Recombination of Human Mitochondrial DNA

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Human mitochondrial DNA (mtDNA) is a 16.5-kb, circular genome essential for the maintenance of mitochondrial function and is present in multiple copies in most cell types. High sequence divergence and maternal inheritance make mtDNA useful in tracing human lineages. Whether recombination occurs between mitochondrial genomes is a long-standing question in mitochondrial biology, human evolution, and population studies (*1*). MtDNA recombination occurs in yeast, and recombinant mtDNA has been found in several animal species (*2*); however, the evidence for recombination between heterologous mtDNA in humans is controversial (*1*).

We searched for mtDNA recombinants in muscle tissue of an individual with paternal inheritance of the mitochondrial genome (*3*), where heterologous (paternal and maternal) mtDNAs are mixed and thus may have an opportunity to recombine. DNA was cleaved (*4*) by a paternal-specific restriction endonuclease at position 14,793 (fig. S1) to exclude a 10:1 excess of paternal mtDNA. It was then subjected to single-molecule polymerase chain reaction (PCR), which avoids in vitro recombination and has been successfully used to isolate nuclear DNA recombinants (*5*). In single-molecule PCR, each PCR product is a clone of identical molecules that originate from a single template. We recovered 450 PCR clones containing a maternal sequence at position 14,793 and screened them for paternal sequences at position 73 (fig. S1). We found 33 such clones. Their sequences revealed alternating maternal and paternal segments, a hallmark of recombination (Fig. 1). Multiple lines of evidence, including a control experiment with a reconstructed 10:1 mixture of paternal and maternal DNA, support an in vivo origin of these recombinants (supporting online text).

The recombinants fall into two structural classes (Fig. 1): class 1, with a short paternal sequence inserted into a mostly maternal molecule, and class 2, with a maternal sequence flanked by paternal sequences. Furthermore, sequence breakpoints in recombinants cluster within distinct hotspots (A, B, and C in Fig. 1), each associated exclusively with one or the other structural class. Breakpoint hotspots appear to coincide with biologically relevant sequences. Hotspot A is located just beyond an alternate light-strand origin of replication, as mapped in the mouse (*6*). The region beyond light-strand origins is a heavy-strand replication-pausing site. Stalling of the replication fork in this region may produce a recombinogenic 3′ end that is capable of invading a neighboring mtDNA [by means of mitochondrial RecA-like] activity (*7*), for example and resuming replication, thus creating a breakpoint. Hotspots B and C colocalize with 5′ ends of 7S DNA, a short nascent strand of DNA formed by the premature termination of mtDNA replication and residing in a displacement loop. This colocalization implies that recombinants may originate from 7S "template switching": If a paternal 7S DNA is released from a displacement loop, invades a maternal mtDNA, and initiates replication, a class 1 recombinant will result. If 7S DNA is partially degraded before strand invasion, recombinants with a shortened paternal region will result, exactly as observed (Fig. 1).

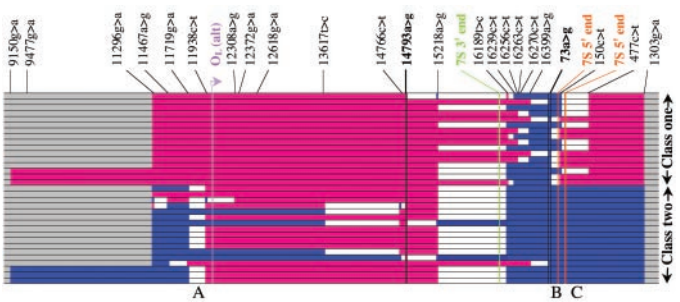

Fig. 1. Structural map of recombinants. Each horizontal row represents a recombinant, with paternal and maternal segments in blue and red, respectively. Maternal to paternal nucleotide changes are shown above the map. Segments that join polymorphisms of different (paternal/maternal) descent, and thus contain sequence breakpoints, are white. Prominent stacks of white segments represent breakpoint hotspots, of which A, B, and C (but not the one at 15,218 to 16,189) are highly statistically significant (*4*). Nonmapped areas are gray. Vertical lines depict restriction sites used for recombinant screening (double black), the secondary origin of light-strand replication (lavender), and 3′ (green) and 5′ (orange) ends of the 7S DNA. Several recombinants contain more than two breakpoints (supporting online text).

The observed frequency of recombinants of ~0.7% of total mtDNA (7% of maternal molecules, which in turn constitute 10% of all mtDNA) requires cautious interpretation. First, mtDNA of different descent may be segregated into separate entities such as cells, mitochondria, or nucleoids (*8*). Recombination between identical mtDNAs within such entities and between sister molecules in replication forks would be undetectable in this study. Second, the frequency of recombinants might have been affected by strong selection for paternal mtDNA in this person's muscle (*3*). Although the probability of inheritance of recombinant genomes is unknown, this demonstration of genetic transfer between paternal and maternal human mtDNA and the identification of breakpoint hotspots is highly relevant for studies of human evolution, mtDNA replication and repair, and generation and propagation of dysfunctional genomes in mitochondrial disease.

References and Notes

1. E. Hagelberg, *Trends Genet.* **19**, 84 (2003).
2. A. Rokas, E. Ladoukakis, E. Zouros, *Trends Ecol. Evol.* **18**, 411 (2003).
3. M. Schwartz, J. Vissing, *N. Engl. J. Med.* **347**, 576 (2002).
4. Materials and methods are available as supporting material on *Science* Online.
5. C. L. Yauk, P. R. Bois, A. J. Jeffreys, *EMBO J.* **22**, 1389 (2003).
6. T. Brown, D. Clayton, in preparation.
7. B. Thyagarajan, R. A. Padua, C. Campbell, *J. Biol. Chem.* **271**, 27536 (1996).
8. H. T. Jacobs, S. K. Lehtinen, J. N. Spelbrink, *Bioessays* **22**, 564 (2000).

9. We thank A. de Grey, M. Frank-Kamanetskii, B. Thilly, and anonymous reviewers for their input, and A. Pluzhnikov for help with statistical analysis. Supported in part by NIH grant nos. ES11343, AG19787, and AG18388 (K.K.).

Supporting Online Material

www.sciencemag.org/cgi/content/full/304/5673/981/DC1
Materials and Methods
SOM Text
Fig. S1
References and Notes

2 February 2004; accepted 11 March 2004

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Science **304** (5673), 981.
DOI: 10.1126/science.1096342