Mitochondria-driven cell elongation mechanism for competing sperms

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Sexual competition has selected a number of extreme phenotypes like the tail ornament of peacock male. Sperm tail of Drosophilidae elongate up to 6 cm as a result of evolutionary selection for reproductive fitness among competing sperms. Sperm elongation takes place post meiotically and can proceed in the absence of an axoneme. Here, we used primary cultures of elongating spermatids of D. melanogaster to demonstrate that sperm elongation is driven by interdependent extension of giant mitochondria and microtubule array that is formed around the mitochondrial surface. This work established that, in addition to functioning as an energy source, mitochondria can serve as internal skeleton for shaping cell morphology.

Varia[on of Sperm Tail Length under Sexual Competition

Fierce competition for better mating partners has been a driving force for evolution of diverse forms and behaviors in sex-related traits. During fertilization, sperms from multiple males compete for the chance of fertilizing a limited number of eggs. This natural experimentation in every single fertilization has selected sperms that work best in their fertilization environment, leading to diversification of sperm morphology as a result of sperm competition. Although tadpole-like sperm morphology in mammals represents a remarkable perfection as swimming cell machinery, extensive diversification of sperm morphology and physiology has been observed across other species, serving as a resource for resolving species phylogenies. Evolution of sperm tail in insects and other arthropods is particularly rapid and diverse, ranging from aflagellate to multi-flagellate, and in some cases develops extraordinary long tails.

During sperm competition, any advantageous traits of sperm to outdo its competitors (better swimmer, longer survival, blocking competitors, dimorphism, etc.,) will directly contribute to fertilization and reproductive success in his descendant. Females are also under a strong evolutionary pressure to gain control over the fertilization process to pick sperms from a particular male of their choice. Among taxa as wide as birds, reptiles and insects, females have sperm storage organs specially adapted for sperm selection. Thus, co-evolution of sperm and the female sperm storage organ has been reported in many species, sometimes leading them to develop bizarre morphology of these organs.

The co-evolution of male and female reproductive traits in Drosophila makes it a unique and powerful model for exploring the evolutionary consequences of post-copulatory sexual selection (Fig. 1A). The Drosophilidae has great variation in sperm length, ranging from 300 μm to 6 cm. The female has two storage organs, seminal receptacle and spermathecae, in which sperms stay in a fertile state waiting for ovulation. It has been shown that the length of the primary storage organ, seminal receptacle, positively correlates with sperm length across 46 Drosophilidae species. The length of seminal receptacle range from 400 μm to 8 cm, and in most of the cases is slightly longer than the sperm length. It was suggested that inside the thin tubular seminal receptacle, sperm from different males are mixed and compete with each other for a chance to reach the ovulated egg, and that a longer tail is...
microscopic analyses revealed that cytoplasmic microtubules are located in the vicinity of mitochondria, arranged in parallel to the longitudinal axis of the sperm tail. Based on live imaging and local drug treatment data, we found that microtubules at the tail tip region with particularly fast turn-over rate and active sliding motion are essential for sperm tail elongation. Thus, we call this region “the growth zone” of sperm tail.

**Dual Role of Mitochondria in Sperm Morphogenesis**

Through genetic perturbation of mitochondrial functions, we discovered that mitochondria play an essential role in sperm tail elongation. In postmeiotic spermatids, a testis-specific Mitofusin, *fuzzy onions*, promotes massive fusion of mitochondria to form two large pieces of

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**Figure 1.** (A) Schematic diagram of long sperm evolution among Drosophilidae species. (B) Time series of spermatid cyst elongation in vitro. Number indicates elapsed time in minutes. (C) Model of mitochondria-dependent elongation of sperm tail. Two giant mitochondria elongate together with microtubules and push cell membrane of elongating sperm tail. Top: sperm heads with nuclei.
mitochondrial derivatives called nebenkern. During spermatid elongation, giant mitochondrial lobes elongate in parallel to the axoneme to fill the entire length of sperm tail (Fig. 1C). When final mitochondrial length was reduced by mutations of fuzzy onions or no mitochondrial derivative, sperm tails failed to elongate to the maximum length. Similar defect in elongation was observed in mutants of Milton-dMiro complex, an adaptor linking mitochondria to microtubule motor protein Kinesin. In addition, we found that the surface of spermatid mitochondria serves as a microtubule-organizing center (MTOC), promoting assembly of microtubule array around themselves. We propose that double membrane architecture of mitochondria combined with cytoplasmic microtubules can serve as structural support for sustainable elongation of the sperm tail. MTOC activity of mitochondria ensures the formation of microtubule arrays as mitochondria elongate. Elongation and sliding of microtubules in the growth zone would stretch folded mitochondria to expose open surface for new microtubule assembly. By repetition of this stretch cycle, giant mitochondria can be a self-promoted structural scaffold, in addition to its original role as energy sources needed for flagellar motion.

**Future Perspectives**

Our work has two important implications in cell biology. First, it demonstrated that mitochondria play a novel role in cell morphogenesis acting as an inner skeleton of sperm tail. In other words, mitochondria, a double membrane organelle with MTOC activity, can actually determine the extracellular morphology of sperm. Another double membrane organelle nucleus showing similar morphological diversification in sperm across species is also likely serving as an alternative shaping tool for sperm morphogenesis. Second, this is a detailed cell biological study on tissue specific mitochondrial morphogenesis, which is poorly understood in the field of mitochondria biogenesis. Typical mitochondria in somatic cells are 3–4 μm in length and 1 μm in diameter moving along microtubules and undergo fusion and fission process. However, in cells of many specialized tissues, great variations of mitochondrial number, size and morphology have been reported. Many of which are suggested to be related to their tissue specific functions. In the case of Drosophila sperm morphogenesis, motor complex (Milton/dMiro/kinesin) used for mitochondrial trafficking in neuronal cells are converted into an elongation machinery of much larger mitochondria. Intriguing questions are whether the conversion of microtubule motor complex from mitochondrial trafficking and mitochondria dependent microtubule nucleation are general mechanisms required for morphogenesis of mitochondria in other type of cells, such as the ordered arrays within muscle fibers of muscle cells and the elongated forms in photoreceptor cells of human retina.

Having outlined the process of sperm tail elongation, we are in the position of starting a comparative analysis of spermatid elongation in *Drosophila melanogaster* strains with long and short sperm. Since sperm length variation appears to occur rapidly during regional separation, it should be possible to search for rapidly evolving genes among genes involved in key steps of spermatid elongation. In addition, gigantic sperms in *Drosophila* appear to have multiple evolutionary origins; it will be of interest to search for the crucial process that may have permitted the appearance of long sperm by asking which step of sperm elongation differs most between closely related species of long and short sperm. Measurement of the size of spermatocyte and nebenkern, speed of elongation, time required to reach full length and requirement of microtubules and mitochondria should clarify whether the mitochondria-driven elongation mechanism is used in other *Drosophila* species with extremely long sperms.

One of the main questions in speciation is the identification of mechanism for reproductive isolation, reducing gene exchange between two populations and enhancing the chance of speciation genes to fix among them. Comparative analysis of sperm morphogenesis may be extended to species other than *Drosophila*. Giant sperm are reported from a range of taxa including the coleopterans *Divales bipustulatus*, *Pitella patella*, hemipteran *Notonecta glauca* and the lepidopteran *Xenosoma geometrina*. Also, large mitochondria are the most frequent feature of sperms among insects and arthropods. Thus, the sperm-elongation mechanism described in this report might be a general system for facilitating sperm-size variation among insects with giant mitochondria, thereby enhancing sexual selection and reproductive isolation. Moreover, recent advance in transgenesis and gene knockout technologies has made genetic analysis in non-model insects more feasible. Therefore, cell biological analyses we performed on *Drosophila melanogaster* may be applied to other species. Taken together, our study revealed a novel mechanism of cell elongation and set a road map for future study to address the genetic basis for sperm competition and reproductive isolation.

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**References**

1. Afzelius BA. The Functional Anatomy of the Spermatozoan. Pergamon Press, Oxford 1975.
2. Baccetti B. Spermatogenesis and phylogeny in orthopteroid insects. In Baccetti B, Ed., Evolutionary Biology of Orthopteroid Insects. Ellis Horwood, Chichester UK 1987; 12-112.
3. Jamieson BGM. The Ultrastructure and Phylogeny of Insect Spermatogenesis. Cambridge University Press, Cambridge UK 1987.
4. Dallas R, Baccetti B, Bernini F, Bigliardi E, Burrini AG, Giusti F, et al. New models of agglutinate arthropod spermatozoa. In Afzelius BA, Ed., The Functional Anatomy of the Spermatozoan. Pergamon Press, Oxford (1975); 279-87.
5. Pinnick S, Markow TA, Spicer GS. Delayed male maturity is a cost of producing large sperm in *Drosophila*. Proc Natl Acad Sci USA 1995; 92:10614-8; PMID:7479851; http://dx.doi.org/10.1073/pnas.92.23.10614.
6. Simmons LW. Sperm competition and its evolutionary consequences in the insects. Princeton University Press 2001.
7. Pinnick S, Markow T, Spicer GS. Evolution of multiple kinds of female sperm-storage organs in *Drosophila*. Evolution 1999; 53:1804-22; http://dx.doi.org/10.2307/2640442.
8. Pattarini JM, Sturmer WT, Bjork A, Pinnick S. Mechanisms underlying the sperm quality advantage in *Drosophila melanogaster*. Evolution 2006; 60:2064-80; PMID:17133863.
9. Miller GT, Pinnick S. Sperm-female coevolution in *Drosophila*. Science 2002; 298:1230-3; PMID:12424377; http://dx.doi.org/10.1126/science.1076968.
10. Noguchi T, Koizumi M, Hayashi S. Sustained elongation of sperm tail promoted by local remodeling of giant mitochondria in *Drosophila*. Curr Biol 2011; 21:805-14; PMID:21549602; http://dx.doi.org/10.1016/j.cub.2011.04.016.
11. Noguchi T, Miller KG. A role for actin dynamics in individualization during spermatogenesis in Drosophila melanogaster. Development 2003; 130:1805-16; PMID:12642486; http://dx.doi.org/10.1242/dev.00406.

12. Basto R, Lau J, Vinogradova T, Gardsel A, Woods CG, Khodjakov A, et al. Flies without centrioles. Cell 2006; 125:1375-86; PMID:16814722; http://dx.doi.org/10.1016/j.cell.2006.05.025.

13. Hales KG, Fuller MT. Developmentally regulated mitochondrial fusion mediated by a conserved, novel, predicted GTPase. Cell 1997; 90:121-9; PMID:9230308; http://dx.doi.org/10.1016/S0092-8674(00)80031-9.

14. Stowers RS, Megeath LJ, Górska-Andrzejak J, Meinertzhagen IA, Schwarz TL. Axonal transport of mitochondria to synapses depends on milton, a novel Drosophila protein. Neuron 2002; 36:1063-77; PMID:12495622; http://dx.doi.org/10.1016/S0896-6278(02)01094-2.

15. Fawcett JW. Mitochondria. In The Cell. Saunders WB company, Philadelphia 1981; 2:410-68.

16. Pitnick S, Miller GT, Schneider K, Markow TA. Ejaculate-female coevolution in Drosophila mojavensis. Proc Biol Sci 2003; 270:1507-12; PMID:12965017; http://dx.doi.org/10.1098/rspb.2003.2382.

17. Markow TA, Reed LK, Kelleher ES. Sperm fate and function in reproductive isolation in Drosophila. Soc Reprod Fertil Suppl 2007; 65:155-73; PMID:17644960.

18. Mazzini M. Giant spermatozoa in Daubes bipustulatus F. (Coleoptera: Cleridae). Int J Insect Morphol Embryol 1976; 5:107-15; http://dx.doi.org/10.1016/0020-7322(76)90033-7.

19. Taylor VA, Luke BM, Lomas MB. The giant sperm of a minute beetle. Tissue Cell 1982; 14:113-23; PMID:7089959; http://dx.doi.org/10.1016/0040-8166(82)90011-8.

20. Axelius BA, Baceri B, Dallas R. The giant spermatozoa of Notonecta. J Submicrosc Cytol 1976; 8:149-61.

21. Morrow EH. Giant sperm in a neotropical moth (Lepidoptera: Arctiidae). Eur J Entomol 2000; 97:281-3.

22. Bogdanove AJ, Voytas DF. TAL effectors: customizable proteins for DNA targeting. Science 2011; 333:1843-6; PMID:21960622; http://dx.doi.org/10.1126/science.1204094.

23. Carroll D. Genome engineering with zinc-finger nucleases. Genetics 2011; 188:773-82; PMID:21828278; http://dx.doi.org/10.1534/genetics.111.131433.