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The sperm mitochondria-specific translocator has a key role in maternal mitochondrial inheritance

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Abstract

The mechanism of maternal mitochondrial inheritance in animals involves the selective elimination of sperm mitochondria by the elimination factor of the egg and the sperm mitochondria-specific factor. In vitro fertilization using sperm from isogenic mice incorporating heterospecific mitochondrial DNA (mtDNA) showed that the number of PCR positives of sperm mtDNA in two-cell embryos was significantly increased following sperm incubation with anti-tetratricopeptide repeat-containing protein involved in spermatogenesis (tpis) protein, anti-translocator of mitochondrial outer membrane (Tom) 22 and anti-Tom40 antibodies. The treatment of fertilized eggs with EGTA and other endonuclease inhibitors increased the sperm mtDNA levels. We conclude that the elimination factor, which is probably an endonuclease, is selectively received by the tpis protein of the sperm mitochondrial outer membrane within the egg. It is then transported into the sperm mitochondria by Tom22 and Tom40, where it destroys the sperm mtDNA, establishing the maternal inheritance of mtDNA.

Keywords: Maternal mitochondrial inheritance; Elimination factor; Sperm factor; Tom complex; *tpis*

1. Introduction

Maternal mitochondrial inheritance occurs in many eukaryotes (Birky, 2001). With the exception of interspecific crosses, strict maternal inheritance is known to take place among mammals (Shitara et al., 1998). During fertilization, the entire spermatozoan, including the mitochondria of the midpiece, enters the egg (Szollosi, 1965; Yanagimachi and Noda, 1970). The sperm mitochondria are selectively destroyed, whereas those of the egg escape destruction (Birky, 2001). Kaneda et al. (1995) showed that sperm mitochondrial DNA (mtDNA) is lost by the late pronucleus stage immediately after incorporation, using intraspecific crosses of isogenic mice that incorporated heterospecific mtDNA (for detection by PCR). The microinjection of spermatid and liver cell mitochondria into eggs resulted in only the spermatid mitochondria being eliminated (Shitara et al., 2000). Moreover, the mtDNA of sperm from mice with a heterospecific mitochondrial and nuclear genome was not eliminated, whereas that from mice in which only the mitochondrial genome was heterospecific was destroyed (Kaneda et al., 1995). These indicate that the sperm mitochondria-specific substance might be incorporated into the mitochondria of spermatogenic cells during spermatogenesis and encoded not by the mitochondrial genome but by the nuclear genome.

As this selective elimination system does not operate in interspecific crosses, in which the sperm mitochondria are not eliminated (Kaneda et al., 1995; Lee et al., 2002;

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Sutovsky et al., 2000), we believe that species specificity exists in this system. Hiraoka and Hirao (1988), using electron microscopy, showed that multivesicular bodies, which are a type of lysosome, gathered around the sperm mitochondria in the fertilized eggs of hamsters at the two-cell stage. Sutovsky et al. (1999, 2000) reported that prohibitin, which seems to exert a regulatory function during membrane protein degradation in the inner mitochondrial membrane (Arnold and Langer, 2002), of sperm mitochondria was ubiquitinated before fertilization in rhesus monkeys and cows. They assumed that an ubiquitin–proteasome system was the elimination factor of the oocyte in mammals, and speculated that proteasome directly recognizes ubiquitinated prohibitin in a species-specific manner.

Mitochondrial proteins are selectively transported into the mitochondrion via the translocator of mitochondrial outer membrane (Tom) complex (Blobel and Dobberstein, 1975; Zimmermann and Neupert, 1980). We suggested that egg mtDNA is not protected from the elimination factor, but that the sperm mitochondria selectively take up the elimination factor followed by the destruction of mtDNA, which would explain the question of selectivity. We predicted the following characteristics of the sperm factor and conducted a search within GenBank: first, the protein's expression is specific to the testis and spermatozoa; second, it is localized in the mitochondrial outer membrane; third, it is a receptor or translocator protein; fourth, based on predictions two and three, the similarity with the Tom complex might be high; fifth, based on prediction one, it might be an antigen of an antisperm antibody in human females; sixth, there is species specificity in binding with the elimination factor; and seventh, the gene for the protein is encoded in the nuclear genome. The tetratricopeptide repeat (TPR)-containing protein involved in spermatogenesis (*tpis*) gene meets predictions one, four and seven.

According to Takaishi and Huh (1999), the gene is expressed as both a skin type (s-*tpis*) (accession code AF181253) and a testis type (t-*tpis*) (accession code AF181252) of cDNA. s-*tpis* codes for 529 amino-acid residues, while t-*tpis* codes for 901 with 372 additional amino-acid residues at the 5′ end. The *tpis* gene is encoded by chromosome 15. The t-tpis protein showed 50% identity with mouse Tom34. In Northern blotting analysis, s-*tpis* transcripts were detected in the cerebellum, tongue, esophagus and forestomach among various adult tissues as well as in the embryonic skin, while t-*tpis* transcripts were detected in the testis. In situ hybridization showed strong signals from *tpis* transcripts in testis spermatogenic cells. The function of *tpis* is unknown. The human homologue of t-*tpis* is assumed to be *HSD-3.8* (67% identity; accession code AF311312). *HSD-3.8* was identified using the serum of an infertile woman containing antisperm antibody (Lin et al., 2001; Zang et al., 1992).

This paper demonstrates that the tpis protein meets our predictions and appears to be the protein involved in the elimination system. We additionally discuss indications of a candidate elimination factor.

2. Materials and methods

2.1. Mouse strains

ICR mice were used for RT-PCR, protein extraction and immunofluorescence staining. Congenic strain C57BL/6J(B6)-mt^{spr} males (Shitara et al., 1998) provided by The Tokyo Metropolitan Institute of Medical Science and B6 females were used for the inhibition assay.

2.2. Detection of tpis mRNA (RT-PCR)

RNA was extracted from the testis, spermatozoa in the epididymis, and the cerebellum of a mature male ICR mouse using the acid guanidinium–phenol–chloroform method. A total of 50 ng of each was measured. Unfertilized eggs were collected by harvesting the mature ovaries of female ICR mice 48 h after injecting them with pregnant-mare's serum gonadotropin (PMSG). One-cell and two-cell embryos were collected following the in vitro fertilization of spermatozoa and unfertilized eggs in the oviduct, which were made to superovulate 24 h after injecting human chorionic gonadotropin (hCG) 48 h after the PMSG injection. Five of each type of egg were collected in the PCR tube. A few whole spermatozoa were sampled from the suspension. Based on the manufacturer's instructions for the OneStep RT-PCR Kit (QIAGEN), RT-PCR was performed by treating the specimen, primer and dNTP at 70 °C for 10 min, and then adding the enzyme mix followed by reverse transcription at 60 °C for 60 min. The primer pair for the t-*tpis*-specific portion comprised the forward primer 5′-AAAACCATCCCGGAGTG GAA-3′ and the reverse primer 5′-CGGCCTGAGCTC GATTGTTA-3′. The primer pair for the s-*tpis* mRNA-specific portion comprised the forward primer 5′-CCGGTGCGCCCTTCTCTAC-3′ and the reverse primer 5′-CTCTCCCGCCATTTTGATCC-3′. A total of 10 μl of RT-PCR products were applied to NuSieve 3:1 agarose gel and electrophoresed in TBE buffer. Gels were stained with ethidium bromide.

2.3. Preparation of anti-tpis, -Tom-complex antibody

Purified rabbit antiserum against tpis protein was kindly provided by Dr. M. Takaishi, Okayama University Graduate School of Medicine and Dentistry, Japan. To prepare the antiserum, cDNAs encoding