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INVITED EDITORIAL
Mutational Hot Spots in the Mitochondrial Microcosm

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Human mitochondria can be seen as a genetic microcosm, located within the macrocosm of the entire cell. In each cell, this microcosm contains a few thousand copies of a circular DNA genome of 16,569 bp that is transmitted between generations, almost exclusively from mother to child. The study of this genetic microcosm holds particular fascination, since it may foreshadow the coming genetics of the nuclear genome. In the mitochondrial microcosm, the "Human Genome Project" was completed 15 years ago, with the publication of the DNA sequence of an entire mitochondrial genome (Anderson et al. 1981). In the microcosm, one is thus well into the "postgenomic era." It therefore may be instructive to ask what the first 15 years of postgenomics has brought within the microcosmic field of mitochondrial genetics. The availability of a complete genome sequence obviously brought great benefits to the study of physiological process in the mitochondria. However, in addition, many insights in the immediately postgenomic phase seem to come from the study of genomic variation. At least four areas come to mind:

First, the human genome sequence was followed by the sequencing of entire mitochondrial genomes of several other species. This has allowed insights into how genomic rearrangements, changes in the genetic code, and other processes affect the genome (see Wolstenholme and Jeon 1992). Second, the study of DNA sequence variation among humans from around the world has led to a model of an African origin of the mitochondrial gene pool as recently as 100,000–200,000 years ago (Cann et al. 1987; Vigilant et al. 1991). This has affected the notions about human origins in society at large and has been widely and hotly debated within the genetic community (e.g., see Templeton 1993) and among anthropologists and paleontologists (e.g., Stringer and Andrews 1988; Thorne and Wolpoff 1992).

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Third, the availability of the mitochondrial genome sequence greatly facilitated the search for mutations involved in human diseases. More than 20 mutations associated with diseases have been identified (Larsson and Clayton 1995), and more are undoubtedly yet to be found.

Fourth, and more mundanely, knowledge about the genomic variation has been put to forensic use to exclude—and, sometimes, to include—an individual as the source of a biological sample (Wilson et al. 1993).

However, impressive as this list may seem, to make real sense of the comparative work, we need knowledge about the frequency and nature of the mutations that affect the genome. For example, estimates of evolutionary relationships among mtDNA sequences, of dates for the common ancestors of mitochondrial sequences, and of the probability that two individuals will share the same mtDNA sequence in forensic applications depend on the models of mutational change used. It is therefore disturbing that these models rely on indirect conjecture, since, with few exceptions, one has not been able to observe mitochondrial mutations directly. In this issue of the *Journal,* Howell et al. (1996) present one of the first studies in which mitochondrial mutations are directly observed in families.

The authors sequenced the mitochondrial control region from members of a family affected with Leber hereditary optic neuropathy, a disease caused by a primary mitochondrial mutation in the NADH-dehydrogenase 6 gene. When 40 members of this family were analyzed, 1 individual was found who carried two mutations in the control region. These mutations (presumably unrelated to the disease) were present on different mtDNA molecules, such that approximately two-thirds of the molecules carried one mutation and ~1 molecule in 10 carried another mutation. From another member of the pedigree, one mutation was found in approximately half of the molecules amplified from a fibroblast cell line. However, this mutation seems to be restricted to some somatic-cell types, since it was not found in blood cells from the same individual.

When Howell et al. studied the first two mutations in relatives of the individual carrying them, they showed that the mutations had arisen three or four generations

earlier. The three types of mitochondrial molecules (the "wild type" and the two mutant forms) were transmitted from mother to offspring in varying proportions. Thus, among five sibs of a mother carrying the three types of mitochondrial molecules, two carry all three types in sizable proportions, one carries equal proportions of the two mutated molecules, one carries predominantly one of the mutated sequences, and a fifth carries only the original, wild-type sequence.

The observation of two germ-line mutations among some 50 individuals studied suggests a mutation rate of 1 event/25 generations in a DNA segment where phylogenetic comparisons to the chimpanzee have suggested a frequency of $\leq$1 event/200 generations. In the same vein, Howell et al. elsewhere have described two mutations in coding regions of the genome in families, and they now describe a third one. This indicates a 200-fold-higher rate of mutations in the mitochondrial structural genes than has been derived from phylogenetic comparisons. These observations receive support from another group, which similarly has found a high rate of mitochondrial mutation (Lederberg et al. 1995), and from reports suggesting that heteroplasmy—that is, the occurrence of two or more types of mitochondrial sequences within an individual—may be more common in humans than previously had been thought (Gill et al. 1994; Comas et al. 1995; Ivanov et al. 1996). When these studies are pooled, the number of mutations observed is still very small, whereas the uncertainty of the rate estimates is correspondingly huge. Nevertheless, the high mutation rate represents an impressive discrepancy with the received wisdom among evolutionists.

In fact, the high mutation rates derived from family studies not only are radically different from the received wisdom, but they are, at first glance, incompatible with everything that we know about human evolution. For example, if the lower "rates" are used, then the ancestral molecule common to all human mitochondrial genomes existed some 150,000 years ago, on the basis of comparisons of whole genomes (Horai et al. 1995). If we believe that the rate on which this was calculated is 200-fold too low, we might conclude that common mitochondrial ancestor lived some 750 years ago—clearly an impossibility. How can these observations be reconciled?

The authors consider the possibility that abnormal mitochondrial metabolism may accelerate the rate of mutations in the families studied. This is possible, particularly given the unlikely clustering of two mutations in one family. However, mutations at two positions have similarly been observed in a heteroplasmic individual not known to suffer from any mitochondrial disease (Comas et al. 1995). An alternative possibility for a clustering of mutations could be that nuclear-gene products involved in mtDNA replication or repair may have alleles that accelerate mutations, thus causing clustering of mutations in certain families. One nuclear locus that causes mitochondrial deletions has recently been mapped to chromosome 10q (Suomalainen et al. 1995). Alternatively, mutations may, as the authors note, occur frequently in the population but be eliminated through selection before reaching fixation (Nachman et al. 1996).

Although these may all play a role, the major factor reconciling these apparently conflicting results is probably that nucleotide positions differ greatly in their tendency to mutate. Phylogenetic studies have suggested that between positions in the control region there is a 15- to 20-fold difference in evolutionary rate (Hasegawa et al. 1993; Wakeley 1993). In these studies, the mutational rates of the most rapidly evolving positions may still be greatly underestimated. If some positions evolve, say, 100-fold faster than others, then a pedigree approach will pick up primarily mutations at such positions, whereas the true number of mutations at the latter positions will be indiscernible to phylogenetic approaches. Thus, if this is true, the rate at the most rapidly evolving positions, detected in pedigrees, cannot be extrapolated to the entire sequence, in which most positions will evolve a lot more slowly. On the other hand, the rates determined from evolutionary comparisons cannot be extrapolated to the whole sequence either, since some sites will change very frequently. The notion that the two control-region positions where Howell et al. have detected mutations are evolving extremely rapid change is compatible with the fact that both these positions are highly polymorphic among humans. Among 526 humans studied, 213 carry the "mutation-like" C at one of the positions. At the other position, 160 carry the mutation-like C, and, in addition, 5 carry a transversion to an A (S. Meyers, personal communication). Among chimpanzees, both these positions also vary (Kocher and Wilson 1991).

Thus, neither the "pedigree rates" nor the "phylogeny rates" would be "right" or "wrong." Rather, they would be useful for estimating dates of different types. For comparisons of sequences that are closely related, perhaps sharing common ancestral sequences on the order of hundreds or thousands of years ago, the pedigree rates would be better suited. For comparisons that go back hundreds of thousands or millions of years, the phylogeny rates would be the better estimates.

The new rates that will emerge when more mutations have been detected in families are therefore not likely to revise our understanding of the origin of the human mitochondrial gene pool dramatically, but they may cause a reinterpretation of more recent events—for example, the population-size expansion that is indicated by a peak in histograms of pairwise sequence differences.

This expansion has been dated to ~40,000 years ago (Rogers and Harpending 1992; Rogers 1995). Here, a faster rate may allow a more adequate approximation of the actual mutations having taken place and may indicate that this expansion is associated with the agricultural revolution that occurred some 10,000–5,000 years ago. Support for this idea comes from observation that the signature of the expansion is absent in groups in India, Scandinavia, and Africa that live from food gathering rather than from food production (Mountain et al 1995; Sajantila et al 1995; Watson et al. 1996).

Certainly, the "direct" observation of mitochondrial mutations will have dramatic consequences for the use of mtDNA in forensics. If 1 person in 25 may carry a DNA sequence different from that of his or her mother, sometimes even at several positions, then the exclusions of a person (as the donor of a sample) on the basis of a different sequence in his or her relatives is questionable. If, furthermore, heteroplasmy is common, and if the extent differs among tissues, as indicated by data from one individual in the report by Howell et al., this has to be taken into account when mtDNA is used to identify individuals on the basis of traces of tissues at a crime scene. In particular, it will be crucial to determine that a PCR amplification from a forensic sample is initiated by a number of initial template molecules that is large enough to reflect accurately the initial population of molecules present in the sample. If the amplification starts from just one or a few molecules, these may represent a rare variant present in a sample. Thus, quantitation of the number of molecules from which an amplification starts (see Handt et al. 1996) will be more important than hitherto has been realized.

Finally, I think that the work of Howell et al. heralds a new phase of postgenomic mitochondrial research. The observation of mutations in families will allow hot spots for mutations to be identified directly. In vitro assays, eventually using purified enzymes involved in mtDNA replication and repair, can then be used to study to what extent the hot spots reflect intrinsic properties of the replication machinery. Thus, the biochemical basis for population variation can be studied, and the relative contributions that mutational processes and selection make to genomic change can be better understood. In this, developments in the mitochondrial microcosm will again be ahead of developments in the more complex macrocosm of the nuclear genome.

References

Anderson S, Bankier AT, Barrell BG, de Bruijn MHL, Coulson AR, Drouin J, Eperon IC, et al (1981) Sequence and organization of the human mitochondrial genome. Nature 290: 457–465

Cann LS, Stoneking M, Wilson AC (1987) Mitochondrial DNA and human evolution. Nature 325:31–36

Comas D, Pääbo S, Bertranpetit J (1995) Heteroplasmy in the control region of human mitochondrial DNA. Genome Res 5:89–90

Gill P, Ivanov PL, Kimpton C, Piercy R, Benson N, Tully G, Evett I, et al (1994) Identification of the remains of the Romanov family by DNA analysis. Nat Genet 6:130–135

Handt O, Krings M, Ward RH, Pääbo S (1996) The retrieval of ancient human DNA sequences. Am J Hum Genet 59: 368–376

Hasegawa M, Di Rienzo A, Kocher TD, Wilson AC (1993) Towards a more accurate time scale for the human mitochondrial DNA tree. J Mol Evol 37:347–354

Horai S, Hayasaka K, Kondo R, Tsugane K, Takahata N (1995) Recent African origin of modern humans revealed by complete sequences of hominoid mitochondrial DNAs. Proc Natl Acad Sci USA 92:532–536

Howell N, Kubacka I, Mackey DA (1996) How rapidly does the human genome evolve? Am J Hum Genet 59:000–000 (in this issue)

Ivanov PL, Wadhams MJ, Roby RK, Holland MM, Weedn VW, Parson TJ (1996) Mitochondrial DNA sequence heteroplasmy in the Grand Duke of Russia Georgij Romanov establishes the authenticity of the remains of Tsar Nicholas II. Nat Genet 12:417–420

Kocher TD, Wilson AC (1991) Sequence evolution of mitochondrial DNA in humans and chimpanzees. In: Osawa S, Honjo T (eds) Evolution of life. Springer, Berlin

Larsson N-G, Clayton DA (1995) Molecular genetic aspects of human mitochondrial disorders. Annu Rev Genet 29: 151–178

Lederberg J, Bashinski J, Budowle B, Gill P, Stoneking M, Wallace D, Weir B, et al (1995) The use of DNA technology for identification of ancient remains. Office of the Under Secretary of Defense for Acquisition and Technology, Washington, DC

Mountain JL, Hebert JM, Bhattacharyya S, Underhill PA, Ottolenghi C, Gadgil M, Cavalli-Sforza LL (1995) Demographic history of India and mtDNA-sequence diversity. Am J Hum Genet 56:979–992

Nachman MW, Brown WM, Stoneking M, Aquadro CF (1996) Nonneutral mitochondrial variation in humans and chimpanzees. Genetics 142:953–963

Rogers AR (1995) Genetic evidence for a Pleistocene population explosion. Evolution 49:608–615

Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise differences. Mol Biol Evol 9:552–569

Sajantila A, Lahermo P, Anttinen T, Lukka M, Sistonen P, Savontaus M-L, Aula P, et al (1995) Genes and languages in Europe: an analysis of mitochondrial lineages. Genome Res 5:42–52

Stringer CB, Andrews P (1988) Genetic and fossil evidence for the origin of modern humans. Science 239:1263–1268

Suomalainen A, Kaukonen J, Amati P, Timonen R, Haltia M, Weissenbach J, Zeviani M, et al (1995) An autosomal locus

predisposing to deletions of mitochondrial DNA. Nat Genet 9:146–151

Templeton, AR (1993) The "Eve" hypothesis: a genetic critique and reanalysis. Am Anthropol 95:51–72

Thorne AG, Wolpoff MH (1992) The multiregional evolution of humans. Sci Am (April), pp 76–83

Vigilant L, Stoneking M, Harpending H, Hawkes K, Wilson AC (1991) African populations and the evolution of human mitochondrial DNA. Science 253:1503–1507

Wakeley J (1993) Substitution rate variation among sites in hypervariable region I of human mitochondrial DNA. J Mol Evol 37:613–623

Watson E, Bauer K, Aman R, Weiss G, von Haeseler A, Pääbo S (1996) mtDNA sequence diversity in Africa. Am J Hum Genet 59:437–444

Wilson MR, Holland MM, Stoneking M, DiZinno JA, Budowle B (1993) Crime Lab Dig 20:68–77

Wolstenholme DR, Jeon KW (eds) (1992) Mitochondrial genomes. International Review of Cytology. Vol 141: Mitochondrial genomes. Academic Press, San Diego