INVITED CORRESPONDENCE
mtDNA Recombination: What Do In Vitro Data Mean?

Neil Howell

Departments of Radiation Oncology and Human Biological Chemistry and Genetics, The University of Texas Medical Branch, Galveston

In a provocative and stimulating study, Thyagarajan et al. (1996) show that extracts of mitochondria from human cells harbor enzymes that catalyze the homologous recombination of DNA plasmids. These authors highlight an important discrepancy. If mitochondria have the enzymatic machinery to carry out homologous recombination in vitro between DNA plasmids, then why hasn't recombination in situ between mtDNA molecules been detected?

Actually, there were early reports, using physical approaches rather than genetic or molecular analyses of marker exchange, of mtDNA recombination in interspecific rodent-human hybrids (Horak et al. 1974), but those results were refuted by subsequent investigations (Zuckerman et al. 1984). Thyagarajan et al. (1996) suggest that recombination does not occur in interspecific hybrid cells because of the extensive sequence divergence between the mtDNAs, but recent data indicate the extreme rarity (and possibly the absence) of intergenomic or reciprocal mtDNA recombination in human cells. Thus, Ohno et al. (1996) described a patient with a multisystem mitochondrial disorder who was heteroplasmic for the MELAS (mitochondrial encephalopathy, lactic acidosis, and strokelike episodes) point mutation at nucleotide 3243. In addition, an mtDNA deletion also was found in the patient's muscle tissue. Significantly, the deletion was found *only* in mtDNA molecules that carried the 3243 mutation and not in those that were the wild-type sequence at this nucleotide. In other words, there was no indication for crossover recombination between the deletion and the pathogenic point mutation. Another triplasmic individual (i.e., having three mtDNA genotypes but, in this instance, involving two point mutations) has been studied recently, and, again, there was no evidence for recombination (Bidooki et al. 1997). In both studies, however, the level of sensitivity was not great, and a low frequency of recombination cannot be excluded. We have analyzed several triplasmic family members from a LHON (Leber hereditary optic neuropathy) pedigree: one putative mtDNA recombination event was observed (with a frequency of ~1% for that individual), but the two markers are separated by only 40 bp. A PCR artifact may be a more likely explanation for this rare crossover event (Howell et al. 1996).

The pathogenic role of mtDNA deletions, in clinical disorders such as Kearns-Sayre syndrome or Pearson syndrome, has been recognized for almost 10 years, but whether such deletions are generated by illegitimate intragenomic recombination or by slipped-strand mispairing during replication is not yet known (reviewed in Larsson and Clayton 1995). One difficulty for either model is that deletion breakpoints are not always associated with short repeat sequences (e.g., see Mita et al. 1990). However, in a small number of cases in which short repeat sequences constituted the breakpoints, the first repeat was retained in the deleted molecule, which is a prediction of the slipped-strand mispairing model (Shoffner et al. 1989; Degoul et al. 1991). On the other hand, Mita et al. (1990) observed that mtDNA deletion breakpoints were often near topoisomerase binding sites, which is an intriguing result that will be discussed below (also see Blok et al. 1995). Furthermore, Poulton et al. (1993) have suggested that mtDNA duplications may be a transient intermediate in the generation of deletions. Both sets of results seem to favor illegitimate recombination rather than slipped-strand mispairing.

Thyagarajan et al. (1996) further speculate that the mammalian mitochondrial homologous-recombination system generates only gene conversion–type events, rather than the reciprocal exchange of sequences. If conversion is limited to small regions of the mtDNA, then one might not detect recombination, either by molecular-genetic analysis of widely spaced markers in triplasmic individuals or by physical approaches such as those of Clayton et al. (1974). Another possible explanation for the apparent lack of a marker exchange–type recombination in mammalian mitochondria is that intergenomic mtDNA recombination requires not only a recombi-

Received December 10, 1996; accepted for publication May 5, 1997.

Address for correspondence and reprints: Dr. Neil Howell, Biology Division 0656, Department of Radiation Oncology, The University of Texas Medical Branch, 301 University Boulevard, Galveston, TX 77555-0656. E-mail: nhowell@mspo3.med.utmb.edu

This article represents the opinion of the author and has not been peer reviewed.

0002-9297/97/6101-0006$02.00

nase, but also a mechanism whereby genotypically distinct mtDNA molecules are brought into sufficient physical proximity for recombination/conversion to occur. It has been reported that mitochondria that carry distinct populations of wild-type and deleted mtDNA molecules mingle freely and rapidly within cells and show complementation at the level of mitochondrial translation (Hayashi et al. 1994; Takai et al. 1997), thereby attaining the homogeneous intracellular distribution that should be permissive for recombination. Those studies, however, conflict with others. Thus, Yoneda et al. (1994) did not observe complementation when mitochondria with different mtDNA genotypes were introduced into the same cell, even after 3 mo in continuous culture, although they postulate that complementation does occur if the mtDNA molecules arise within the same population of organelles (also see Shoubridge 1994). Recent measurements of mtDNA replication in situ reveal a complex and dynamic intracellular process in which replication is localized to the perinuclear region of cells and in which the newly replicated mtDNA molecules then are distributed rapidly throughout the mitochondrial network (Davis and Clayton 1996). What these results mean for complementation and intermitochondrial exchange of mtDNA molecules is not yet clear, but they are a salutary reminder that all aspects of mitochondrial genetics will be constrained by the structural organization of the mtDNA molecules within the organelles and by the dynamics of this organization.

However, even if there is intermitochondrial complementation within cells, this process still may not be sufficient to bring mtDNA molecules into the physical proximity that is necessary for recombination. In mammalian cells, mtDNA molecules do not form a freely mixed, or panmictic, pool; instead, they are sequestered into clusters, or nucleoids, of 2–10 molecules that are attached to the inner mitochondrial membrane (e.g., see Nass 1969; Satoh and Kuriowa 1991). If the exchange of mtDNA molecules among different nucleoids is slow, then there will be limited opportunity for recombination to occur, even if organelle fusion/fission occurs at a high frequency (which apparently is the normal situation at some stages of mammalian oogenesis and embryogenesis; reviewed in Smith and Alcivar 1993). It is interesting to note that mtDNA recombination has not been detected in *Paramecium tetraurelia,* probably as a result of inefficient mitochondrial fusion (Adoutte et al. 1979).

It may be worthwhile to step back from the issue of the detection of mtDNA recombination and to ask, instead, how else might mammalian mitochondria utilize an enzymatic machinery that can catalyze recombination. Mammalian mtDNA is maternally transmitted and is thus, from the population-genetics perspective, a system of small, sexually isolated demes, or clonal lineages. As a consequence, there is almost no opportunity for mitochondrial "sex," and, therefore, mammalian mtDNA faces the possibility of evolutionary extinction through the operation of Muller's ratchet, because there is no efficient mechanism for the removal of deleterious mutations from the lineage's mitochondrial gene pool (reviewed in Howell 1996). There may be a very low level of *paternal* mtDNA transmission in mammals (e.g., see Gyllensten et al. 1991), but it stretches credulity that evolution would sustain a system to foster genetic exchange among maternal mtDNA and those exceedingly rare paternal mtDNA molecules. Although recombination is almost a universal genetic process, natural selection will act against recombination if the advantages of the maintenance of sets of coadapted genes outweigh those gained through recombination (Maynard Smith 1977). In view of the extraordinarily high mtDNA mutation rate and of the unusually compact genetic organization of mammalian mtDNA, one easily can imagine a scenario in which reciprocal recombination would be disadvantageous. In fact, the lack of recombination may be one mechanism that has evolved to slow Muller's ratchet in mammalian mitochondrial genomes.

Thyagarajan et al. (1996) also suggest that the homologous-recombination activity functions in DNA repair, possibly involving a recA-like enzyme. As is discussed in their article, the general view on this issue has shifted from "no repair" to "some repair." However, mammalian mtDNA is a high-copy number system (thousands of copies/cell), and, therefore, such cells seemingly have the luxury of forgoing extensive repair systems (e.g., see Clayton et al. 1974), because the failure to replicate of a small proportion of damaged mtDNA molecules imposes little cost. On the other hand, if only a subpopulation of mtDNA molecules, such as those localized in the perinuclear region, are replicated (Davis and Clayton 1996), then the evolutionary advantage of a repair system becomes more tenable.

One suggestion that seems to tie together several features of mammalian mitochondrial genetics is that the homologous-recombination activity detected by Thyagarajan et al. (1996) may be part of a topoisomerase/resolvase complex that functions to separate daughter monomers, at the termination of replication, and then to introduce superhelical turns into these monomers (Clayton 1982). It has been reported that mammalian mitochondria contain a topoisomerase that has different properties from those of its nuclear homologue (Topcu and Castora 1995). The termination of bacterial genome replication involves a tightly integrated process of recombination, topological resolution of daughter molecules, and partitioning (e.g., see Lobner-Olesen and Kuempel 1992; Louarn et al. 1994). However, in con-

trast to the high-fidelity resolvase system that governs chromosome partitioning and nucleoid organization, in bacteria, the enzymatic machinery that catalyzes segregation or separation of daughter mtDNA molecules is imprecise, and there is a substantial fraction of complex mtDNA forms, either unicircular or concatenated oligomers, in many types of mammalian cells (Clayton and Smith 1975; Howell et al. 1984). Both mtDNA duplications and mtDNA deletions (discussed above) ultimately may arise through "mistakes" of the topoisomerase/resolvase complex. Lockshon et al. (1995) have shown that, in yeast mitochondria, increased levels of topologically complex mtDNA species (generated in their system by a failure to resolve recombination junctions) alter the pattern of mtDNA segregation, by reduction of the number of segregation units. These results suggest that a resolvase function may be important not only for proper replication and partitioning of human mtDNA, but also for the control of segregation.

The results of the study by Thyagarajan et al. (1996) are convincing, important, and tantalizing. However, the physiological significance of this recombinase remains inaccessible, because there is still neither any evidence for reciprocal recombination of mtDNA molecules nor any apparent reason that such an activity would be beneficial. When we resolve this paradox, we will have obtained some key insights into human mitochondrial biogenesis and evolution. However, this is a genetic system that "plays by its own rules," and we should anticipate further surprises along the way.

References

Adoutte A, Knowles JK, Sainsard-Chanet A (1979) Absence of detectable mitochondrial recombination in Paramecium. Genetics 93:797–831

Bidooki SK, Johnson MA, Chrzanowska-Lightowlers Z, Bindoff LA, Lightowlers RN (1997) Intracellular mitochondrial triplasmy in a patient with two heteroplasmic base changes. Am J Hum Genet 60:1430–1438

Blok RB, Thorburn DR, Thompson GN, Dahl H-H M (1995) A topoisomerase II cleavage site is associated with a novel mitochondrial DNA deletion. Hum Genet 95:75–81

Clayton DA (1982) Replication of animal mitochondrial DNA. Cell 28:693–705

Clayton DA, Doda JN, Friedberg EC (1974) The absence of a pyrimidine dimer repair mechanism in mammalian mitochondria. Proc Natl Acad Sci USA 71:2777–2781

Clayton DA, Smith CA (1975) Complex mitochondrial DNA. Int Rev Pathol 14:1–65

Davis AF, Clayton DA (1996) In situ localization of mitochondrial DNA replication in intact mammalian cells. J Cell Biol 135:883–893

Degoul F, Nelson I, Amselem S, Romero N, Obermaier-Kusser B, Ponsot G, Marsac C, et al (1991) Different mechanisms inferred from sequences of human mitochondrial DNA deletions in ocular systems. Nucleic Acids Res 19:493–496

Gyllensten U, Wharton D, Josefsson A, Wilson AC (1991) Paternal inheritance of mitochondrial DNA in mice. Nature 352:255–257

Hayashi J-I, Takemitsu M, Goto Y, Nonaka I (1994) Human mitochondria and mitochondrial genome function as a single dynamic cellular unit. J Cell Biol 125:43–50

Horak I, Coon HG, Dawid IB (1974) Interspecific recombination of mitochondrial DNA molecules in hybrid somatic cells. Proc Natl Acad Sci USA 71:1828–1832

Howell N (1996) Mutational analysis of the human mitochondrial genome branches into the realm of bacterial genetics. Am J Hum Genet 59:749–755

Howell N, Huang P, Kolodner RD (1984) Origin, transmission, and segregation of mitochondrial DNA dimers in mouse hybrid and cybrid cell lines. Somat Cell Mol Genet 10:259–274

Howell N, Kubacka I, Mackey DA (1996) How rapidly does the human mitochondrial genome evolve? Am J Hum Genet 59:501–509

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

Lobner-Olesen A, Kuempel PL (1992) Chromosome partitioning in *Escherichia coli*. J Bacteriol 174:7883–7889

Lockshon D, Zweifel SG, Freeman-Cook LL, Lorimer HE, Brewer BJ, Fangman WL (1995) A role for recombination junctions in the segregation of mitochondrial DNA in yeast. Cell 81:947–955

Louarn J, Cornet F, Francois V, Patte J, Louarn J-M (1994) Hyperrecombination in the terminus region of the *Escherichia coli* chromosome: possible relation to nucleoid organization. J Bacteriol 176:7524–7531

Maynard Smith J (1977) Why the genome does not congeal. Nature 268:693–696

Mita S, Rizzuto R, Moraes CT, Shanske S, Arnaudo E, Fabrizi GM, Koga Y, et al (1990) Recombination via flanking direct repeats is a major cause of large-scale deletions of human mitochondrial DNA. Nucleic Acids Res 18:561–567

Nass MMK (1969) Mitochondrial DNA. I. Intramitochondrial distribution and structural relations of single- and double-length circular DNA. J Mol Biol 42:521–528

Ohno K, Yamamoto M, Engel AG, Harper CM, Roberts LR, Tan GH, Fatourechi V (1996) MELAS- and Kearns-Sayre-type commutation with myopathy and autoimmune polyendocrinopathy. Ann Neurol 39:761–766

Poulton J, Deadman ME, Bindoff L, Morten K, Land J, Brown G (1993) Families of mtDNA re-arrangements can be detected in patients with mtDNA deletions: duplications may be a transient intermediate form. Hum Mol Genet 2:23–30

Satoh M, Kuriowa T (1991) Organization of multiple nucleoids and DNA molecules in mitochondria of a human cell. Exp Cell Res 196:137–140

Shoffner JM, Lott MT, Voljavec AS, Soueidan SA, Costigan DA, Wallace DC (1989) Spontaneous Kearns-Sayre/chronic external ophthalmoplegia plus syndrome associ-

ated with a mitochondrial DNA deletion: a slip-replication model and metabolic therapy. Proc Natl Acad Sci USA 86:7952–7956

Shoubridge EA (1994) Mitochondrial DNA diseases: histological and cellular studies. J Bioenerg Biomembr 26:301–310

Smith LC, Alcivar AA (1993) Cytoplasmic inheritance and its effects on development and performance. J Reprod Fertil Suppl 48:31–43

Takai D, Inoue K, Goto Y, Nonaka I, Hayashi J-I (1997) The interorganellar interaction between distinct human mitochondria with deletion mutant mtDNA from a patient with mitochondrial disease and with HeLa mtDNA. J Biol Chem 272:6028–6033

Thyagarajan B, Padua RA, Campbell C (1996) Mammalian mitochondria possess homologous DNA recombination activity. J Biol Chem 271:27536–27543

Topcu Z, Castora FJ (1995) Mammalian mitochondrial DNA topoisomerase I preferentially relaxes supercoils in plasmids containing specific mitochondrial DNA sequences. Biochim Biophys Acta 1264:377–384

Yoneda M, Miyatake T, Attardi G (1994) Complementation of mutant and wild-type human mitochondrial DNAs coexisting since the mutation event and lack of complementation of DNAs introduced separately into a cell within distinct organelles. Mol Cell Biol 14:2699–2712

Zuckerman SH, Solus JF, Gillespie FP, Eisenstadt JM (1984) Retention of both parental mitochondrial DNA species in mouse–Chinese hamster somatic cell hybrids. Somat Cell Mol Genet 10:85–91