

## Supporting Materials and Methods

**H-Strand Nascent Chain Identification and Analysis.** mtDNA contained in total cell DNA (3  $\mu$ g) and was utilized as a template for carrying out a primer extension on all the nascent H-strand chains, using 1 pmol of either of the two following gel-purified 5'-<sup>32</sup>P-labeled primers: 5'-GAGCTCTCCATGCATTTGGT (L-strand positions 36-55), or, less frequently, 5'-CTCCATGCATTTGGTATTTTCG (L-strand positions 41-62), both primers corresponding to sequences within the D-loop. The DNA and the chosen primer were combined in 40  $\mu$ l of a reaction mixture consisting of 10 mM Tris•HCl, pH 8.9 (25°C); 40 mM NaCl; 2 mM MgSO<sub>4</sub>; 0.2 mM of each dNTP; 2 units Vent DNA polymerase (New England Biolabs); and 0.01% gelatin (1). The DNA in the reaction mixture was denatured at 95°C for 5 min; the primer was then annealed at 60°C for 30 min, and subsequently the Vent DNA polymerase reaction was performed at 76°C for 15 min. For detection of the extended products, they were run in a 7 M urea/10% polyacrylamide gel. The gel was dried and, after appropriate exposure, analyzed in a Phosphor-Imager (Molecular Dynamics) or subjected to autoradiography. As a control to exclude artifacts of the primer extension reaction, a total DNA sample from AL4.27, a  $\rho^{\circ}$ 206 cell transformant carrying 100% wild-type mtDNA, was subjected to the primer extension reaction, and an equivalent sample of DNA was first subjected to PCR amplification of the AccI 2,818-bp mtDNA fragment (positions 15,255-1,504 nt), encompassing the whole main control using Taq polymerase and appropriate primers, and then the primer extension reaction was applied to the PCR product.

For the identification of the 5'-ends of the nascent chains, a nondried gel was exposed for autoradiography; the bands of interest were excised, and the DNA was eluted

overnight in 10 mM Tris•HCl, pH 8.0/0.1% SDS, ethanol precipitated, and dissolved in 35 µl of 10 mM Tris•HCl, pH 8.5. For the purpose of intramolecular ligation of the 5'- and 3'-ends of each extended primer, a 20-µl reaction mixture consisting of 2 µl of 500 mM Tris•HCl, pH 7.8; 100 mM MgCl<sub>2</sub>; 100 mM DTT; 10 mM ATP; 17.5 µl of eluted DNA sample; and 0.5 µl of T4 RNA ligase (10 units, New England Biolabs) was incubated at 22°C for 16 h. The circularized molecules were then subjected to PCR amplification, using two adjacent primers [H-strand: 5'-CAGCGTCTCGCAATGCTATC (positions 100-81); L-strand: 5'-GAGCCGGAGCACCTATGTC (positions 101-121)] divergent from a position about one-third to one-half of the way around the circle from the original 3'-end of the extended primer. The PCR products were purified by native PAGE, dissolved in 10 µl of 10 mM Tris•HCl, pH 8.5, cloned in *Escherichia coli*, using a TA vector (TA vector kit, Promega), and 25 to 50 plasmids carrying each PCR product were then sequenced. Analysis of the sequences revealed which of the cloned PCR products had the appropriate structure expected from the circularization of the primer extension products. The sequences thus selected were used for analysis. To verify the accuracy of determination of 5'-ends of the primer-extended products by the approach described above, a synthetic oligodeoxynucleotide 75 nt long covering the segment of the L-strand from position 81 to position 155 was purified by SDS/PAGE, its size verified by mass spectrometry, and the oligonucleotide intramolecularly ligated with T4 RNA ligase. The ligated construct was then subjected to PCR amplification; the PCR product, purified by native PAGE, was cloned in *E. coli* as described above, and 39 plasmids were sequenced.

1. Kang, D., Miyako, K., Kai, Y., Irie, T. & Tageshige, K. (1997) *J. Biol. Chem.* **27**, 15275–15279.