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Breakthroughs and Views

New patterns of inheritance in mitochondrial disease

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Abstract

With the identification of a patient with mutated mitochondrial DNA (mtDNA) of paternal origin, it has been unequivocally proven that not only does paternal mtDNA survive in the zygote, but it can also contribute substantially to the mtDNA pool of adult, human skeletal muscle. The questions are: how often does paternal mtDNA inheritance occur and what mechanisms are involved? In this paper, we will review current knowledge on the fate of sperm mitochondria after fertilization and discuss the impact paternal inheritance may have on our understanding of mitochondrial biology.

Mammalian mitochondria are present in virtually all cells of the body. Each mitochondrion contains 1–10 mitochondrial DNA (mtDNA) molecules. The mammalian mitochondrial genome is a small circular double-stranded DNA with 16,568 bp, encoding 22 tRNAs, two rRNAs, and 13 polypeptides. The 13 polypeptides are all subunits of the mitochondrial enzyme complexes involved in oxidative phosphorylation [1]. mtDNA has a high rate of mutation due to the lack of histones and to damage from oxygen radicals generated by the respiratory chain [2].

It is generally believed that mitochondria are inherited exclusively from the mother, and that their inheritance is clonal, meaning that all mtDNA copies are identical (homoplasmy) [3–5]. In mitochondrial diseases caused by mutations of mtDNA, wild-type (normal) and mutated mtDNA usually coexist in the same cells, a condition called heteroplasmy [6].

Studies of large pedigrees and phylogenetic studies have documented a clear maternal inheritance of mitochondria in humans [7–10]. From these studies it has been inferred that mtDNA mutations in sporadic cases of mitochondrial disease also arise on a maternal background, although mtDNA is almost never analyzed in detail and a paternal mtDNA haplotype therefore cannot be ruled out.

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In the following, we will review the evidence for paternal inheritance of mtDNA and discuss the possible implications for genetic counseling and anthropology.

Dilution of paternal mtDNA in the embryo

The sperm contributes to the fertilized oocyte's pool of mitochondria (see Fig. 1). During fertilization, the mitochondria-rich mid-piece of the sperm tail also enters the egg. The number of mtDNA molecules within single spermatozoa is much lower than the mtDNA copies in the oocyte. It has generally been believed that the number of mtDNA copies in each spermatozoon is in the range of 50–100 [11,12]. However, the number varies considerably in the literature, probably due to different methods used. In more recent papers, where mtDNA in human sperm was determined with more sensitive techniques, spermatozoa were found to contain on average 1200 mtDNA copies per spermatozoon [13]. The human oocyte contributes approximately 100,000 mtDNA molecules [14,15].

Gyllensten and co-workers [16] were the first to prove that some of this paternal mtDNA may survive in the embryos of interspecific mitochondrial congenic mice generated by several successive backcrosses between *Mus musculus* and *Mus spretus*. The authors showed that 0.1–0.01% of the mtDNA in all tissues of the offspring was of paternal origin. They concluded that

leakage of paternal mtDNA does occur in animals and that previous failure to detect paternal mtDNA was due to the use of insensitive methods. This idea has been supported mathematically by Milligan [17], who determined the probability of falsely negating paternal inheritance, i.e., accepting that $P = 0$ (where P is the probability of paternal inheritance) when in fact $P > 0$. He calculated that a sample of 300 progeny or more with no detected paternal mtDNA is required to exclude a paternal contribution greater than 1%. Very large samples of progeny are required to prove a strict maternal inheritance and therefore studies involving simple progeny testing may be misleading.

Specific destruction of sperm mitochondria

The finding by Gyllensten and co-workers of paternal mtDNA in mouse offspring has been questioned. Kaneda et al. [18] developed an assay capable of detecting sperm mtDNA in a single mouse embryo. They showed that in intraspecific hybrids of *M. musculus*, the paternal mtDNA disappeared after the pronucleus stage. In contrast, in interspecific hybrids between *M. musculus* and *M. spretus*, paternal mtDNA was detectable from the pronucleus stage to the newborn mouse. In the interspecific hybrids, the leaked paternal mtDNA was not distributed to all tissues nor was it transmitted from the females to the following generation [19]. They concluded that species-specific exclusion of paternal mtDNA is stringent and that the mouse oocyte cytoplasm can recognize nuclear DNA-encoded factors in sperm mitochondria of the same species, but not the sperm mtDNA per se or products encoded by mtDNA. It was later shown [20] that only sperm or spermatid mitochondria and not liver mitochondria have these factors that render them susceptible to selective elimination from embryos, emphasizing the unique role of sperm mitochondria.

Ubiquitination

One of the factors believed to be involved in the destruction and elimination of sperm mitochondria is the ubiquitination of sperm mitochondria. Ubiquitination is one of the processes in which a protein is tagged for further breakdown through proteolysis. This is achieved by covalent binding of the protein ubiquitin to the ε-amino group of the substrate's lysine residues [21]. In several reports by Sutovsky and co-workers it has been documented that sperm mitochondria are tagged with ubiquitin in the oocyte cytoplasm and subsequently eliminated by proteolysis [22–24]. They showed that the destruction of the ubiquitinated sperm could be prevented by microinjection of anti-ubiquitin antibodies or by inhibiting the activity of lysosomal proteases. The ubiquitination and destruction were not seen in hybrid embryos between domestic cow eggs and sperm of wild cattle, supporting the above hypothesis of a species-specific recognition apparatus. Ubiquitinated sperm has now been detected in the mitochondrial sheathing of several other species (i.e., mouse, rhesus monkey, and humans) than cattle.

Ubiquitination is also believed to play a role in the recognition of defective sperm for destruction [25].

In vitro fertilization

Intracytoplasmic sperm injection (ICSI) is widely used in assisting reproduction. With this technique, a single spermatozoon is injected into the oocyte. To study the fate of the sperm mtDNA in humans, Danan et al. [26] investigated the mtDNA in 27 neonates born after ICSI. The techniques used allowed paternal mtDNA to be detected with a sensitivity level between 0.01% and 2%, using the highly polymorphic D-loop as a target for distinguishing paternal from maternal mtDNA. Following this procedure, they found no evidence for transmission of paternal mtDNA. They concluded that the ICSI technique, using mature spermatozoa, does not circumvent the maternal inheritance of mtDNA. However, they emphasized that the results were obtained using mtDNA from blood and therefore could not rule out the possibility that paternal mtDNA might be found in other tissues. In support of paternal transmission of mtDNA, St. John et al. [27] detected paternal mtDNA after both in vitro fertilization and ICSI in abnormal embryos. They analyzed 32 human polyploid embryos and found paternal mtDNA at the two-cell stage, the blastocyst stage, and even past the eight-cell stage. The data from ICSI and in vitro fertilization are thus equivocal concerning paternal mtDNA inheritance, but at least suggest that in association with an altered genetic environment, paternal mtDNA may survive in the human embryo.

Recombination

Recombination of mtDNA is a phenomenon that has been heavily disputed over the years [28–34]. Recombination would imply either mitochondrial fusion or uptake of leaked and released paternal mtDNA into maternal mitochondria, and the existence of enzymes allowing recombination. The existence of homologous DNA recombination activity in mitochondrial extracts is documented, although it may only serve as part of an mtDNA repair system [35–37]. Fusion of mitochondria seems to be rare and there is apparently no rapid exchange between mtDNA molecules within a cell [38]. So far there has been no proven evidence of recombination in human mitochondrial DNA.

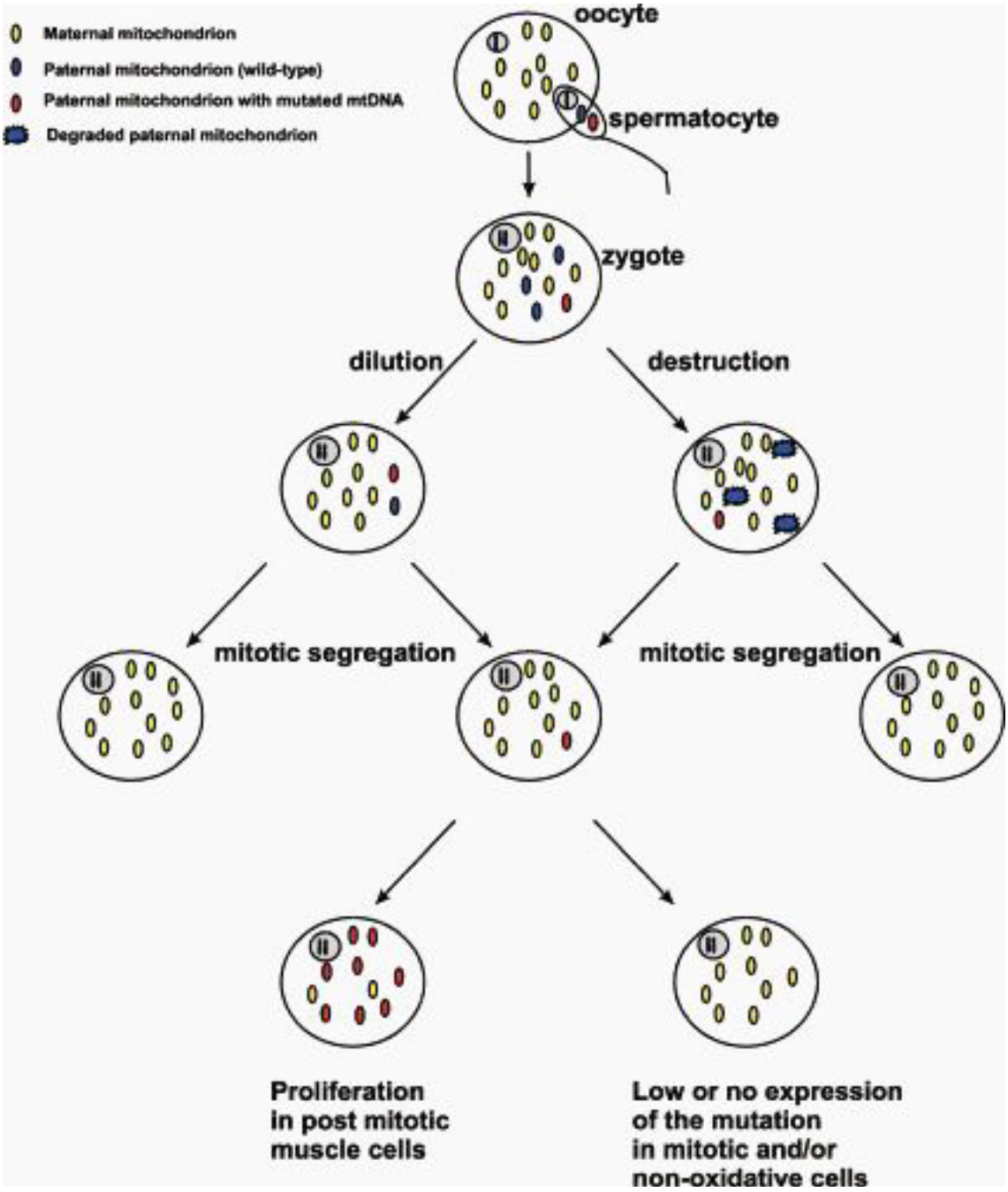

Fig. 1. Illustration of possible different pathways leading to the accumulation of paternally derived mutated mitochondria in muscle. In the left scenario the paternal mitochondria will disappear due to simple dilution and mitotic segregation. The right scenario illustrates specific destruction of the imprinted (ubiquitinated) paternal mitochondria. This destruction may not always be complete, leading to the survival of single paternal mitochondria. In both scenarios, a single paternal mitochondrion will by chance end up in a post-mitotic muscle fiber. Due to the high energy demand in these cells, the mutated mitochondria may have a proliferative, replicative advantage in contrast to mitochondria in actively replicating cells with lower oxidative rates.

Paternal inheritance does exist!

With the identification of a patient in whom mutated mtDNA in muscle was of paternal origin, it has been proven that it is possible for paternally derived mtDNA to contribute substantially (90%) to the mtDNA pool of adult human skeletal muscle [39]. The paternal mtDNA in the patient harbored a 2-bp deletion in the ND2 gene, thus introducing a frame-shift and a premature stop codon close to the mutation. As a result, complex I activity was decreased below 5% of normal in the patient's muscle. Paternal mtDNA could not be detected in blood, fibroblasts, and hair roots.

The 2-bp deletion probably arose de novo in early embryogenesis or in the paternal germ line, since the patient's father was healthy and did not carry the mutation in blood. The clinical feature of the patient and the restriction of mutated mtDNA to skeletal muscle resemble the more common patients with single large-scale deletions of mtDNA associated with chronic progressive ophthalmoplegia and the rare point mutations or microdeletions of mtDNA [40–44]. The origin of the mutation in these cases has so far been thought to arise de novo in maternal mtDNA, either in the germ line or in early embryogenesis. However, in none of these cases was the possibility of paternal inheritance considered. Haplotype studies of these groups of patients are therefore warranted.

It is still obscure by what mechanisms the paternal mtDNA ended up constituting 90% of mtDNA in muscle. It could have been the result of survival of a single or a few sperm mitochondria that would probably have been diluted out and never been recognized, had the pathogenic mutation not conferred the mitochondria a selective proliferative advantage [45]. However, it is also possible that the disease was a coincidence, which led to the detection of the paternal mtDNA in muscle. Ubiquitination and the selective destruction of sperm mitochondria in the oocyte could also have been impaired by the altered mtDNA genotype or by a mutation in a nuclear gene involved in the recognition process of paternal mitochondria, thus allowing paternal mtDNA to survive. Furthermore, if the number of mtDNA copies is 10 times higher than generally reported [13], the chance that a few paternal mtDNA molecules could propagate in intact mitochondria would increase.

Another case of paternal inheritance has recently been reported in birds [46]. Among 27 birds sampled in a zone where two subspecies groups of the great tit, *Parus major* and *Parus minor* live, one bird of *major* phenotype had both *minor* and *major* mitochondrial haplotypes. The two haplotypes differed from each other at 36 mtDNA positions. The differences were therefore most likely not due to somatic mutations, but rather the result of a paternal leakage of mtDNA. However, for obvious reasons, it was not possible to study whether the mtDNA had leaked from the father of the bird or whether such leakage had occurred in previous generations, with maternal transmission of the heteroplasmy. In any case, this is a further demonstration of the survival of paternally derived mtDNA in cross-species hybrids.

Findings during the last few years have unequivocally proved that paternal transmission of mtDNA does occur to offspring in animals and humans. The big question still remains as to how frequent this phenomenon is in humans. The single case reported so far in humans, and even a few more future such cases, will not negate the general rule of maternal inheritance of mtDNA in humans. If the case is not unique and paternal inheritance occurs with more than 1% frequency, then the debate of mtDNA recombination will be reopened and the anthropological research on the evolution and migrations of modern man, based on mtDNA analyses, must be reconsidered. Furthermore, genetic counseling, which is already very complex in mitochondrial disorders, will have to consider paternal contribution to the inheritance as well. Systematic haplotype analyses of large cohorts of patients with sporadic mtDNA mutations and healthy individuals are therefore warranted to unravel this enigma.

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