybridization therefore involves base pairing of an undetermined number of sequences that must include those at the aminoacyl end of the tRNA. This hybridization system has proven to be quite specific and has been effective in the discrimination of T_4 and *E. coli* tRNAs (Weiss et al., 1968), as well as mitochondrial and cytoplasmic isoaccepting tRNA species in yeast (Cohen et al., 1972, Casey et al., 1972), *Tetrahymena* (Chiu et al., 1974), and rat liver (Buck and Nass, 1968). Since the tRNA is labeled only in the aminoacyl group, comparison of hybridization with a variety of mtDNAs at truly saturating conditions provides information only about the relative number of leucyl-tRNA cistrons present in each mitochondrial genome, and not about the relative sequence homology of the tRNA cistron. If each of the various mtDNAs contained one leucyl-tRNA cistron, or equal numbers of cistrons, hybridization levels would be identical as long as sequence differences were small enough to allow saturation. It is likely that the lower hybridization levels we obtained with the heterohybrids was due to our inability to achieve saturation of the available leucyl-tRNA cistrons. Our heterohybridization reactions were probably rate limited because of the base sequence divergence in the various leucyl-tRNAs. Sequence mismatching may considerably decrease the rate of hybridization (Sutton and McCallum, 1971; McCarthy and Farquhar, 1972; Bonner et al., 1973), as well as the stability of the hybrids, and thus lead to substantial depression in the observed levels of hybridization. However, the different levels of hybridization observed probably do reflect differences in sequence divergence since the rate of hybridization is related to fidelity of base pairing. The extent of sequence divergence, however, is probably better measured by the depression in melting temperatures of the heterohybrids as discussed above.

Another possible explanation for the various levels of hybridization observed is that there are several leucyl-tRNA cistrons coding for isoaccepting mitochondrial leucyl-tRNA species, which have different degrees of cross-homology in the different organisms. Isoaccepting mitochondrial tRNA species have been reported in *Tetrahymena* (Chiu et al., 1974), and we have established separate cistrons for two glutamyl mitochondrial tRNA species in yeast (N. Hinckley and M. Rabinowitz, unpublished observation). This possibility seems less likely in higher organisms like the rat if indeed HeLa cells are representative of these organisms in containing only 12 mitochondrial tRNA cistrons in its mtDNA (Wu et al., 1972). Recently, however, Wallace and Freeman (1974) have separated four Met-tRNAs from rat liver mitochondria, but it has not been established that they are derived from separate mitochondrial cistrons. In any case, we may conclude that there is considerable conservation of the mitochondrial leucyl-tRNA sequences in the rat, mouse, and guinea pig.

The conservation of the mitochondrial leucyl-tRNA sequences in the species studied (chicken may be an exception) appears to be greater than that of the overall conservation of mtDNA sequences measured by DNA-DNA hybridization, as presented in the accompanying paper (Jakovcic et al., 1975). Only 20-70% of the sequences in the mammalian and chicken mtDNAs examined hybridized with rat mtDNA (T_m −35°), and a 18-24° T_m depression was observed in the heteroduplexes formed. However, the sequence divergence observed for mitochondrial tRNA cistrons appears to be considerably greater than that noted for cytoplasmic rRNA (Brown et al., 1972), or for hemoglobin mRNA (Gummerson and Williamson, 1974), where sequences are conserved to a remarkable degree in widely divergent species.

Limited data derived from sequence analysis are available concerning the conservation of nonmitochondrial tRNA sequences in different organisms (reviewed by Holmquist et al., 1973). Considerable sequence divergence is obtained in tRNAs of different species, although in some cases there is sufficient similarity to indicate a common evolutionary origin. Striking sequence homology has been observed, however, in some isoaccepting species of tRNA in a given organism (Yaniv and Barrel, 1969; Egan et al., 1973). Sufficient information is not available to compare quantitatively the sequence divergence in cytoplasmic and mitochondrial tRNAs of the organisms studied in this investigation.

It may be of considerable practical interest that, when suitable hybridization conditions are selected, there is essentially no cross-hybridization between rat mitochondrial leucyl-tRNA and monkey mtDNA. Therefore this system may prove useful in locating rodent or primate mitochondrial leucyl-tRNA cistrons in mouse-human or rat-human cell hybrids.

Acknowledgments

We greatly appreciate the able technical assistance of Miss Rufina Nepomuceno, and the stimulating discussions and critical review of the manuscript by Dr. Godfrey S. Getz.

References

Abadom, P. N., and Elson, D. (1970), *Biochim. Biophys. Acta 199,* 528.

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

Avital, S., and Elson, D. (1969), *Biochim. Biophys. Acta 179,* 297.

Bendich, A., and McCarthy, B. J. (1970), *Proc. Natl. Acad. Sci. U.S.A. 65,* 349.

Bernardi, G. (1971), *Methods Enzymol.* 95.

Blüthmann, H., Brück, D., Hübner, L., and Schöffski, A. (1973), *Biochem. Biophys. Res. Commun. 50,* 91.

Bonner, T. I., Brenner, D. J., Neufeld, B. R., and Britten, R. J. (1973), *J. Mol. Biol. 81,* 123.

Brown, D. D., Wensink, P. C., and Jordan, E. (1972), *J. Mol. Biol. 63,* 57.

Buck, C. A., and Nass, M. M. K. (1968), *Proc. Natl. Acad. Sci. U.S.A. 60,* 1045.

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. W., Hsu, H. J., Getz, G. S., Rabinowitz, M., and Fukuhara, H. (1974), *J. Mol. Biol. 88,* 735.

Chiu, N., Chiu, A. O. S., and Suyama, Y. (1974), *J. Mol. Biol. 82,* 441.

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

Cohen, M., and Rabinowitz, M. (1972), *Biochim. Biophys. Acta 281,* 192.

Dawid, I. B. (1969), *Symp. Soc. Exp. Biol. 24,* 227.

Dawid, I. B., and Chase, J. W. (1972), *J. Mol. Biol. 63,* 201.

Egan, Z. B., Weiss, J. F., and Kelmers, A. D. (1973), *Biochem. Biophys. Res. Commun. 55,* 320.

Gillespie, S., and Gillespie, D. (1971), *Biochem. J. 125,* 481.

Gordon, P., and Rabinowitz, M. (1973), *Biochemistry 12,* 116.

Gummerson, K. S., and Williamson, R. (1974), *Nature (London) 274,* 265.

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

Holmquist, R., Jukes, T. H., and Pangburn, S. (1973), *J. Mol. Biol. 78,* 91.

Hudson, B., Upholt, W. B., Devinny, J., and Vinograd, J. (1969), *Proc. Natl. Acad. Sci. U.S.A. 62,* 813.

Jakovcic, S., Casey, J., and Rabinowitz, M. (1975), *Biochemistry,* following paper in this issue.

Jakovcic, S., Haddock, J., Getz, G. S., Rabinowitz, M., and Swift, H. (1971), *Biochem. J. 121,* 341.

Kirby, K. S. (1965), *Biochem. J. 96,* 266.

Laird, C. D., McConaughy, B. L., and McCarthy, B. J. (1969), *Nature (London) 224,* 149.

Mason, T. L., and Schatz, G. (1973), *J. Biol. Chem. 248,* 1355.

McCarthy, B. J., and Farquhar, M. N. (1972), *Brookhaven Symp. Biol. 23,* 1.

McConaughy, B. L., Laird, C. D., and McCarthy, B. J. (1969), *Biochemistry 8,* 3289.

Nass, M. M. K., and Buck, C. A. (1969), *Proc. Natl. Acad. Sci. U.S.A. 62,* 506.

Nass, M. M. K., and Buck, C. A. (1970), *J. Mol. Biol. 54,* 187.

Radloff, R., Bauer, W., and Vinograd, J. (1967), *Proc. Natl. Acad. Sci. U.S.A. 57,* 1514.

Reijnders, L., and Borst, P. (1972), *Biochem. Biophys. Res. Commun. 47,* 126.

Reijnders, L., Kleisen, C. M., Grivell, L. A., and Borst, P. (1972), *Biochim. Biophys. Acta 272,* 396.

Rifkin, M. R., Wood, D. D., and Luck, D. J. L. (1967), *Proc. Natl. Acad. Sci. U.S.A. 58,* 1025.

Sebald, W., Weiss, H., and Jackl, G. (1972), *Eur. J. Biochem. 30,* 413.

Sinclair, J. H., and Brown, D. D. (1971), *Biochemistry 10,* 2761.

Sutton, W. D., and McCallum, M. (1971), *Nature (London), New Biol. 232,* 83.

Tzagaloff, A., and Meagher, P. (1972), *J. Biol. Chem. 247,* 594.

Wallace, R. B., and Freeman, K. B. (1974), *Biochem. Biophys. Res. Commun. 60,* 1440.

Weiss, H. (1972), *Eur. J. Biochem. 30,* 469.

Weiss, S. B., Hsu, W.-T., Foft, J. W., and Scherberg, N. H. (1968), *Proc. Natl. Acad. Sci. U.S.A. 61,* 114.

Wu, M., Davidson, N., Attardi, G., and Aloni, Y. (1972), *J. Mol. Biol. 71,* 81.

Yaniv, M., and Barrel, B. G. (1969), *Nature (London) 222,* 278.