

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.

Variation 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,¹⁻³ 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,⁴ and in some cases develops extraordinary long tails.⁵

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.⁶

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,⁷ 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

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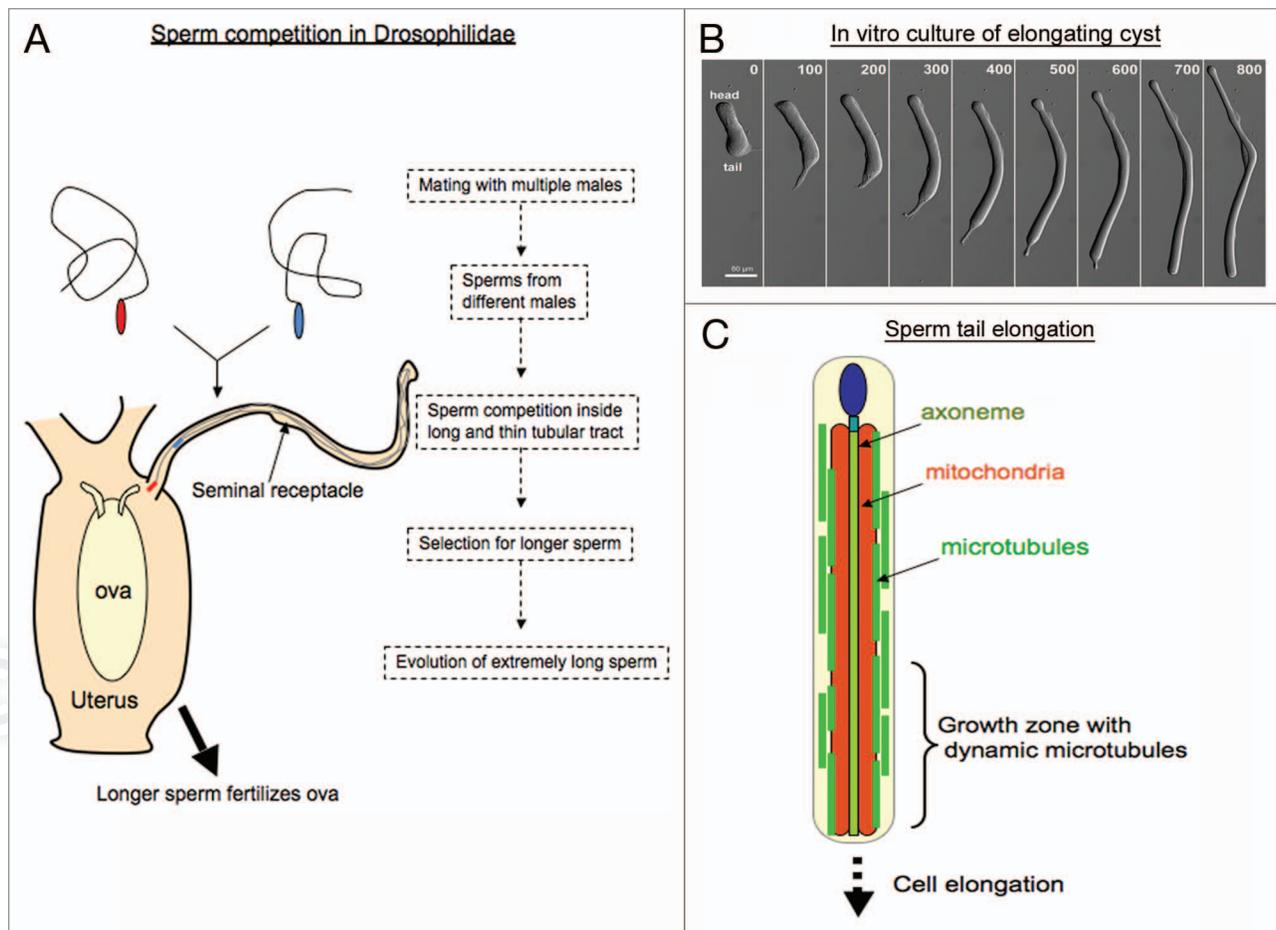


Figure 1. (A) Schematic diagram of long sperm evolution among *Drosophilidae* species. (B) Time series of spermatid cyst elongation in vitro. Number indicates elapsed time in minutes. (C) Model of mitochondria-dependent elongation of sperm tail. Two giant mitochondria elongate together with microtubules and push cell membrane of elongating sperm tail. Top: sperm heads with nuclei.

helpful to position the sperm head closer to the exit from the seminal receptacle.⁸ Indeed, it is empirically demonstrated that a longer sperm tail is advantageous for reproduction.⁹ It was hypothesized that the length of seminal receptacle and sperm tail have co-evolved, and the length incompatibility suppresses fertilization efficiency, thereby promoting reproductive isolation. In order to understand the genetic basis for sperm competition in *Drosophila*, knowledge of the cellular basis for sperm tail elongation is necessary. We have undertaken a cell biological approach to identify the mechanism of sperm tail elongation in *Drosophila*.¹⁰

Localized Microtubule Activities Drive Sustained Sperm Elongation

Study of sperm morphogenesis has been slow due to lack of both genetic tool and

in vitro culture system for detailed observation of spermatogenesis. Noguchi and Miller¹¹ have previously shown that carefully dissected spermatids (64 cell cyst) from model animal *Drosophila melanogaster* testes can be cultured in vitro and elongated spermatids separated into single sperms through individualization.¹¹ We used this system to study details of sperm tail elongation (Fig. 1B).¹⁰

Axoneme, a major structure of sperm tail which propels the swimming motion, is dispensable for tail elongation because spermatids of axoneme-less mutants (*Dsas-4*) elongated reasonably well in vitro, consistent with the previous observation of immobile but well-elongated sperms in testes of the mutants.¹² However, microtubule inhibitor experiments demonstrated that yet another microtubule-based structure is essential for sperm tail elongation. Fluorescent microscopy and electron

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

mitochondrial derivatives called nebenkern.¹³ 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.¹⁴ 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.³ 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.¹⁵

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,¹⁶ it should be possible to search for rapidly evolving genes among genes involved in key steps of spermatid elongation. In addition, gigantic sperms in *Drosophilidae* 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 *Drosophilidae* 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.¹⁷ Comparative analysis of sperm morphogenesis may be extended to species other than *Drosophilidae*. Giant sperm are reported from a range of taxa including the coleopterans *Divales bipustulatus*,¹⁸ *Prinella patella*,¹⁹ hemipteran *Notonecta glauca*²⁰ and

the lepidopteran *Xenosoma geometrina*.²¹ 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.^{22,23} 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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