Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 7952–7956, October 1989
Genetics

Spontaneous Kearns–Sayre/chronic external ophthalmoplegia plus syndrome associated with a mitochondrial DNA deletion: A slip–replication model and metabolic therapy

(oxidative phosphorylation/polymerase chain reaction/coenzyme Q10/succinate)

JOHN M. SHOFFNER*, MARIE T. LOTT†, ALEXANDER S. VOLJAVEC‡, SHAWKE A. SOUEIDAN*, DONAL A. COSTIGAN*, AND DOUGLAS C. WALLACE*†§

Departments of *Neurology, †Biochemistry, and ‡Nephrology, Emory University School of Medicine, Atlanta, GA 30322

Communicated by Richard M. Krause, June 5, 1989

ABSTRACT **The muscle mitochondria of a patient with Kearns–Sayre/chronic external ophthalmoplegia plus syndrome were found to be completely deficient in respiratory complex I activity and partially deficient in complex IV and V activities. Treatment of the patient with coenzyme Q10 and succinate resulted in clinical improvement of respiratory function, consistent with the respiratory deficiencies. Restriction enzyme analysis of the muscle mtDNA revealed a 4.9-kilobase deletion in 50% of the mtDNA molecules. Polymerase chain reaction analysis demonstrated that the deletion was present in the patient's muscle but not in her lymphocytes or platelets. Furthermore, the deletion was not present in the muscle or platelets of two sisters. Hence, the mutation probably occurred in the patient's somatic cells. Direct sequencing of polymerase chain reaction-amplified DNA revealed a 4977-base-pair deletion removing four genes for subunits of complex I, one gene for complex IV, two genes for complex V, and five genes for tRNAs, which paralleled the respiratory enzymes affected in the disease. A 13-base-pair direct repeat was observed upstream from both breakpoints. Relative to the direction of heavy-strand replication, the first repeat was retained and the second repeat was deleted, suggesting a slip–replication mechanism. Sequence analysis of the human mtDNA revealed many direct repeats of 10 base pairs or greater, indicating that this mechanism could account for other reported deletions. We postulate that the prevalence of direct repeats in the mtDNA is a consequence of the guanine-cytosine bias of the heavy and light strands.**

Kearns–Sayre and chronic external ophthalmoplegia plus (KS/CEOP) syndrome have been associated in muscle and other tissues with deleted mtDNAs maintained in a population with normal mtDNAs (heteroplasmy) (1–6). The oxidative phosphorylation enzymes are encoded by both nuclear DNA and mtDNA. The mtDNA codes for the mitochondrial ribosomal and transfer RNAs as well as for 13 OxPhos polypeptides (7). The deletions in KS/CEOP syndromes reported to date remove 13–42% of the mutant mtDNA genome without crossing either of the origins of heavy (O_H) or light (O_L)-strand replication (1–6). Because transfer or ribosomal RNAs are usually deleted, normal mtDNA is required to sustain mitochondrial protein synthesis.

We have characterized the mtDNA deletion of a proband with KS/CEOP syndrome. Knowledge of the deletion and associated biochemical defect permitted design of a specific metabolic therapy that improved the proband's condition. Sequence analysis of the breakpoint has permitted us to propose a mechanism by which these mtDNA deletions may occur.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Muscle Biopsy and Pathological Analysis. After informed consent had been obtained, deltoid or quadriceps muscle biopsies done under local anesthesia (2% lidocaine hydrochloride) were taken for histochemistry, electron microscopy, mitochondrial isolation, and oxidative phosphorylation enzyme analysis (8). Histochemical processing included Gomori-modified trichrome, hematoxylin and eosin, NADH-tetrazolium, ATPase (pH 9.2 and 4.7), oil-red-O, and myophosphorylase.

Mitochondrial Preparation and Oxidative Phosphorylation Enzymology. Mitochondrial isolation and enzymology of oxidative phosphorylation complexes I–V were performed as described (8). The oxidative phosphorylaton enzyme activities of the patient specimen were compared with that of six adult control specimens, all collected as above. No evidence of neuromuscular disease was found in control specimens by histochemistry and electron microscopy. The mean ($\bar{x}$), SD, and 95% confidence limits were computed for each assay (9).

mtDNA Isolation, Extraction, and Analysis. Approximately 1 g of muscle was used for mtDNA extraction as described (8). DNA fragments generated by *Bam*HI, *Hae* II, *Hin*dIII, and *Xba* I were separated on 0.9% or 1.2% agarose gels. The gels were vacublotted (American Bionetics, Hayward, CA) onto nylon membrane (Pall Biodyne, ICN), and the filters were hybridized to a random primer-extended probe (10, 11) generated from purified mtDNA (12). mtDNA was quantitated by scanning autoradiographs of the Southern blots with a Bio-Rad model 620 videodensitometer.

Polymerase Chain Reaction (PCR). PCR (13) was used to identify normal and deleted mtDNAs with synthetic oligonucleotides used as primers (Microchemical Facility for Molecular Biology, Emory University). Forward (→) primers were located at nucleotides (nt) 7392–7410 and 8282–8305, and reverse (←) primers were located at nt 9244–9225 and 13950–13932 of the published human mtDNA sequence (7). The nt 7392(→) and nt 9244(←) primer pair amplified a 1852-base-pair (bp) fragment from wild-type DNA template. The nt 7392(→) and nt 13950(←) primer pair amplified a 1581-bp fragment from the deleted molecule. The 100-μl PCR reactions contained 200 μM of each dNTP (dATP, dTTP, dCTP, and dGTP), 50 mM KCl, 10 mM Tris·HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.01% (wt/vol) gelatin, 30 pmol of each primer, and 2.5 units of *Thermus aquaticus* (*Taq*) polymerase (Perkin–Elmer/Cetus). When a forward primer was paired with

Abbreviations: KS/CEOP syndrome, Kearns–Sayre/chronic external ophthalmoplegia plus syndrome; nt, nucleotide(s); O_H, origin of heavy-strand replication; O_L, origin of light-strand replication; DR1 and DR2, direct repeat 1 and 2, respectively.

§To whom reprint requests should be addressed at: 109 Woodruff Memorial Building, Department of Biochemistry, Atlanta, GA 30322.

two reverse primers, 60 pmol of the forward primer and 30 pmol of each reverse primer were used. PCR-generated DNA was visualized on 1% agarose gels with ethidium bromide.

Sequencing. Asymmetrically PCR-amplified DNA for dideoxynucleotide chain-termination sequencing was prepared by adding one primer in 100-fold excess of the other (14). The thermal profile for the asymmetric PCR consisted of 35 cycles of 93°C for 30 sec, 55°C for 1 min, and 72°C for 1 min. The standard PCR reaction was modified to include each dNTP at only 20 μM, 0.2 pmol of forward primer, 20 pmol of reverse primer and was performed without gelatin or the standard oil overlay. Single-stranded DNA was precipitated with isopropanol/ammonium acetate and hybridized with 0.5 pmol of the forward primer in 25 mM Tris (pH 8.8)/3.5 mM $MgCl_2$ at 70°C for 3 min, then incubated at 55°C for 10 min, and quenched on ice. Primer-extension reactions contained 0.5 μl of ^{35}S-labeled dATP (1000 Ci/mmol, Amersham; 1 Ci = 37 GBq); 5 μl of 1.5 μM dGTP, dTTP, and dCTP; and 2 μl of cloned *Taq* polymerase (Amplitaq, Perkin–Elmer/Cetus) diluted to 1 unit/μl and were allowed to proceed for 5 min at 42°C. Termination mixes were added containing each dNTP at 20 μM and either 60 μM ddGTP, 800 μM ddATP, 800 μM ddTTP, or 400 μM ddCTP. After incubation at 70°C for 5 min, tubes were transferred to room temperature to equilibrate for 5 min before adding 4 μl of the stop mixture containing 90% (wt/vol) formamide/20 mM EDTA, pH 8.0/0.05% xylene cyanol/0.05% bromophenol blue. Samples were then loaded on 6% polyacrylamide/8 M urea sequencing gel.

RESULTS

Case Summary. The proband, a 61-year-old female who is the ninth (II-9) of 10 siblings experienced the onset of ptosis and external ophthalmoplegia in her late 20s. Proximal muscle weakness progressed slowly. By her late 50s she experienced respiratory insufficiency manifested by dyspnea at rest.

At age 61, the proband had respiratory failure requiring mechanical ventilation. At that time, a physical exam confirmed the presence of ophthalmoplegia, ptosis, and respiratory distress, without ataxia, deafness, dementia, or pigmentary retinopathy. Respiratory failure could not be ascribed to cardiac disease, infection, or intrinsic lung disease. An electrocardiogram showed a left anterior fascicular conduction block. During hospitalization, the proband experienced an episode of narrow-complex supraventricular tachycardia at 250 beats per min, suggesting that an occult bypass tract could be present.

Cerebrospinal fluid protein was mildly elevated to 57 mg/ml (normal levels are 15–45 mg/ml) but contained normal levels of glucose, lactate, and pyruvate. A generalized increase in cerebrospinal fluid amino acids was present. Serum lactate, pyruvate, and amino acid levels were normal. Analysis of urine organic acids showed increased lactate (0.123), acetoacetate (0.279), and citrate (5.927) (normal levels for all <0.1 mg/mg of creatinine). Analysis of urine amino acid levels revealed a generalized increase, suggesting impaired proximal renal tubule function.

The proband's father, two brothers, and three sisters were found to be clinically normal. The mother died at age 60 of a stroke. One of the proband's sisters (II-8) died at 18 months after a 6-month illness characterized by poor feeding, loss of walking, poor growth, and dry skin. A second sister (II-10) died at day 1 of unknown causes. No consanguinity was present.

Muscle Biopsy. Histochemistry and electron microscopy showed ragged-red fibers with paracrystalline inclusions. These findings are consistent with type-I fiber pathology associated with mitochondrial myopathy. The muscle biopsies of sisters II-1 and II-6 were normal.

Biochemical Analysis. The oxidative phosphorylation enzyme activities for complexes I–V were determined in the proband (II-9) and two sisters (II-1, II-6) (Table 1). The complex I activity of the proband was virtually undetectable in both assays and close to the lower 5–95% confidence limits of the controls. Complexes IV and V were also reduced but to a lesser degree. The complex IV activity of the proband for freeze-thawed mitochondria was ≈65% of control activity. However, this enzyme complex was destabilized such that brief sonication reduced the activity to 9% of control levels. We measure the lability of complex IV as the ratio of the specific activities of sonicated versus freeze-thawed mitochondria and refer to this parameter as the fragility index (8); a low fragility index indicates an unstable complex IV. The proband's fragility index was 13% that of controls.

Metabolic Therapy. The severe deficiency in respiratory complex I activity suggested that the respiratory capacity of the proband might be improved using succinate to bypass the complex I block. Succinate contributes electrons to the electron transport chain by means of complex II. The instability of complex IV suggested that a mitochondrial-membrane stabilizing agent, such as coenzyme Q, might also have a therapeutic effect. Accordingly, the proband was treated with succinate (6 g/day) and coenzyme Q10 (Twin Labs, Ronkonkoma, NY) (300 mg/day).

Throughout the hospitalization of the proband, her vital capacity was monitored as an indicator of respiratory muscle

Table 1. Biochemical characterization of Kearns–Sayre syndrome

Oxidative phosphorylation complex	Assay	Patient			Control (n = 6)	
		II-9	II-6	II-1	$\bar{x}$ ± SD	5–95%
I	NADH-DB	40	211	151	214 ± 68	25–403
I+III	NADH-Cyt *c*	10	184	204	309 ± 151	0–728
II+III	Succ-Cyt *c*	405	525	572	756 ± 170	284–1228
III	DBH_2-Cyt *c*	814	1792	1544	1822 ± 677	0–3701
IV(S)	Cyt Oxidase	146	1926	1301	1552 ± 183	1044–2060
IV(FT)		936	—	—	1438 ± 502	44–2832
IV(S)/(FT)		0.16	—	—	1.21 ± 0.48	0–2.54
V	F_1 ATPase	198	430	256	479 ± 104	190–768

KS/CEOP oxidative phosphorylation analysis. NADH-DB, NADH-*n*-decyl coenzyme Q oxidoreductase; NADH-Cyt *c*, NADH-cytochrome *c* oxidoreductase (rotenone-sensitive fraction); Succ-Cyt *c*, succinate-cytochrome *c* oxidoreductase; DBH_2-Cyt *c*, reduced *n*-decyl coenzyme Q-cytochrome *c* oxidoreductase; S, 6-sec sonication; FT, freeze-thaw; IV(S)/IV(FT), fragility index; F_1 ATPase, activity in the F_1 subunit of mitochondrial ATP synthase. Patient II-9 is the proband; II-1 and II-6 are sisters. Enzyme activity is reported in nmol of substrate used per mg of mitochondrial protein per min.

strength. While mechanically ventilated (hospital days 3–27), mean vital capacity was 635 ml (n = 31; range, 290–1000 ml). From days 28–30, she was taken off the ventilator and had a mean vital capacity of 860 ml (n = 5; range, 600–1200 ml). Treatment with succinate and coenzyme Q10 was instituted on day 31 and coincided with subsequent rise in vital capacity over days 31–38 to a maximum value of 1500 ml (mean = 1083 ml; n = 9; range, 750–1500 ml).

The proband remained stable at home for the next 2 months until stopping her medications (on approximately day 100). Within 2 weeks she experienced respiratory failure requiring mechanical ventilation. Vital capacity on admission was 600 ml with a mean vital capacity while on the ventilator of 610 ml (n = 10; range, 600–800 ml). Reinstitution of the succinate and coenzyme Q10 was associated with resolution of the respiratory failure over 4 days. Vital capacity measured 4 mo after this episode was 1250 ml. She has subsequently remained on succinate and coenzyme Q10 without recurrence of the respiratory failure. The marked decline in the respiratory capacity of the proband after voluntary withdrawal of the medication and her subsequent recovery when medication was readministered indicates a direct association between treatment regime and improved clinical status of the patient.

mtDNA Deletion Mapping. To clarify the molecular basis of the disease, the muscle mtDNA of the proband and two sisters (II-1 and II-6) was analyzed by Southern blot hybridization by using total human mtDNA as a probe (Fig. 1). Digestion with *Bam*HI revealed that the two sisters had two mtDNA fragments of 14.4 and 2.2 kilobases (kb). The proband had these fragments but also had an additional 9.5-kb fragment. Hence, the proband's muscle appeared to contain two mtDNAs, a normal 16.6-kb molecule having two *Bam*HI sites and a smaller deleted molecule. Further restriction enzyme analysis revealed that the deleted mtDNA in the proband lacked *Hae* II sites at nt 9052 and nt 13177, *Hin*dIII sites at nt 11680 and nt 12570, and an *Xba* I site at nt 10256 (Fig. 1). This pattern indicated that >4 kb of the proband's mtDNA had been deleted between the *MTATP6* (ATPase subunit 6) (nt 9052) and *MTND5* (nt 13177) genes. Densitometry analysis indicated that ≈50% of the proband's muscle mtDNA molecules were of the deleted type.

To characterize this deletion further, PCR primers were chosen to selectively amplify the normal and deleted mtDNAs. To amplify the normal mtDNA, we used primers at nt 7392(→) and nt 9244(←). Amplification between these

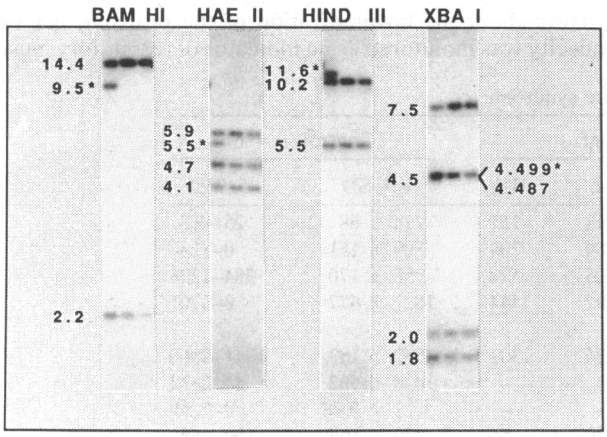

FIG. 1. Southern analysis of muscle mtDNA in KS/CEOP syndrome. For each restriction endonuclease (*Bam*HI, *Hae* II, *Hin*dIII, *Xba* I) lanes: 1, proband (II-9); 2, sister II-1; and 3, sister II-6. Abnormally sized bands (see text) are marked with an asterisk. For the *Xba* I digest, the proband's abnormal doublet at 4.499 and 4.487 kb (lane 1) is marked at right.

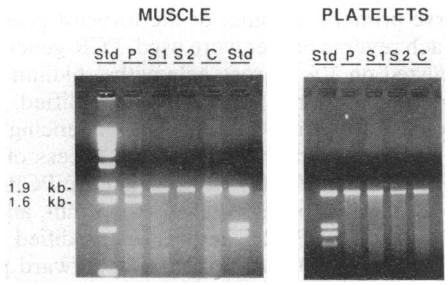

FIG. 2. PCR amplification across deletion in mtDNA isolated from muscle and platelets from proband (P) (II-9) and her sisters S1 (II-1) and S2 (II-6). 1.9-kb fragment, normal mtDNA; 1.6-kb fragment, amplification across deletion; C, control; Std, size standard.

primers could only occur from normal mtDNA molecules and would yield a 1.9-kb fragment. For amplification of the deleted mtDNA, we used primers that flanked the deleted region [nt 7392(→) and nt 13950(←)]. Only the deleted mtDNA could be amplified with these primers, producing a 1.6-kb fragment. The normal mtDNA PCR fragment would be 6.5 kb, which is too large to be amplified.

Amplification of the proband's muscle mtDNA with a mixture of three primers [nt 7392(→), nt 9244(←), and nt 13950(←)] yielded two fragments of 1.9 and 1.6 kb. This result confirmed the presence of both normal and deleted mtDNA molecules in proband muscle (Fig. 2). Similar amplification from muscle mtDNAs of the two sisters yielded only the 1.9-kb fragment, confirming that their muscles lack the deleted mtDNA. Application of this strategy to platelet and lymphocyte mtDNAs of all three women only detected wild-type mtDNAs. Hence, the deleted mtDNA appears not to be transmitted through the maternal lineage and probably appeared during the proband's somatic cell development.

Deletion-Breakpoint Sequencing. To determine the sequence at the breakpoints of the mtDNA deletion, the junction fragment was asymmetrically amplified by using primers at nt 8282(→) and nt 13950(←). The normal mtDNA in this region was similarly amplified using nt 8282(→) and nt 9244(←) primers. Single-stranded DNA sequencing identified the breakpoints as the adenine at nt 13446 and the thymidine at nt 8469. The breakpoint joined the center of the complex I subunit 5 gene with the complex V (ATPase) subunit 8 gene. The intervening genes for complex I (NADH dehydrogenase) subunits 4, 4L, and 3; complex IV (cytochrome *c* oxidase) subunit III; complex V (ATPase) subunit 6; and five tRNAs (leucine, serine, histidine, arginine, and glycine) were deleted. A 13-bp direct repeat (break box) was located 1 bp upstream from both breakpoints.

An alignment of the deleted and wild-type mtDNA sequences (Fig. 3) indicates that the deleted molecule maintains homology with the normal mtDNA from the displacement loop (D loop) (nt 1, nt 16569) down through nt 13446. Homology then switches to nt 8468 at the common cytidine and continues. This fact means that the break box at nt 13447–13459 was retained, whereas the break box at nt 8470–8482 was deleted.

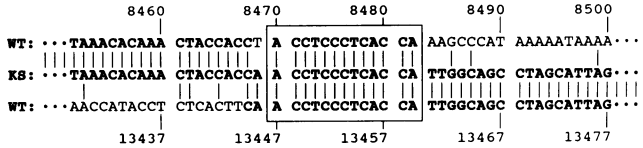

FIG. 3. Sequence alignment of wild-type (WT) mtDNA with deleted mtDNA (KS). Sequence homology is indicated by vertical bars and is in boldface. The 13-bp break box is outlined. Nucleotide positions are numbered according to the published sequence (7).

DISCUSSION

Genetics of Spontaneous KS/CEOP Syndrome. The 4.9-kb deletion in the muscle mtDNA seen in this KS/CEOP syndrome patient does not appear to be familial. No evidence of ophthalmoplegia was found in any other family member, and clinical and biochemical analysis of the proband's two elder sisters (II-1 and II-6) were normal. Further, the muscle from the sisters did not contain the deleted mtDNAs. Hence, the deleted molecules do not appear to have been maternally inherited (6), and the deletion probably arose during the proband's somatic development.

Because PCR analysis of the proband's blood cell mtDNA also failed to reveal the deleted mtDNA, the mtDNA deletion may have occurred after divergence of the muscle and hematopoietic lineages. Alternatively the mutant mtDNAs could have been eliminated from hematopoietic cells by selection (3). The spontaneous occurrence within a pedigree of most KS/CEOP patients would be consistent with their being somatic cell mutations.

Biochemical Implications of mtDNA Deletion. Direct sequencing across the breakpoint (Fig. 3) revealed that the deletion was 4.9 kb, involving all or parts of four complex I subunits, a single complex IV subunit, two complex V subunits, and five intervening tRNAs. The more severe reduction of complex I-specific activity and the lesser reduction of complex IV and V activities roughly parallels the number of genes deleted for each complex. However, this association may be more apparent than real. It is equally possible that the reduced protein synthesis and respiratory capacity of the mutant mitochondria destabilized the respiratory complexes in proportion to their complexity and the number of mtDNA subunits they contain. By this logic complex I would be the most labile followed by complexes IV, V, and III.

Consistent with the hypothesis that the primary defect in this patient was in complex I, treatment with succinate and coenzyme Q10 resulted in a marked improvement in her ventilation. Succinate should donate electrons directly to complex II, bypassing the complex I block. The effectiveness of this treatment is supported by a report that the stroke-like episodes of a patient with mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) decreased when treated with succinate at 6 g/day.¶ It is equally possible, however, that the therapeutic effect might primarily be the result of coenzyme Q10. Coenzyme Q has been hypothesized to stabilize the mitochondrial inner membrane by improving membrane fluidity, decreasing the effects of free-radical oxidation, and transferring electrons from complex I to III and from complex II to III (15–17). Treatment with high doses of coenzyme Q10 alone has been shown to have a therapeutic effect in KS/CEOP patients (18–23) and in a MELAS patient (24, 25).

mtDNA Deletion Mechanism. Identification of a perfect direct repeat flanking the mtDNA breakpoints suggests that these deletions may occur through a slip–replication mechanism. The retention of the adenosine at nt 13446 demonstrates that of the 13-bp direct repeats that flank the deleted region [nt 13447–13459 (direct repeat 1, DR1) and nt 8470–8482 (direct repeat 2, DR2)], only DR1 was retained in the deleted molecule. This retention suggests a scenario in which the deletion occurred during mtDNA replication (Fig. 4).

Replication of the mtDNA is a slow asynchronous process, initiating in the D loop and proceeding around the mtDNA light strand (Fig. 4*A*). This movement displaces the original heavy strand, starting at nt 109 and moves progressively

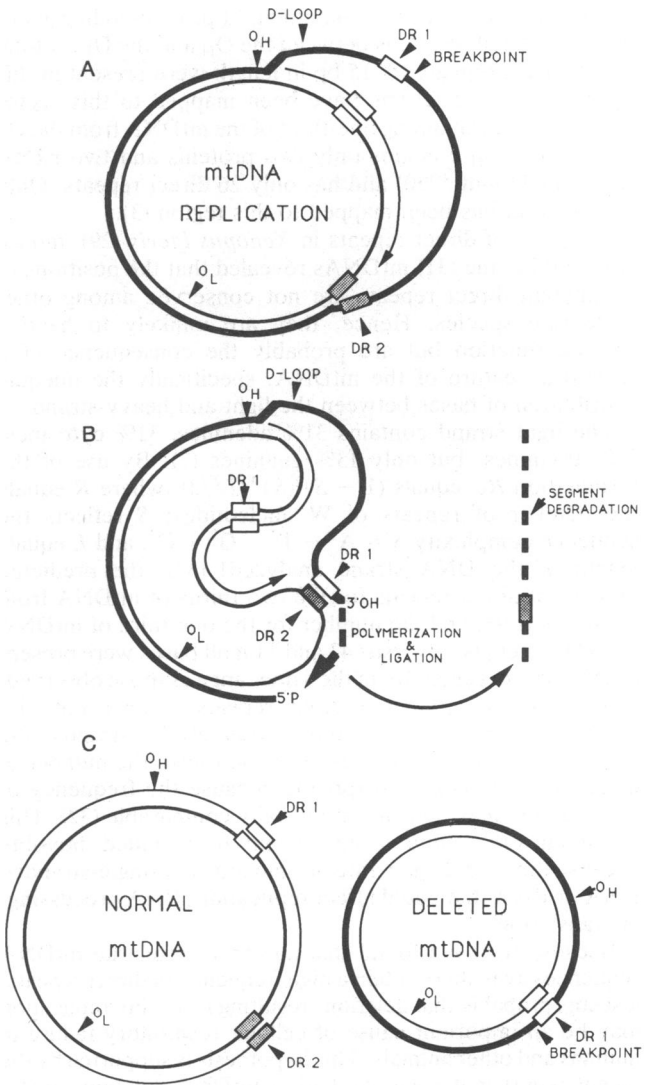

FIG. 4. Hypothetical slip–replication mtDNA deletion mechanism. ▭, DR1; ▩, DR2; thick line, parent heavy strand; medium line, parent light strand; fine line, daughter heavy strand.

across nt 1 and 16569 and toward O_L. Replication of the light strand does not begin until heavy-strand synthesis has proceeded about two-thirds the circumference of the molecule (26).

For this patient, we hypothesize that while the heavy strand was displaced, the upstream DR1 of the parental heavy strand could have base paired with the downstream light-strand DR2 exposed by the replicating fork (Fig. 4*B*). If a break occurred in the parent heavy strand downstream from DR1 and after the cytidine at nt 13445 (Fig. 4*B*), a base-paired template with a 3′-hydroxyl would be created that would permit further replicative extension of the heavy strand along the light-strand template. Degradation of the free heavy strand back to the double-stranded DNA molecule and subsequent ligation of its 5′-phosphate with the 3′-hydroxyl of the extended heavy strand would generate the observed deletion (Fig. 4*B*). Completion of replication would then generate a normal mtDNA from the original parent light strand and a deleted mtDNA from the slip-replicated heavy strand (Fig. 4*C*).

In support of this mechanism, a computer search revealed that direct repeats were common in the mtDNA sequence (7, 27). A regional analysis of the protein-coding regions of the mtDNA revealed that direct repeats are prevalent in the

¶Kobayashi, M., Morishita, H., Okajima, K., Sugiyama, N. & Wada, Y., Fourth International Congress of Inborn Errors of Metabolism, May 23–30, 1987, Sendai, Japan, p. 148 (abstr.).

two-thirds of the mtDNA containing 11 protein-coding genes (nt 5763–nt 110) which is between the O_H and the O_L. A total of 158 direct repeats 10–15 bp in length were present in this region, and 25 deletions have been mapped to this sector (1–6, 28). The remaining one-third of the mtDNA from the O_L back to the O_H contains only two proteins and two rRNA genes (nt 110–nt 5730) and has only 26 direct repeats. Only one deletion has been mapped to this region (3).

A survey of direct repeats in *Xenopus laevis* (29), mouse (30), and bovine (31) mtDNAs revealed that the positions of the human direct repeats are not conserved among other vertebrate species. Hence, they are unlikely to have a specific function but are probably the consequence of a structural feature of the mtDNA, specifically the unequal distribution of bases between the light and heavy strand.

The light strand contains 31% adenines, 31% cytosines, 25% thymines, but only 13% guanines (7). By use of the formulation R_W equals $(1 - S)^2(S)^W(L^2/2)$ [where R equals the number of repeats of W nucleotides; S reflects the sequence complexity $S = A^2 + T^2 + G^2 + C^2$; and L equals length of the DNA strand analyzed] (32), the predicted number of direct repeats for the two-thirds of mtDNA from O_H to O_L is 93, and the number for the one-third of mtDNA from O_L to O_H is 25 versus 43 and 11 if all bases were present at 25%. These values are in the same range as those observed.

Although the number of direct repeats is consistent with predictions for the base composition of the strands, the number of indirect repeats is only one-fourth the number of direct repeats. This is surprising because the frequency of direct and indirect repeats are usually comparable (32). This fact implies that indirect repeats may be excluded, possibly because they would generate hairpin loops in single-stranded DNA or RNA that could affect replication, RNA processing, or translation.

Because we have found that all other vertebrate mtDNA sequences available contain a high frequency of direct repeats, it seems probable that deletions resulting from slip–replication may be an important cause of cellular respiratory failure in humans and other animals. This hypothesis is supported by the recent report that mice can harbor mtDNA deletions analogous to that seen in the patient analyzed in our study (33).

Note. Subsequent to submission of this paper, two other reports have identified deletions flanked by the same set of 13-bp repeats. This deletion was found in 11 of 29 ocular myopathy patients (34) and in one Pearson syndrome patient (35). In these cases, the breakpoints occurred within or immediately adjacent to the direct repeats, and therefore it was not possible to determine which repeat was retained. Consequently, homologous recombination was favored over slip–replication. The high frequency of this deletion suggests that this pair of direct repeats is particularly prone to interaction, possibly due to the presence of A+T-rich sequences surrounding the downstream repeat (34).

We thank Dr. Linton Hopkins, Department of Neurology, Emory University for his helpful discussion and consultations and the manuscript reviewers for their critical review and helpful theoretical suggestions. This work was supported by National Institutes of Health Grant NS21328-05 and a Muscular Dystrophy Clinical Research grant awarded to D.C.W.

1. Lestienne, P. & Ponsot, G. (1988) *Lancet* **i**, 885.
2. Zeviani, M., Moraes, C. T., DiMauro, S., Nakase, H., Bonilla, E., Schon, E. A. & Rowland, L. P. (1988) *Neurology* **38**, 1339–1346.
3. Holt, I. J., Harding, A. E. & Morgan-Hughes, J. A. (1988) *Nature (London)* **331**, 717–719.
4. Holt, I. J., Cooper, J. M., Morgan-Hughes, J. A. & Harding, A. E. (1988) *Lancet* **i**, 1462.
5. Johns, D. R., Drachman, D. B. & Hurko, O. (1989) *Lancet* **i**, 393–394.
6. Ozawa, T., Yoneda, M., Tanaka, M., Ohno, K., Sato, W., Suzuki, H., Nishikimi, M., Yamamoto, M., Nonaka, I. & Horai, S. (1988) *Biochem. Biophys. Res. Commun.* **154**, 1240–1247.
7. Anderson, S., Bankier, A. T., Barrell, B. G., de Bruijn, M. H. L., Coulson, A. R., Drouin, J., Eperson, I. C., Nierlich, D. P., Roe, B. A., Sanger, F., Schreier, P. H., Smith, P. H., Staden, R. & Young, I. G. (1981) *Nature (London)* **290**, 457–465.
8. Wallace, D. C., Zheng, X., Lott, M. T., Shoffner, J. M., Hodge, J. A., Kelley, R. I., Epstein, C. M. & Hopkins, L. C. (1988) *Cell* **55**, 601–610.
9. Lentner, E. C. (1982) *Geigy Scientific Tables* (CIBA–Geigy, Greensboro, NC), Vol. 2, pp. 51, 191, 205.
10. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132**, 6–13.
11. Feinberg, A. P. & Vogelstein, B. (1984) *Anal. Biochem.* **137**, 266–267.
12. Giles, R. E., Stroynowski, I. & Wallace, D. C. (1980) *Somatic Cell Genet.* **6**, 543–554.
13. Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A. & Arnheim, N. (1985) *Science* **230**, 1350–1354.
14. Innis, M. A., Myambo, K. B., Gelfand, D. H. & Brow, M. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 9436–9440.
15. Lenaz, G. & De Santis, A. (1985) in *Coenzyme Q: Biochemistry, Bioenergetics and Clinical Applications of Ubiquinone*, ed. Lenaz, G. (Wiley, New York), pp. 83–105.
16. Landi, L., Cabrini, L., Sechi, A. M. & Pasquali, P. (1984) *Biochem. J.* **222**, 463–466.
17. Takeshige, K., Takayanagi, R. & Minakami, S. (1980) in *Biomedical and Clinical Aspects of Coenzyme Q*, eds. Yamamura, Y., Folkers, K. & Ito, Y. (Elsevier, New York), Vol. 2, pp. 15–25.
18. Ogasahara, S., Yorifuji, S., Nishikawa, Y., Takahashi, M., Wada, K., Hazama, T., Nakamura, Y., Hashimoto, S., Kono, N. & Tarui, S. (1985) *Neurology* **35**, 372–377.
19. Ogasahara, S., Nishikawa, Y., Yorifuji, S., Soga, F., Nakamura, Y., Takahashi, M., Hashimoto, S., Kono, N. & Tarui, S. (1986) *Neurology* **36**, 45–53.
20. Bet, L., Bresolin, N., Binda, A., Nador, F., Ferrante, C., Comi, G. & Scarlato, G. (1987) *Neurology* **37**, Suppl. 1, 202 (abstr.).
21. Bresolin, N., Bet, L., Binda, A., Moggio, M., Comi, G., Nador, F., Ferrante, C., Carenzi, A. & Scarlato, G. (1988) *Neurology* **38**, 892–899.
22. Desnuelle, C., Pellissier, J., Serratrice, G. & Pouget, J. (1988) *Neurology* **38**, Suppl. 1, 120 (abstr.).
23. Shoffner, J. M., Voljavec, A. S., Costigan, D. A., Hopkins, L. C. & Wallace, D. C. (1989) *Neurology* **39**, Suppl. 1, 404 (abstr.).
24. Yamamoto, M., Sato, T., Anno, M., Ujike, H. & Takemoto, M. (1987) *J. Neurol. Neurosurg. Psychiatry* **50**, 1475–1481.
25. Goda, S., Hamada, T., Ishimoto, S., Kobayashi, T., Goto, I. & Kuroiwa, Y. (1987) *J. Neurol.* **234**, 62–63.
26. Clayton, D. A. (1982) *Cell* **28**, 693–705.
27. Queen, C. & Korn, L. J. (1984) *Nucleic Acids Res.* **12**, 581–599.
28. Rotig, A., Colonna, M., Blanche, S., Fischer, A., Le Deist, F., Frezal, J., Saudubray, J.-M. & Munnich, A. (1988) *Lancet* **ii**, 567–568.
29. Roe, B. A., Ma, D.-P., Wilson, R. K. & Wong, J. F.-H. (1985) *J. Biol. Chem.* **260**, 9759–9774.
30. Bibb, M. J., Van Etten, R. A., Wright, C. T., Walberg, M. W. & Clayton, D. A. (1981) *Cell* **26**, 167–180.
31. Anderson, S., deBruijn, M. H. L., Coulson, A. R., Eperon, I. C., Sanger, F. & Young, I. G. (1982) *J. Mol. Biol.* **156**, 683–717.
32. Moore, G. P., Moore, A. R. & Grossman, L. I. (1984) *J. Theor. Biol.* **108**, 111–112.
33. Boursot, P., Yonekawa, H. & Bonhomme, F. (1987) *Mol. Biol. Evol.* **4**, 46–55.
34. Schon, E. A., Rizzuto, R., Moraes, C. T., Nakase, H., Zeviani, M. & Dimauro, S. (1989) *Science* **244**, 346–349.
35. Rotig, A., Colonna, M., Blanche, S., Fischer, A., Le Deist, F., Frezal, J., Saudubray, J. M. & Munnich, A. (1989) *Lancet* **i**, 902–903.