Modification Defect at Anticodon Wobble Nucleotide of Mitochondrial tRNAs\textsuperscript{Leu(UUR)} with Pathogenic Mutations of Mitochondrial Myopathy, Encephalopathy, Lactic Acidosis, and Stroke-like Episodes\textsuperscript{*}

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The mitochondrial tRNAs\textsuperscript{Leu(UUR)} (R = A or G) gene possesses several hot spots for pathogenic mutations. A point mutation at nucleotide position 3243 or 3271 is associated with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes and maternally inherited diabetes with deafness. Detailed studies on two tRNAs\textsuperscript{Leu(UUR)} with the 3243 or 3271 mutation revealed some common characteristics in cybrid cells: (i) a decreased life span, resulting in a 70% decrease in the amounts of the tRNAs in the steady state, (ii) a slight decrease in the ratios of aminoacyl-tRNAs\textsuperscript{Leu(UUR)} versus uncharged tRNAs\textsuperscript{Leu(UUR)}, and (iii) accurate aminoacylation with leucine without any misacylation. As a marked result, both of the mutant tRNA molecules were deficient in a modification of uridine that occurs in the normal tRNAs\textsuperscript{Leu(UUR)} at the first position of the anticodon. The lack of this modification may lead to the mis-translation of leucine into non-cognate phenylalanine codons by mutant tRNAs\textsuperscript{Leu(UUR)}, according to the mitochondrial wobble rule, and/or a decrease in the rate of mitochondrial protein synthesis. This finding could explain why two different mutations (3243 and 3271) manifest indistinguishable clinical features.

Point mutations in mitochondrial tRNA genes are frequently found in mitochondrial diseases leading to neuromuscular disorders (1). One of the major clinical subgroups of the mitochondrial encephalomyopathies, mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS),\textsuperscript{1} is caused by a single base replacement in the tRNAs\textsuperscript{Leu(UUR)} gene corresponding to the UUR (R = A or G) leucine codons (tRNAs\textsuperscript{Leu(UUR)}) (2). The majority (80%) of MELAS patients have an A to G transition at nucleotide position (np) 3243 (3, 4), whereas in about 10%, a T to C transition is observed at np 3271 (5). Mutations at np 3243 and 3271 have also been found to be associated with diabetes subgroup named maternally inherited diabetes with deafness (6, 7). Since approximately 1% of patients with diabetes carry 3243 mutant mitochondrial DNAs (mtDNA) in a heteroplasmic manner (8), the mitochondrial tRNAs\textsuperscript{Leu(UUR)} gene appears to be critically involved not only in neuromuscular disorders but also in some more generalized diseases.

Despite the fact that the two mutations occur in different regions of the tRNAs\textsuperscript{Leu(UUR)} gene (np 3243 is in the D-loop at G-14, and np 3271 is in the anticodon stem at C-40), the clinical symptoms they give rise to appear identical. In contrast, point mutations in the tRNAs\textsuperscript{Leu(UUR)} gene are responsible for another subgroup of the mitochondrial encephalomyopathies, myoclonus epilepsy associated with ragged-red fibers (MERRF), which is distinguishable from MELAS by certain differences in its clinical presentation (9, 10). Thus, the clinical features of mitochondrial encephalomyopathies depend on the species of the tRNA gene in which the mutation is located. It has yet to be clarified why point mutations at different locations in the same tRNA gene should bring about indistinguishable symptoms. It also remains unknown why the phenotypes are distinct, corresponding to particular tRNA genes with point mutations. To answer these questions, the effects arising from different mutations in the same tRNA gene need to be investigated at the molecular level.

Mutations in the mitochondrial genome are themselves directly responsible for decreased respiratory chain activity or oxygen consumption without nuclear gene involvement. This has been demonstrated by constructing cybrid cell lines in which mutant mtDNAs derived from patients are intercellularly transferred into human cells lacking mtDNA (\textsuperscript{11–13}). Unusual RNA processing (14) or a termination of RNA synthesis (15) have been proposed as possible outcomes arising directly from mitochondrial mutations, which would in turn cause a decrease in respiratory activity. However, there is as
yet no conclusive evidence to support these proposals.

In the case of MERRF, it has been shown that premature polypeptides are generated by the point mutation in the tRNA^{A99} gene (16). In MELAS, on the other hand, a decrease in protein synthesis was observed in cybrid cells containing more than 95% mtDNA mutated at np 3243; however, the extent of the deficiency in protein synthesis did not seem to parallel the decline in enzymatic activity (17, 18). Similarly, in cybrid cells with homoplasmic mtDNA mutated at np 3271, complex I activity was severely reduced, but protein synthesis was only slightly lower than normal (19). Since the decreases in protein synthesis are relatively modest in both mutations, they cannot in themselves explain the marked reductions observed in respiratory enzymatic activities. That is, the modest decrease in protein synthesis observed in cells with exclusively mutant mtDNA does not appear to be the direct cause of the clinical symptoms presented by the disease.

The possibility remains that abnormalities in tRNA molecules with pathogenic mutations are the cause of mitochondrial diseases; it could be that these tRNAs induce the misincorporation of amino acids. However, this line of investigation has been little pursued because a chemical amount of the mutant tRNA has not been purified, probably due to technical difficulties. We have succeeded in purifying the mutant tRNA{Leu(UUR)} molecules in a chemical amount by taking advantage of the solid phase probing method we have developed (20), revealed that a modification of the uridine at the first position of the anticodon is missing in both the mutants. This common abnormality might provide an insight into the pathogenesis of MELAS at the translational level.

**Experimental Procedures**

**Cybrid Cell Lines—**Cybrid cell lines were constructed by intercellular transfer of patient mtDNA to HeLa cells (EB8) and isolation of cybrid clones as described (13). Two cybrid cell lines were constructed by fusing EB8 cells with enucleated fibroblasts from a MELAS patient with the heteroplasmic T3271C mutation: the ML5-1-13 cell line exclusively containing mtDNA with the 3271 mutation and the ML5-15 cell line containing only normal mtDNA as a control cybrid cell line (19). Mitochondrial DNA with the 3243G mutation and 500 mtDNA molecules from a MELAS patient with the 3243 mutation was transferred to EB8 cells, and the cybrid cell line ML2-2 possessing more than 90% of the A3243G-mutated mtDNA was obtained. The FT2-11 cell line for use as a wild-type control was obtained by fusing EB8 cells with enucleated fetal human fibroblasts. The cybrid cells were cultured in normal medium (Dulbecco’s modified Eagle’s medium/F-12 (1:1) (Life Technologies, Inc.), 10% fetal calf serum).

**Quantification of tRNAs Using Northern Blot Analysis—**Total RNA from semiconfluent cultured cybrid cells was isolated by treatment with 1 M NaCl and fractionated on a DEAE-Sepharose Fast Flow column (1 × 45 cm) with a linear gradient of NaCl and MgCl2 between 8 and 16 M. tRNAs were monitored by dot hybridization. tRNA Leu(UUR) was labeled with [32P]ATP and dephosphorylated by treatment with RNase P1 (Seikagaku Kogyo). The resultant 5′-labeled fragments was then digested completely by RNase T1 (Amersham Pharmacia Biotech), U2 (Seikagaku Kogyo, Tokyo, Japan), T4 (Amersham Pharmacia Biotech) and T4 polynucleotide kinase (Toyobo, Osaka, Japan). The nucleotide-specific RNases used for restricted digestion of tRNA were RNase T1 (Amersham Pharmacia Biotech), U2, T4, (Seikagaku Kogyo, Tokyo, Japan), PhyM (Amersham Pharmacia Biotech), and CL2 (Roche Molecular Biochemicals). For the Kuchino et al. method (25), the homogeneous tRNA was labeled at the 5′ terminus with [γ-32P]ATP (110 TBq/mmol, Amersham Pharmacia Biotech) and T4 polynucleotide kinase (Toyobo, Osaka, Japan). The nucleotide-specific RNases used for restricted digestion of tRNA were RNase T1 (Amersham Pharmacia Biotech), U2, T4, (Seikagaku Kogyo, Tokyo, Japan), PhyM (Amersham Pharmacia Biotech), and CL2 (Roche Molecular Biochemicals). For the Kuchino et al. method (25), the homogeneous tRNA was heated at 95 °C for 1.5 min in H2O for random cleavage at a single site. The 3′ half fragments of the single-site-digested tRNA were labeled with [γ-32P]ATP and T4 polynucleotide kinase and electrophoresed in 12 or 15% polyacrylamide denaturing gel. The ladders of 32P-5′-labeled fragments were cut out one by one and eluted from the gel. Each of the 5′-labeled fragments was then digested completely by RNase P1 (Seikagaku Kogyo). The resultant 5′-labeled nucleotides were analyzed one by one by two-dimensional thin-layer chromatography with two different solvent systems. Solvent system A consisted of isobutyric acid/concentrated ammonia/H2O (66:1:33 by volume) in the first dimension and 2-propanol/HCl/H2O (70:15:15 by volume) in the second dimension. Solvent system B was used as described above. Nucleoside Analysis of tRNA{Leu(UUR)} by LC/MS Using Electrospray Ionization (ESI)/Ion Trap Mass Spectrometry—A Finnigan LCQ ion trap mass spectrometer (Thermoquest) equipped with an ESI source.
Modification Defect at Wobble Position in Mitochondrial tRNA

RESULTS
Decreased Life Spans of Both Mutant tRNAsLeu(UUR)-(A3243G) and tRNAsLeu(UUR)/tRNAsLeu(A3243G)—Resulting in Lower Steady-state Amounts—We first quantified the steady-state amounts of the mutant tRNAsLeu(UUR) by Northern blotting; the values were normalized by the amount of nuclear-encoded 5S rRNA as an internal standard. The steady-state amounts of the wild-type tRNAsLeu(UUR) with the 3243 mutation (tRNAsLeu(UUR)/tRNAsLeu(A3243G)) and of tRNAsLeu(UUR) with the 3271 mutation (ML5-15) and mutants 3271 (ML-5-1-13) and 3243 (ML2-2) were estimated to be 77%, 68%, and 65%, respectively. Since, as noted above, the mutant tRNAsLeu(UUR) amount to approximately 30% of the wild-type tRNA, the total mutant aminocyl-tRNAs were estimated to comprise less than 30% of the wild-type aminocyl-tRNA.

Defect in the Modification at the Anticodon First Position of Both Mutant tRNAsLeu(UUR)/(A3243G) and tRNAsLeu(UUR)/tRNAsLeu(A3243G)—We purified the wild-type and mutant tRNAsLeu(UUR) in amounts sufficient for structural analysis from mass cultures of cybrid cells and determined the nucleotide se-
sequences, including modified nucleotides, by combining the methods of Donis-Keller (24) and Kuchino et al. (25). Sequence analysis by the Donis-Keller method (24) of the wild-type and two mutant tRNAsLeu(UUR) labeled at their 5'-ends revealed that tRNA^{Leu(UUR)} from the A3243G mutant had the A to G transition in the anticodon stem (Fig. 3, a and b), whereas that from the T3271C mutant had the U to C transition in the D-loop, suggesting that it is probably not a usual uridine but a modified uridine (U*) observed in the wild-type tRNA Leu(UUR) (Fig. 3b). Absence of the modified uridine from the mutant tRNA with the 3271 mutation was also confirmed by the LC/MS analysis (Fig. 4, bottom panel).

In the case of the ML2-2 (A3243G) mutant cells, which possess both the mutant tRNA and a small amount of the wild-type (Fig. 3a, upper panel), both unmodified and modified uridine were detected at the wobble position (Fig. 3a, lower panel and 3b). This modified uridine was identical to that found recently by our group at the wobble position of bovine mitochondrial tRNA^{Leu(UUR)} (27, 28) - but the chemical structure is unknown. Although no digestion was observed at position U33 with PhyM, the alkaline ladder was normal and this residue was confirmed to be unmodified uridine by two-dimensional TLC analysis (not shown). The resistance against PhyM digestion was probably due to the influence of the modification at U34, since such a phenomenon is sometimes observed around a sequence containing a modified nucleotide(s).

This novel modified uridine (U*) was characterized by nucleoside analysis of the wild-type tRNA^{Leu(UUR)} by LC/MS using ESI/ ion trap mass spectrometry. Positive and negative ions were scanned throughout the separation, since it has been reported that uridine derivatives are difficult to detect as positive ions using electrospray ionization (29). The modified uridine (U*) at the wobble position was detected at 22.95 min as a negative ion with an m/z of 380.1 (Fig. 4, a and c). From this, its molecular weight is estimated to be 381.1Da, which is identical to that found recently by our group at the wobble position of bovine mitochondrial tRNA^{Leu(UUR)} (27), the largest among the modified uridines reported so far (27, 30).

The fact that digestion of both the mutant tRNAs^{Leu(UUR)} with PhyM resulted in discrete bands at the wobble position in the sequence ladders suggests that they have unmodified uridine at this position. Additionally, the spot observed at the wobble position of the mutant tRNA^{Leu(UUR)}(T3271C) in the two-dimensional TLC analysis clearly corresponds to unmodified uridine (Fig. 3b). These results lead us to conclude that the mutant tRNA^{Leu(UUR)} with the 3271 mutation has no modification at the wobble position, leaving the anticodon as UAA. Absence of the modified uridine from the mutant tRNA^{Leu(UUR)}(T3271C) was also confirmed by the LC/MS nucleoside analysis. As shown in Fig. 4, a and b, positive ions of 1-methylguanosine (m^G) and 2-methylguanosine (m^2G) were respectively detected in both the wild-type and mutant tRNAs^{Leu(UUR)} with the intensities expected from the amounts of the tRNAs subjected to the analysis (0.05 and 0.02 \( \Delta_{260} \) units of the wild-type and mutant tRNAs, respectively). In contrast, the modified uridine (U*) observed in the wild-type tRNA^{Leu(UUR)} was not detected in the mutant tRNA^{Leu(UUR)} (Fig. 4b, bottom panel).

Modification Defect at Wobble Position in Mitochondrial tRNA
position (U34) had only G14 at np 3243, while two bands corresponding to A and G were observed in the fragment with modified U34 (Fig. 3c). This finding demonstrates that the tRNA\textsubscript{Leu(UUR)} with the wild-type sequence (A14) had the fully modified U34 and that the unmodified U34 originated only from the mutant tRNA\textsubscript{Leu(UUR)}. Thus, the minor spot corresponding to the modified uridine is mainly derived from the coexisting wild-type tRNA\textsubscript{Leu(UUR)} (Fig. 3b).

To compare other modified nucleotides of the wild-type and mutant tRNAs\textsubscript{Leu(UUR)}, we further analyzed all the nucleo-
Modification Defect at Wobble Position in Mitochondrial tRNA

Mutation Defect at Wobble Position in Mitochondrial tRNA

DISCUSSION

We found that both the mutant tRNA<sup>Leu</sup>(UUR)(A3243G) and tRNA<sup>Leu</sup>(UUR)(T3271C) were markedly unstable in the respective cybrid cells despite there being only one nucleotide substitution (Fig. 5), resulting in significantly decreased steady-state amounts of these tRNAs (Fig. 1). In addition, the extent of aminocytosine of the mutant tRNAs was relatively low (Fig. 2b). The total amounts of leucyl-tRNAs<sup>Leu</sup>(UUR) with the mutations were estimated to be less than 30% that of the wild-type counterpart. In the case of the mutant tRNA<sup>Leu</sup>(UUR) in MERRF, it has been proposed that an approximately 50% reduction of aminocytosine of mitochondrial tRNA<sup>Leu</sup>(UUR) by collision-induced dissociation analysis. In contrast, this uridine modification is absent in the tRNA<sup>Leu</sup>(UUR) with a mutation at either 3243 or 3271 (Fig. 5). Helm et al. (28) recently analyzed human tRNA<sup>Leu</sup>(UUR) and quantitatively compared the modified nucleotides by labeling tRNA with [32P]phosphate in cybrid cells containing the 3243 mutation. They reported that a nonreproducible result was obtained for the quantification of the modified nucleotide at the wobble position in the cultured cells, although their sequence agrees with ours, including the other modified nucleotides. This discrepancy may stem from culturing the cells in a low concentration of phosphate in order to label the tRNAs isotope. In contrast, we purified chemical amounts of tRNAs from mass cultures of the respective cybrid cells with a fresh and complete medium. It is interesting to note that both the mutant tRNAs<sup>Leu</sup>(UUR) are deficient in the modification at the wobble position despite having mutations at different positions. This is the first observation of a common modification defect affected by different point mutations within a single tRNA gene.

The deficiency in uridine modification at the wobble position in the mutant tRNA<sup>Leu</sup>(UUR) strongly suggests mistranslation by these mutant tRNAs according to the mitochondrial wobble rule. All or most family boxes of mitochondria (34–38) and Mycoplasma spp. (39, 40) contain only a single tRNA species with the anticodon sequence UNN, the first nucleotide U being unmodified. This suggests that the unmodified U at this position pairs with all four bases, A, G, C, and U, at the third position of the codons. Indeed, in vitro translation experiments have shown that Mycoplasma tRNAs with an unmodified uridine at the wobble position translate all the corresponding four synonymous codons (41, 42). Thus, it is suggested that unmodified uridine at the wobble position of mutant tRNAs<sup>Leu</sup>(UUR) can recognize all four nucleotides at the third position of codons, giving rise to the translation of not only the usual UUR (R = A or G) leucine codons but also the unusual UUY (Y = C or U) phenylalanine codons in mitochondria of MELAS patients, which may eventually lead to the incorporation of leucine into phenylalanine sites at a certain frequency. Because the modification deficiency is observed for both the 3243 and 3271 mutations, it is likely that the lack of the modification at the wobble position leads to the particular MELAS phenotype through mistranslation.
Results obtained with a MELAS patient provide evidence in support of our hypothesis; the phenylalanine residue at position 251, a highly conserved amino acid in the carboxyl terminus of cytochrome c oxidase subunit III, was converted to leucine, which was found to arise from a point mutation in the cytochrome c oxidase subunit III gene, regardless of the mutation in the tRNA^Leu(UUR) (43, 44). This finding strongly supports the idea that the replacement of a phenylalanine residue by leucine at a certain position of a mitochondrial residue of MELAS is mainly considered due to the lack of the modifi-
atic activities of the complex(es). Therefore, the pathogenesis of MELAS is mainly considered due to the lack of the modification and the resultant mistranslation, which could explain why different point mutations in the same tRNA manifest indistinguishable clinical features. Work is now in progress on a detailed analysis to verify this proposition by purifying the proteins and directly determining the misincorporation by the mutant tRNAs^Leu(UUR).

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REFERENCES

1. Schon, E. A., Bonilla, E., and DiMauro, S. (1997) J. Bioenerg. Biomembr. 29, 131–149.
2. Kobayashi, Y., Momoi, M. Y., Tominaga, K., Shimoizumi, H., Nihei, K., Yanagisawa, M., Kagawa, Y., and Ohta, S. (1991) Am. J. Hum. Genet. 49, 590–599.
3. Kobayashi, Y., Momoi, M. Y., Tominaga, K., Momoi, T., Nihei, K., Yanagisawa, M., Kagawa, Y., and Ohta, S. (1990) Biochem. Biophys. Res. Commun. 173, 816–822.
4. Goto, Y., Nonaka, I., and Horai, S. (1990) Nature 348, 651–653.
5. Goto, Y., Nonaka, I., and Horai, S. (1991) Biochim. Biophys. Acta 1097, 238–240.
6. Van den Ouweland, J. M. W., Lemkes, H. H. P., Ruitenbeek, W., Sandkuijl, L. A., de Vilder, M. F., Struybreg, P. A. A., Van de Kemp, J. J. P., and Maassen, A. J. J. (1992) Nat. Genet. 1, 368–371.
7. Suzuki, Y., Tsukada, K., Atsumi, Y., Goto, Y., Hosokawa, K., Ashina, T., Nonaka, I., Matsuoka, K., and Oka, Y. (1996) Diabetes Care 19, 1304–1305.
8. Kadowaki, T., Kadowaki, H., Mori, Y., Toke, T., Sakuta, R., Suzuki, Y., Tanabe, Y., Sakura, H., Awata, T., Goto, Y., Hayakawa, T., Matsuoka, K., Kawamori, R., Kanuda, T., Horai, S., Nonaka, I., Hagura, R., Akamizu, Y., and Yazaki, Y. (1994) N. Engl. J. Med. 330, 962–968.
9. Shoffner, J. M., Lott, M. T., Lezza, A. M. S., Seibel, P., Ballinger, S. W., and Wallace, D. C. (1990) Cell 61, 931–937.
10. Silvestri, G., Moraes, C. T., Shanske, S., Oh, S. J., and DiMauro, S. (1992) Am. J. Hum. Genet. 51, 1213–1217.
11. King, M. P., and Attardi, G. (1989) Science 246, 500–503.
12. Chomyn, A., Meola, G., Bresolin, N., Lai, S. T., Scarlato, G., and Attardi, G. (1991) Mol. Cell. Biol. 11, 2336–2344.
13. Hayashi, J-I., Ohta, S., Kikuchi, A., Takemitsu, M., Goto, Y., and Nonaka, I. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 10614–10618.
14. King, M. P., Koga, Y., Davidson, M., and Schon, E. A. (1992) Mol. Cell. Biol. 12, 236–239.
15. Hess, J. F., Parisi, M. A., Bennett, J. L., and Clayton, D. A. (1991) Nature 351, 47–55.
16. Chomyn, A., Martinuzzi, A., Yonedo, M., Daga, A., Hurko, O., Lai, S. T., Nonaka, I., Angelini, C., and Attardi, G. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 4221–4225.
17. Dunbar, D. R., Moenie, P. A., Zeviani, M., and Holt, J. (1996) Hum. Mol. Genet. 5, 125–129.
18. Hayashi, J-I., Ohta, S., Takai, D., Miyabayashi, S., Sakuta, R., Goto, Y., and Nonaka, I. (1995) Biochem. Biophys. Res. Commun. 197, 1049–1055.
19. Watanabe, Y., Watanabe, N., and Nishikawa, S. (1992) J. Biol. Chem. 266, 24712–24718.
20. Suzuki, T., Ueda, T., and Watanabe, K. (1996) FEBS Lett. 381, 195–198.
21. Donis-Keller, H. (1980) Nuclear Acids Res. 8, 3133–3142.
22. Kuchino, Y., Hanyu, N., and Nishimura, S. (1987) Methods Enzymol. 155, 379–396.
23. Keith, G. (1995) Biochimie (Paris) 77, 142–144.
24. Suzuki, T., Suzuki, T., Ueda, T., and Watanabe, K. (1999) J. Mass Spectrom. Soc. Jpn. 47, 168–176.
25. Helm, M., Florentz, C., Chomyn, A., and Attardi, G. (1999) Nuclear Acids Res. 21, 756–763.
26. Banks, J. F. Jr., Shen, S., Whitehouse, C. M., and Fenn, J. B. (1994) Anal. Chem. 66, 406–414.
27. Sprinzl, M., Curn, B., Brown, M., Loundavitch, A., and Steinberg, S. (1998) Biochemicals. Biochem. Acta 26, 148–153.
28. Deleted in proof.
29. Flier, A., Reichmann, H., and Seibel, P. (1997) J. Biol. Chem. 272, 21789–21796.
30. Suzuki, T., Ueda, T., and Watanabe, K. (1997) EMBO J. 16, 1122–1134.
31. Barrell, B. G., Anderson, S., Bankier, A. T., de Bruijn, M. H., Chen, C., Coulson, A. R., Dringin, J., Eperon, I. C., Nierlich, D. P., Roe, B. A., Sanger, F., Schreier, P. H., Smith, A. J. H., Staden, R., and Young, I. G. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 3161–3166.
32. Heckman, J. E., Sarnoff, J., Alzner-DeWeerd, B., Yin, S., and RajBahrdi, U. L. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 3159–3163.
33. Watanabe, K., and Osa, S. (1995) in RNA: Structure, Biosynthesis, and Function (Still, D., and RajBahrdi, U. L., eds) pp. 225–250, American Society for Microbiology, Washington, D.C.
34. Matsuymaya, S., Ueda, T., Crain, P. F., McCloskey, J. A., and Watanabe, K. (1998) J. Biol. Chem. 273, 3363–3368.
35. Ueda, T., and Watanabe, K. (1998) Biochim. Biophys. Acta 1399, 78–82.
36. Andachi, Y., Yamao, F., Iwami, M., Muto, A., and Osawa, S. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 7398–7402.
37. Andachi, Y., Yamao, F., Muto, A., and Osawa, S. (1989) J. Mol. Biol. 209, 37–54.
38. Samuelsson, T., Arberg, T., Boren, T., and Lagerkvist, U. (1985) J. Biol. Chem. 259, 13178–13184.
39. Inagaki, Y., Kojima, A., Bessho, Y., Hori, H., Ohama, T., and Osawa, S. (1995) J. Mol. Biol. 251, 486–492.
40. Manfredi, G., Schon, E. A., Moraes, C. T., Bonilla, E., Berry, G. T., Sladky, J. T., and DiMauro, S. (1995) Neuromusc. Disorders 5, 391–398.
41. Mischel, W. H., and Rottenberg, H. (1996) FEBS Lett. 433, 93–97.
42. Anderson, S., Bankier, A. T., Barrrell, B. G., de Bruijn, M. H. L., Coulson, A. R., Dringin, J., Eperon, I. C., Nierlich, D. P., Roe, B. A., Sanger, F., Schreier, P. H., Smith, A. J. H., Staden, B., and Young, I. G. (1981) Nature 290, 457–465.