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Heavy traffic at a dual-purpose human mitochondrial tRNA gene.

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Editorial

The concept that dysfunctional mitochondria are a significant problem in humans is long standing, but only in about the last decade has our level of understanding of the basics of human mitochondrial DNA (mtDNA) structure and function permitted an assignment of mutations that cause human disease. Studies that began in the late 1960s and continuing through the 1970s developed the fundamental working models of human mtDNA replication (1) and transcription (2, 3). In the 1980s great progress was made in determining the genetic content of human mtDNA and defining the exact nature of gene expression with respect to transcript mapping and the important *cis*-acting elements that define promoters and origins of replication. Current work, using in vitro systems, has been aimed at identifying nuclear gene–encoded *trans*-acting factors that interact with human mtDNA sequences in order to achieve DNA replication and gene expression inside the organelle network. To date, only two of the human proteins involved in replication and transcription have been sequenced in their entireties; these are the major, if not only, transcriptional activator (mtTFA) and the human mitochondrial single-strand DNA binding protein.

Mutations in human mtDNA fall into two general categories: deleted or sometimes duplicated forms of the genome, and point mutations in the coding regions for RNAs and proteins. Situations where the mtDNA is altered in physical form are usually sporadic in appearance, and patients harboring such mutations display a rather complex array of neuromuscular disorders. In contrast, some point mutations in human mtDNA have been documented to be transmitted through the maternal lineage (a reflection of the fact that, in general, mtDNA is always maternally inherited).

There are likely lethal defects relevant to mitochondrial metabolism that are not observed in the natural population. These would include impairment of basic components of the replication or transcription machinery, such as polymerases or any other absolutely required protein component, or loss of mtDNA origins or promoters. Thus, the mutations that we see are probably leaky in the sense that they exhibit a milder phenotype. There is a wide range of frequency of mutation depending on the particular patient and disease; interestingly, in most cases, even as little as 10% wild-type mtDNA in the cell is sufficient to confer a protective situation, presumably by complementation within the mitochondrial system.

In the current report by Moraes et al. (4) the focus is on a particularly intriguing region of human mtDNA. This site, the gene for one of the two leucyl-tRNA species, is located immediately adjacent to the 3′ end of the gene for 16S rRNA and the 5′ end of the gene for the subunit 1 protein of complex I (nicotinamide adenine dinucleotide, reduced form [NADH]/ubiquinone oxidoreductase). Importantly, it is an example of a DNA sequence that has evolved for at least two critical and quite different purposes. One is to encode this particular tRNA species. The other is to sponsor transcription termination at the end of the rRNA gene region. It is known that a small (13-nucleotide) portion of the tRNA gene is the minimal element to effect termination, and this process occurs by the binding of a 34-kD protein to this target sequence (5, 6). The simplest explanation for the nature of transcripts produced both in vivo and in vitro is that when the target sequence is occupied by this protein it presents a barrier to the transcription machinery, thereby terminating transcription at this site.

Moraes et al. (4) have identified two additional human mtDNA mutations in their work, and one of these is in the aforementioned leucyl-tRNA gene. They note that when one assesses all of the currently identified human mtDNA point mutations, this gene, with a summary total of nine, exhibits the largest number of mutations within a localized region of the genome. It is for this reason that they postulate that the gene may be a "hot spot" for mutation in humans.

The points raised by Moraes et al. (4) for why this may be the case are reasonable. It is known that the rRNA genes are heavily transcribed, and the process of transcription might interefere with proper mtDNA replication that approaches with the opposite polarity. They also argue that the natural occurrence of termination factor binding to DNA at this site may cause special problems that prevent the DNA polymerase machinery from operating properly and with a normal degree of accuracy of nucleotide incorporation. To date, however, these concepts remain speculative and have not been tested directly.

Is the heavy traffic of nucleic acid synthesis the key to mutation at this leucyl-tRNA/transcription terminator? It is possible that there is something unique about this particular tRNA that simply causes or permits the occurrence of more point mutations in the heteroplasmic disease state. However, it seems to me more likely that mutations are consequential because multiple pathways of RNA metabolism occur here. In addition to transcription termination, RNA processing is required to produce correctly the tRNA species and the immediately adjacent messenger RNA. Thus, the demands on correct sequence in this gene may reduce the number of potential neutral mutations. This, in turn, would result in a higher frequency of functional problems for mutations in this specialized tRNA gene and would not necessarily require an intrinsically higher rate of mutation within it. Principal clues should come from further studies on the basics of DNA and RNA metabolism at this region of active regulation.

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