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Mitochondria: transportation, distribution and function during spermiogenesis*

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ABSTRACT

Spermiogenesis is a dynamic process which includes organelle reorganization and new structure formation. The morphology and distribution of the mitochondria in germ cells change to accommodate the cellular requirement. Multiple molecular motors and related proteins participate in carrying and anchoring mitochondria to the midpiece during spermiogenesis and this process is regulated precisely. Energetic metabolism provides energy for cellular activity and influences sperm survival and motility directly. Ubiquitination of mitochondria takes place during spermiogenesis, which has been implicated in sperm quality control and mitochondrial inheritance. In light of the essential roles of mitochondria in energy production, calcium homeostasis and apoptosis, mitochondria dysfunction cause severe human diseases, such as male infertility. The present study paves a way for a more detailed exploration of the biology of mitochondria during spermiogenesis.

Keywords: Spermiogenesis; Mitochondria; Molecular Motors; Sperm Motility; Ubiquitination; Infertility

1. INTRODUCTION

Spermiogenesis is the last phase of spermatogenesis, where spherical spermatids differentiate into elongated spermatozoa in mammals [1,2]. Dynamic and pronounced changes occur in the process of spermiogenesis, including the formation of a condensed and elongated nucleus in the head, a well shaped acrosome flattened along the large portion of the nucleus, as well as a long flagellum. The flagellum is composed of cytoskeletal components and mitochondria provide the motors for sperm motility.

Mitochondria have a significant role in energy production, calcium homeostasis and apoptosis. Both sperm biosynthesis and motility require ATP. Recent findings indicate that two metabolic pathways are providing ATP for normal sperm function: oxidative phosphorylation in the midpiece and glycolysis in the principal piece [3-7]. Mitochondria are dynamic both in their morphological transitions and distribution. Several kinds of molecular motors have been indentified to be responsible for transporting mitochondria, including microtubule-dependent kinesins and dyneins, and microfilament-dependent myosins [8-14]. Several related proteins also function in transporting and docking of mitochondria [15]. Mitochondria provide maternal inheritance, and ubiquitination of mitochondria takes place to facilitate their degradation after fertilization [15]. Due to the indispensable role of mitochondria during fertilization, defect of sperm mitochondria or associated proteins can lead to male infertility.

In this review we mainly focus on the morphology and distribution of mitochondria, as well as the functions of this organelle during spermiogenesis. Furthermore, based on the transporting mechanisms within somatic cells, we attempt to define how mitochondria migrate to the midpiece, and to describe some related proteins that have been revealed in the mitochondria anchoring in the midpiece. Then, we review briefly about two energetic metabolisms that provide ATP for sperm motility, the ubiquitin modification during spermiogenesis, and finally talk about some mitochondria-related male infertility.

2. DISTRIBUTION AND MORPHOLOGICAL CHANGES OF MITOCHONDRIA DURING SPERMIOGENESIS

Spermiogenesis is a complicated morphogenesis occurring in the seminiferous epithelium of the testis during which the haploid spermatid is transformed into the finely shaped spermatozoon. Spermiogenesis has been divided into four phases in mammals: Golgi phase, cap phase, acrosome phase and maturation phase [1,16-19].

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The different stages show their own features. At the early stages of spermiogenesis (Golgi and acrosome phase), the spermatids are round; vesicles originated from the Golgi apparatus migrate towards the pole where the pre-acrosomal vesicle locates; the axoneme emerges from the distal centriole and grows actively. At the acrosome phase, the nucleus initiates to elongate and condense, the mitochondria begin to migrate to the opposite pole of the pre-acrosome. During the maturation phase, the ODFs (outer dense fibers) grow rapidly outside of the axoneme, the mitochondria begin to elongate and condense to form the mitochondrial sheath; at the same time, the acrosome is well developed, the spermatids discard most of the cytoplasm defined as the residual body to become mature spermatozoa.

The flagellum of the mammalian spermatozoon consists of four distinct regions: the connecting piece, the mid piece, the principal piece, and the short end piece. In the central part of the mid piece, the axoneme is composed of a "9 + 2" array of microtubules, nine ODFs surround the axoneme, and mitochondria are positioned in four helices along the ODFs [19-21]. The axoneme and seven ODFs surrounded by the fibrous sheath designate as the principal piece, while the end piece only includes the axoneme. During spermiogenesis, the mitochondria undergo dramatic changes both in morphology and location. In rat, the normal mitochondria with lamellar cristae are oval in shape. We observed the main events that happened during spermiogenesis in rat which supported the conclusion by De Martino *et al.* (1979) [22]. Our data show that, during the Golgi phase, cap phase and the early acrosome phase of spermiogenesis, the small, round or oval condensed mitochondria are distributed at the cell periphery, with its inner space flattened (**Figures 1** and **2**). The number of the mitochondria increases considerably. Therefore, the mitochondria are efficient to produce sufficient energy for exchanging metabolites between the adjacent Sertoli cells and spermatids or between the nucleus and the cytoplasm. The mitochondria exhibit convoluted cristae at the late acrosome phase and early maturation phase. We found that part of them move to the flagellum, whereas the remaining start to aggregate (**Figure 3**). These aggregated mitochondria are discarded within the residual bodies which are disposed by phagocytosis through the Sertoli cells or by autolysis in the mature spermatids [23]. Our data show that, at the late maturation phase, the mitochondria begin to condense and to elongate, and finally show a crescent shape along the ODFs in the mid piece (**Figures 4** and **5**). Mitochondria are supposed to generate ATP efficiently and rapidly. The length of the midpiece is similar in the same species, whereas it is obviously different among different species [24]. Possibly

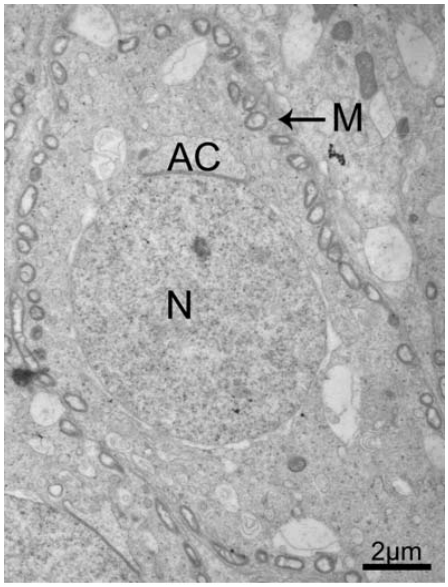

Figure 1. Mitochondria distribute at the cell periphery at the cap phase during rat spermiogenesis (black arrows in 1). M *mitochondria*, AC *acrosome*, N *nucleus*.

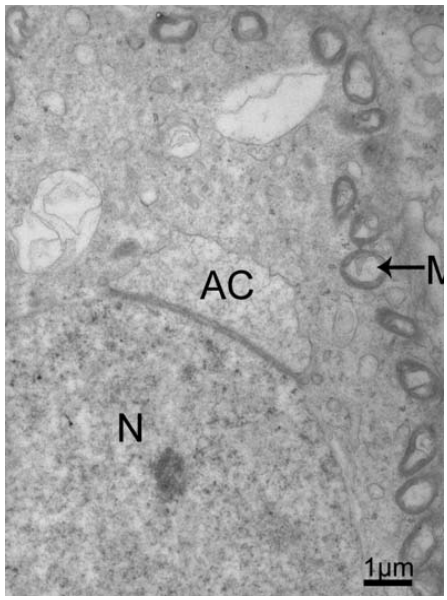

Figure 2. The magnification of partial **Figure 1**.

the whole length of the midpiece is responsible for energy supply when the fertilization process begins. A recent work by Ho *et al.* (2007) proposed a new three dimensional model to illustrate the mitochondrial arrangement in the mid piece in mouse. Initially, four dextral mitochondrial helices surround the outer dense fibers at stage 1. Subsequently, two mitochondria of the opposing arrays form circular structures at stage 2. Then, staggered mitochondria form the sinistral double helix at

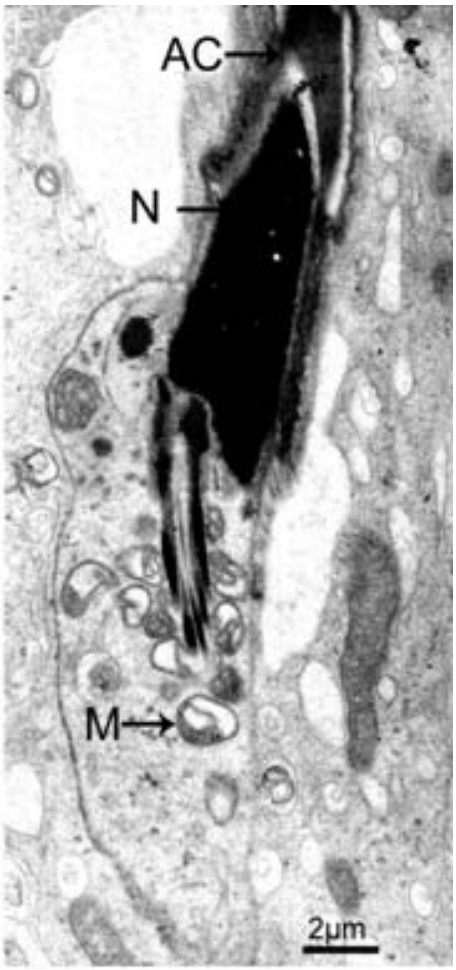

Figure 3. Part of mitochondria has been transported to the midpiece at the maturation phase.

stage 3. At the last stage, the crescent-shaped mitochondria transform to a rod-shaped form [25]. These observations provoke the question how the number, morphology and distribution of the mitochondria are controlled during spermatid differentiation.

Ultrastructure of the spermatozoa displays special features to adapt to the particular environment where fertilization takes place. In marine and freshwater animals, the spermatozoa morphology is different from mammals, exemplified by mollusk and fish.

Most of the marine bivalve mollusk spermatozoa are aquasperm, with a slightly curved or round or short-rodnucleus, a kind of species special acrosome (usually of conical shape), the midpiece consisting of one to six spherical mitochondria (four to five generally, such as *Scapharca broughtoni*, **Figures 6** and **7**) arranged in a circle that are surrounding a pair of centriole or a single large mitochondrion derived from fusion of several mitochondria, and a classical "9 + 2" flagellum [24,26-31]. Some cephalopod spermatozoa have a characteristic

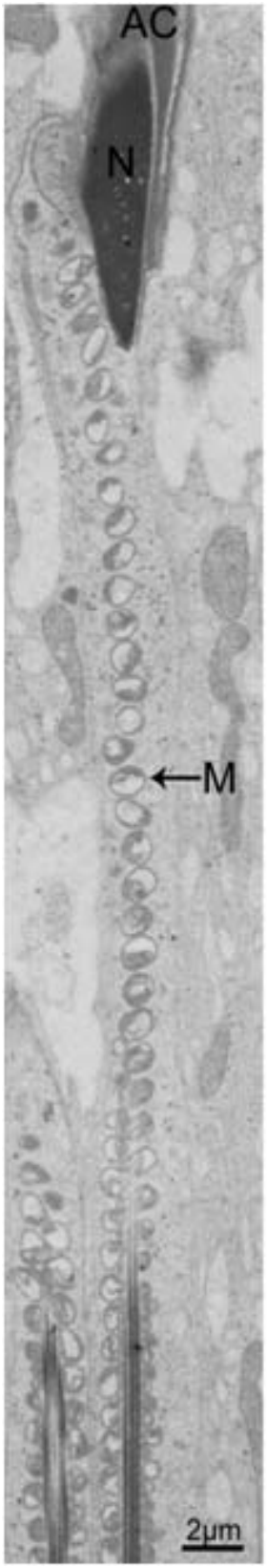

Figure 4. The mature spermatozoon of rat through head and midpiece.

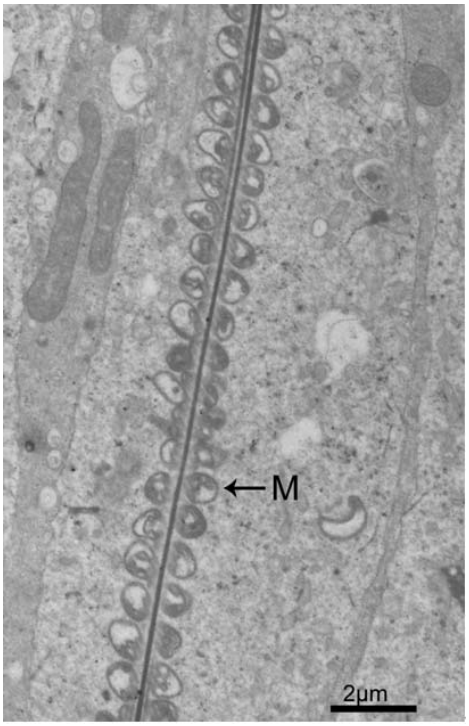

Figure 5. Mitochondrial localization in midpiece.

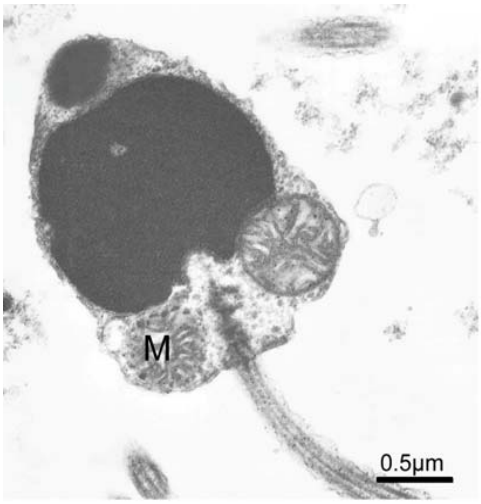

Figure 6. Typical spermatozoon of *Scapharca broughtoni*, two mitochondria visible.

acrosome with periodic and conical bands, and the mitochondrial number ranges from 9 to 11 in the midpiece, exemplified by *Octopus tankahkeei* [32-35] (**Figures 8** and **9**). Some kinds of teleostean spermatozoa belong to the aquasperm. Taking bony fish of the Perciformes as an example, their spermatozoa have a round head with a spherical nucleus, showing no acrosome, but a short midpiece with a cytoplasmic channel, and a flagellum-with typical axonemes [29,36,37]. Gusmão-Pompiani *et al.* (2005) found that in the marine teleost family Sciae-

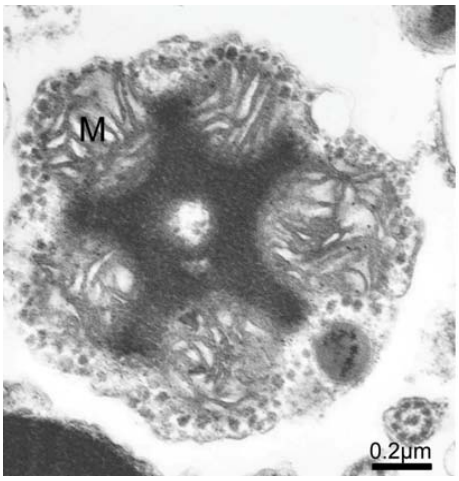

Figure 7. Cross section of midpiece in spermatozoon of *Scapharca broughtoni*, five mitochondria visible.

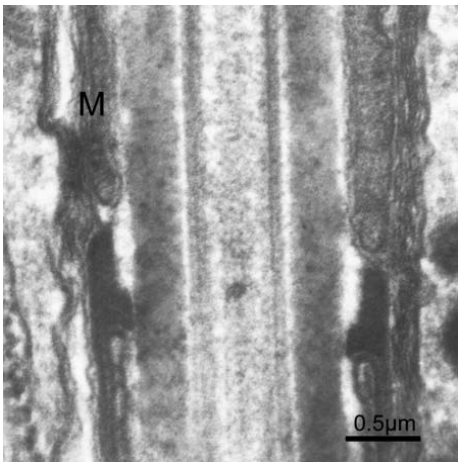

Figure 8. Joint between the midpiece and principal piece of the spermatozoon in *Octopus tankahkeei*.

nidae, there are less than ten spherical or elongate mitochondria which are located close to the nucleus and are arranged in one or two layers at the basal part of the mid piece [37]. Some exceptions exist in the Polynemidae, which are characterized by an arched nucleus surrounded by a large ring of mitochondria with a "C" shape in cross section [37]. The number of mitochondria in the midpiece in mollusks and teleostei is less prominent than in mammals.

Gage (1998) demonstrated that the flagellum length and the head length show a positive association with the mid piece length, these components may have co-evolved to provide effective fertilization [38]. The sperm swimming speed shows a positive ratio with sperm length, males with high sperm motility have higher fertilization success in mammals [39]. Mollusk and teleost employ external or internal fertilization. Sperm competition in the

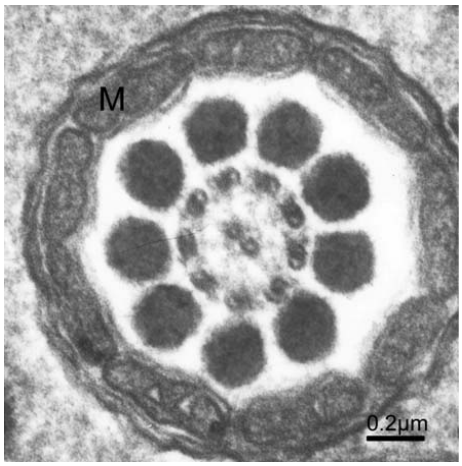

Figure 9. Cross section of the spermatozoon at the midpiece of the tail, showing 9 mitochondria.

course of external fertilization is mainly provided through the sperm number. Sperm longevity is negatively related to sperm length. Mean sperm length is shorter in free spawners compared to teleost with internal fertilization [40].

Sperm ultrastructure also reflects an adaption of the fertilization pattern to the environment. The flagellum becomes a longer mid piece containing a higher number of mitochondria and a fibrous sheath as an adaptation to internal fertilization. Such structural differences will enhance sperm motility which is necessary for successful fertilization.

3. THE TRANSPORT OF MITOCHONDRIA

The interaction between mitochondria and the cytoskeleton is essential for mitochondrial movement within cells. It was demonstrated that mitochondria move along the cytoskeleton depending on various molecular motors [12]. Different motors associate with mitochondria by docking intermediates such as adaptor proteins. Microtubule-dependent motors and actin-dependent motors are responsible for mitochondrial movement and docking. The head domain of two kinds of motors consists of a track binding site and an ATP catalytic site, on the other hand, the tail domain is responsible for recognizing and binding to diverse cargoes.

The microtubules show polarity within cells, with the minus end pointing to the centrosome and the plus end pointing to the cell periphery. Mitochondria move along the microtubule in a bidirectional manner, they usually stop and change directions under regulation to satisfy the cell metabolism. Kinesins and dyneins are microtubule dependent motors. The kinesin superfamily includes three classes of kinesins: N-terminus (KIN-N), C-terminus (KIN-C) and M-terminus (KIN-M). KIN-N moves toward the plus end of the microtubules and is responsible for anterograde transport in axons, whereas the cytoplasmic dyneins and KIN-C move to the minus end and are in charge of retrograde transport [41,42]. KIF1B and KIF5B belonging to KIN-N have been proposed to be involved in mitochondrial anterograde transport in axons [11]. KIF1B belonging to the kinesin 3 family is a monomeric motor participating in mitochondrial transport [10]. KBP (KIF1 binding protein) can regulate mitochondrial movement by increasing the motility of KIF1Bα by a mechanism that remains unknown as yet, which is clear that it does not serve as an adaptor [43]. Mitochondria aggregate around the nucleus when kif5B (a member of kinesin-1) is knocked out, which implicates that kif5B takes part in anterograde transport [9]. Various adaptors have been indentified in coupling kinesins to mitochondria, including the kinesin light chain (KLC), miro and milton complex, syntabulin, APLIP1, Miro and Grif-1 complex [44-48]. Different KLC isoforms target kinesins to different cargoes, and one type of KLC functions as an adaptor between mitochondria and kinesin-1 [44]. However, it was recently suggested that KLC is not necessary for kinesin-1 to transport mitochondria when miro-milton complexes are present in cultured cells [47]. It is therefore possible that KLC and miro-milton function together to transport mitochondria, each can substitute another when it is defective in a functional compensation mechanism. Miro anchors the mitochondria outer membrane by its tail domain, recruiting kinesin to form a functional complex in an indirect or direct manner. Glater *et al.* (2006) found that miro and milton form a complex linking mitochondria to kineisn-1 in *Drosophila*. In addition, milton also has a role in regulating the transport through diverse splicing patterns, by providing various modifications or by changing the state of miro [47]. In mammalian neurons, miro associates with Grif-1 to mediate mitochondrial motility in a signaling dependent way [48]. There are other findings in support of this. Miro contains GTP and Ca^{2+} binding domains and it functions as a linker. Miro also serves as a signal integrator and Ca^{2+} sensor, which has a role in regulating mitochondrial shape and transportation through interaction with cell signal transduction [49-51]. On the other hand, miro can bind kinesin independently [50]. Moreover, miro can prolong the process of kinesin and dynein on microtubules, by which it facilitates the mitochondria anterograde and retrograde transport [51]. Syntabulin, a membrane associated protein, acts as a kinesin-1 adaptor involved in mitochondria transport in mammalian neurons [45]. Whether syntabulin targets to mitochondria membranes directly or through other proteins requires further research. APLIP1, a kind of c-Jun N-terminal kinase (JNK) interacting protein (JIP1), has been demonstrated to affect both kinesin and dynein based mitochondria transport, maybe acting as both kinesin and

dynein linkers [46]. JNK signaling pathway possibly influences mitochondria transport by dissociating kinesin and APLIP1 or other related proteins [52].

Whether these different kinds of kinesin-adaptors work collectively to transport mitochondria, or in an individual manner by responding to different signaling pathways remain to be studied. The same holds for the molecular mechanisms that regulate motors to perform their action in the right sequence and the right place.

The main molecular motor responsible for minus-end transport along the microtubule is cytoplasmic dynein. It is involved in retrograde mitochondrial movement in axons. Dynein and dynactin form a complex to facilitate mitochondria transport [8]. In addition to control the distribution of mitochondria, dynein/dynactin complex also influence the Drp 1 recruitment to mitochondria. It takes part in the maintenance of normal mitochondrial morphology [53]. When Arp1 (a subunit of dynactin) is silent in *Drosophila* axons, the level of the kinesin heavy chain associating with the mitochondria is reduced which impair the anterograde and retrograde movement severely [54]. Conspicuously, similar to miro, dynactin has a role in coordinating bi-directional transport. However, the mechanism of dynactin function is not well understood. More interestingly, the retrograde movement of mitochondria is limited with kinesin-1 inhibition. Further experiments demonstrate that kinesin-1, dynein and dynactin form a complex to associate with the mitochondria [55]. There is also evidence that KIF5 interacts with dynein directly and specifically with KLCs. It also interacts with the dynein intermediate chain [56]. Based on these observations, it is speculated that KIF5 and dynein may serve as each other's cargo. Another possibility is that kinesin and dynein may form a complex to coordinate the bi-directional transport. It is still unknown whether they function independently to facilitate the transport in two opposite direction, or there exist other mechanisms to regulate the transport along the microtubules.

The activity of many proteins can be regulated by phosphorylation and dephosphorylation through protein kinases. Some signal pathways have been involved in mitochondria movement. Kinesins can bind to cargoes through kinesin light chains (KLC). KLC are hyperphosphorylated in the TNF signal pathway, then the phosphorylated KLC represses the activation of kinesin and leads to clusters of mitochondria around the nucleus [57]. NGF and PI3-kinase pathways also influence the mitochondrial distribution. NGF serves as a stop signal in neurons [58]. When the axons are stimulated by NGF, only the mitochondria begin to aggregate at the stimulated place [58]. Further experiments demonstrated that this process is regulated through PI 3-kinase by affecting the membrane receptors, a NGF downstream pathway [59]. The JNK pathway has been demonstrated to influence mitochondrial transport by segregating the linkers and the cargoes [46,52].

However, whether different kinesin motors are walking in the same direction and binding to the cargoes simultaneously? What is the molecular mechanism to regulate the motors activities preciously? All these remain to be studied further.

While cargoes make long range transport on microtubules, actin filaments and myosins are responsible for short range transport within cells. Myosins are actin-based molecular motors, which are fundamental in cellular transport. Their motor domain is responsible for actin binding, and the tail domain for cargo binding. In budding yeast, myo2, a member of the myosin V family, plays a major role in mitochondrial motility and morphology, the transporting and docking of mitochondria on the actin filaments without myo2 are abolished [13]. Ypt11p, a small rab GTPase, may serve as an adaptor for myo2 to bind to mitochondrial membranes [60]. Recently, a novel class myosin, myo19 has been proposed to associate with mitochondria in human [14]. It is the first myosin discovered to be involved in the transportation of mitochondria in vertebrate cells. Further characterization of more myosin candidates and linking proteins may help us to understand mitochondria transport on the microfilaments. Considering all these results, it is reasonable to assume that molecular motors work together to dispatch the mitochondria in a way that their function can optimally be performed. We propose a model for mitochondria transport in somatic cells based on the available evidence (**Figure 10**). Several aspects need to get refined. These include the interaction and regulatory mechanism among multiple adaptors, molecular motors and the mitochondria. How various motors coordinate to facilitate the transport, and how the motors are transported to the location where they become functional remain unknown.

3. TRANSPORT AND ANCHORING OF MITOCHONDRIA DURING SPERMIOGENESIS

Similar to somatic cells, mitochondria transport during spermiogenesis has not been investigated in detail. How the mitochondria are delivered to the developing sperm tail is not clear as yet. Intramanchette transport (IMT) and intraflagellar transport (IFT) are involved in the sperm head and tail development, both of which are related to molecular motors associating with cargoes that are walking along the cystoskeletal elements [61]. The manchette, a transient structure, appears when the nucleus begins to elongate and disappears when the spermatid is well formed. IMT consists of both microtubule based and F-actin based transport. Kinesin II, dynein, myosin

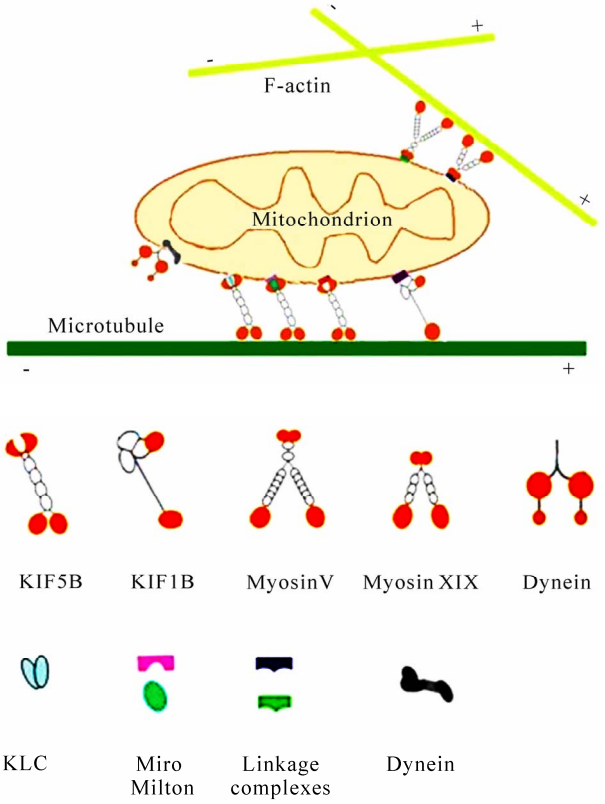

Figure 10. Diagrammatic representation of mitochondrial transport along the cystoskeletal elements in mammalian somatic cells. Motor proteins target mitochondria through special linkage complex. For instance, KIF5B associated mitochondria by KLC, Miro and Grif-1; Dynein and Dynactin form a complex to carry mitochondria. Myosin V and Myosin XIX transport mitochondria walk along the F-actin.

Va have been indentified to localize on the manchette, kinesin II is also involved in intraflagellar transport [62-66]. Further evidence supports that motors cooperate to facilitate intraflagellar transport in *Caenorhabditis elegans* [67,68]. IMT and IFT deliver materials for the tail development during spermiogenesis, thus, it is plausible to speculate that mitochondria migrate to the midpiece by IMT and IFT (**Figure 11**).

Some proteins take part in mediating mitochondria morphology and their localization has been uncovered. Nectin-2, Spergen-1 and KLC are three identified proteins that associate mitochondria to ODFs [69,70]. Nectin-2, a kind of cell adhesion molecule, localizes in the middle piece in mature spermatozoa. In nectin-2 depleted mice, the mitochondria exhibit abnormal distribution: some spermatozoa have disorganized mitochondrial sheaths, and some mitochondria dislocalize on the head [69]. Spergen-1 is only expressed in late spermiogenesis from the elongating spermatid to the mature spermatozoon. It is localized at the mitochondrial surface in the

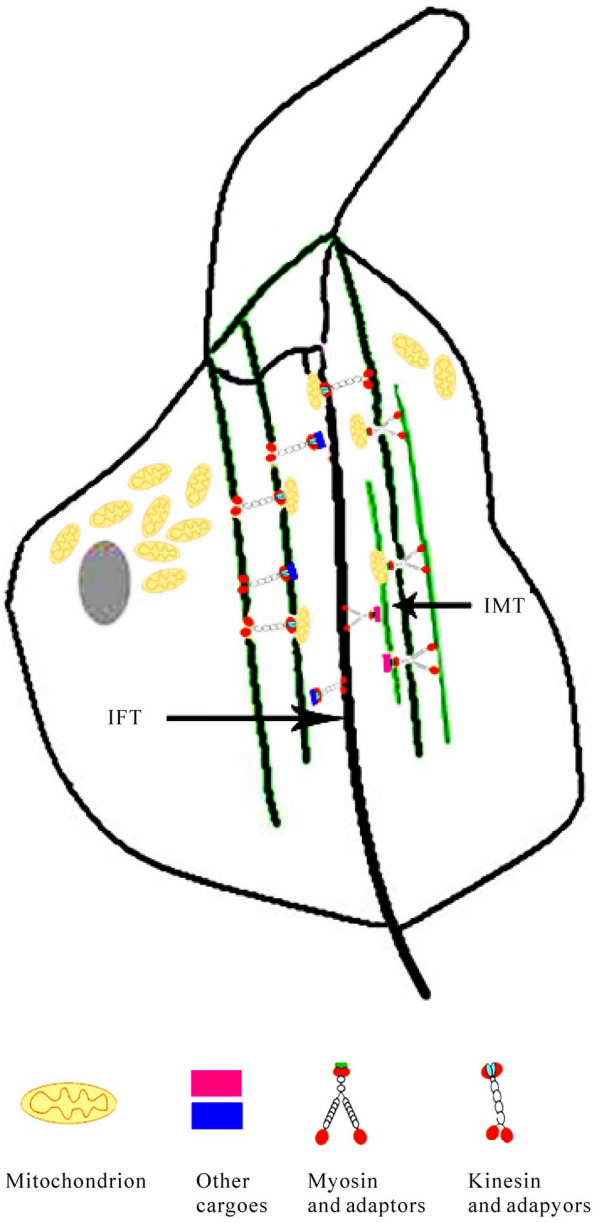

Figure 11. Diagrammatic representation about mitochondria transport at the early maturation phase in rat spermiogenesis. Kinesins and myosins work together to transport mitochondria and other cargoes to the tail through IMT and IFT.

mitochondria in cultured cells, it also contributes to couple mitochondria to ODFs during spermiogenesis [70-73]. Another spermatid specific protein is Kinesin Light Chain 3 (KLC3) and it has been found to connect to mitochondria through its TPR domain in the process of midpiece formation, more interestingly, KLC3 binds to ODFs using the HR domain in mature spermatozoa [72,73]. It is therefore possible that it links mitochondria to ODFs in a microtubule-independent way. Whether these linker proteins interact with each other or function

alone at particular time remains to be determined. In GOPC or PICK1 knock out mice, the mitochondrial sheath arranges abnormally, which is the main feature of globozoospermia [74-76]. PICK1 locates at the mitochondrial membrane and recruits PKCα implicated in phosphorylating some proteins [77]. Moreover, the PICK1/PKCα complex has been demonstrated to be involved in anti-apopotosis by affecting the Bcl2 factor [78]. In Pick1$^{-/-}$ mice, the mitochondrial sheath is abnormal, and some mitochondria migrate to the deformed nucleus, resulting in low sperm motility [76]. However, the detailed mechanism of PICK1 involved in mitochondria sheath formation is unclear. Some observations revealed that the PKCα signaling pathway may modulate the activity of motors responsible for mitochondrial movement and participate in mitochondria mediated apoptosis of the abnormal sperm. Sertoli cells produce some paracrine mitochondrial maturation factor (PMMF) such as activin and follistatin, which regulate the shape and distribution of mitochondria during spermiogenesis in a paracrine way [79]. Accordingly, the mitochondrial membrane in the midpiece contains estrogen receptor β and androgen receptors, indicating that hormones may influence the mitochondrial motility directly [80].

Further identification and characterization of related molecular motors and proteins can help us to understand more about the mechanism of spermiogenesis. Sperm cell culture and spermatogenic stem cell induction are the limiting factors, but these seem to be effective in investigating these issues.

4. SPERM MOTILITY AND ENERGETICS

Mammalian sperm need to sustain motility to reach and fertilize the egg. Mitochondrial aerobic respiration and glycolysis are two metabolic pathways to generate ATP for sperm movement and protein phosphorylation in sperm. Some evidence supports the notion that mitochondria are the only energy source for sperm motility. In mammal sperm, the overall mitochondrial volume shows a positive correlation with the flagellum length, hence, sperm motility is highly dependent on the number and size of mitochondria [4,81]. The sperm even keep their motility when the media contain no glucose or the metabolic pathway of glycolysis is inhibited [7]. The ATP being produced by mitochondria diffuse to the distal end to meet the high energy demand through shuttle mechanisms such as mediated by creatine kinase, adenylate kinase and phosphoglycerate kinase shuttles [5, 82]. However, different species may use different mechanism to deliver ATP produced by oxidative phosphorylation to the distal sperm tail.

Conversely, Malo *et al.* (2006) found that the longer the midpiece is, the slower the sperm moves; sperm motility also positively correlates with the head length and the length of the remaining tail [83]. Other mechanisms are possible to provide energy for sperm motility. Many observations reveal multiple glycolytic enzymes localized in the sperm fibrous sheath. Glycolysis contributes largely to sperm motility. In addition, glycolysis is sufficient to maintain sperm motility when oxidative phosphorylation is inhibited [5,6]. A newly developed technology combining laser and microscopic systems provides a useful tool to study the relation between sperm motility and energy metabolism [84]. With this tool, Nascimento *et al.* (2008) discovered that glycolysis provides more ATP molecules than oxidative phosphorylation to maintain sperm motility in mammals [85]. The Ca^{2+} pathway and cAMP/PKA pathway are two important ways of signal transduction involved in sperm motility control [86-88]. Some other proteins such as protease activated receptor 2, the epidermal growth factor receptor, and pathways including the PI3-K cascade mediate sperm motility [89,90].

Both mitochondrial respiration and glycolysis provide energy during spermiogenesis. Whether these two pathways are stage specific, or might compensation each other remains unclear. It also remains open for future studies to what extent the two pathways are required in different species.

5. UBIQUITINATION OF SPERM MITOCHONDRIA

Ubiquitination, a significant post-translated modification, has multiple biological effects, involved in protein degradation, endocytosis and signaling, activation of transcriptional factors, cell cycle, cell differentiation, immune responses and others [91-94]. In this process, ubiquitin or ubiquitin-like proteins target the substrate through different modifications, such as by different numbers of ubiquitin conjugated, and by various types of linkers among ubiquitins. For instance, monoubiquitin usually results in the target degradation by proteosome or lysosomes.

Mitochondria are ubiquitinated during spermiogenesis. The defective mitochondria will be degraded after ubiquination. In normal mitochondria in the midpiece, cross-linking disulfide bonds cover the ubiquitin tag so as not to be proteolyzed until fertilization, which appears to be a mechanism to maintain the mitochondrial maternal inheritance [15]. Prohibitin is a constitutive protein localized on the mitochondrial membrane in the midpiece, which serves as an ubiquitinated substrate and determines the mitochondrial destination [95]. Defective sperm exhibit high level ubiquitination of mitochondria, they are discarded during spermiogenesis or in the epididymis. Ubiquitination of sperm mitochondria is useful in indentifying sperm quality and act as a criterion for infertility diagnosis. Deep and extensive studies on sperm mitochondria ubiquitination process will provide us with a better understanding of the molecular mecha-

nisms involved, as well as develop more feasible strategies and drugs in clinical trials.

6. MITOCHONDRIA IN MALE INFERTILITY

Defects in mitochondrial transport processes, ion channels and metabolic pathways cause various diseases, such as Parkinson's and Alzheimer's disease. Asthenozoospermia defined by low sperm motility and oligospermia characterized by reduced sperm number are two main causes for male infertility. The mitochondrial sheath dysfunction is the main cause for asthenozoospermia [96].

Mitochondria produce ATP for sperm survival and fertilization. Defective mitochondria cause low sperm motility [97]. Mitochondrial DNA mutations also affect sperm motility [98]. Both point mutation and fragment deletions may lead to abnormal mitochondrial structure and function, which in turn may cause male infertility [97-101]. Quantitative conventional PCR and mutant mice models are useful in the identification of genes related to male infertility [75,102,103]. Mitochondria are a major source of reactive oxygen species (ROS) generation, leading to apoptosis, and excessive ROS is a cause of male infertility. Antioxidant compounds are beneficial to improve fertility, and may serve as preventive drugs [104]. From this point of view, we may employ genetic therapy that combined with other therapeuticas to cure diseases at their place of origin in the future.

Some chemical compounds are toxic to mitochondria and interfere with male fertility. An environmental contaminant, TCCD, induces oxidative stress, with the consequence of reduced male fertility [105]. Parabens, a kind of food preservatives, may affect mitochondrial respiration and apoptosis that is mediated by mitochondria [106]. Toxicity to mitochondria in testes may serve as a criterion for the estimation of chemical side effects in the future.

7. CONCLUSIONS AND PERSPECTIVE

Mitochondria have effects on cell metabolism, cell signaling and apoptosis. Considering this, the intracellular localization of mitochondria is a critical factor. Under related cell signal transduction and regulatory mechanisms, some molecular motors transport the mitochondria from the cell periphery to the midpiece, and then form the mitochondrial sheath consisting of four mitochondria helices in mammals. Comparing numbers, mitochondria are much less in molluscs and teleosts than in mammals, which imply an adaption to the internal fertilization. Energy for sperm motility is provided by different mechanisms in the different parts of the spermatozoon. Whereas oxidative phosphorylation takes place within mitochondria that are located in the midpiece, glycolysis takes place along the principal piece. Ubiquitination assumes the responsibility for sperm quality control and maternal inheritance. To a great extend is male infertility caused by defective sperm and low sperm motility.

Since mitochondria more and more turned out to be relevant for human health, research on mitochondria deserves increased attention. Dynamics of mitochondrial structure, motility and function during spermiogenesis have become an exciting field of research. What kinds of molecular motors are involved, how these are coordinated to transport mitochondria, how oxidative phosphorylation and glycolysis are regulated, and the underlying ubiquitination mechanisms requires to be clarified. The same holds for mitochondria related pathogenic mechanisms in human diseases. Investigating these issues provide an intriguing approach to future studies, particularly at the molecular level.

8. ACKNOWLEDGEMENTS

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