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Review:

Actin-based dynamics during spermatogenesis and its significance*

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Abstract: Actin can be found in all kinds of eukaryotic cells, maintaining their shapes and motilities, while its dynamics in sperm cells is understood less than their nonmuscle somatic cell counterparts. Spermatogenesis is a complicated process, resulting in the production of mature sperm from primordial germ cell. Significant structural and biochemical changes take place in the seminiferous epithelium of the adult testis during spermatogenesis. It was proved that all mammalian sperm contain actin, and that F-actin may play an important role during spermatogenesis, especially in nuclear shaping. Recently a new model for sperm head elongation based on the acrosome-acroplaxome-manchette complex has been proposed. In *Drosophila*, F-actin assembly is supposed to be very crucial during individualization. In this mini-review, we provide an overview of the structure, function, and regulation characteristics of actin cytoskeleton, and a summary of the current status of research of actin-based structure and movement is also provided, with emphasis on the role of actins in sperm head shaping during spermiogenesis and the cell junction dynamics in the testis. Research of the Sertoli ectoplasmic specialization is in the spotlight, which is a testis-specific actin-based junction very important for the movement of germ cells across the epithelium. Study of the molecular architecture and the regulating mechanism of the Sertoli ectoplasmic specialization has become an intriguing field. All this may lead to a new strategy for male infertility and, at the same time, a novel idea may result in devising much safer contraception with high efficiency. It is hoped that the advances listed in this review would give developmental and morphological researchers a favorable investigating outline and could help to enlarge the view of new strategies and models for actin dynamics during spermatogenesis.

Key words: Testis, Actin, Spermatogenesis, Nuclear shaping, Sertoli cell
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INTRODUCTION

Spermatogenesis is the production of sperm from primordial germ cell, which goes through a highly orchestrated series of stages of generating spermatogonium, primary spermatocyte, secondary spermatocyte, spermatid, and finally mature sperm. This complicated developmental process continues throughout nearly the whole lifetime of animals (Gilbert, 2000). During spermatogenesis, significant structural and biochemical changes take place in the seminiferous epithelium of the adult testis (Sanders and Debuse, 2003), and the gradual differentiation process and function of sperm for fertilization is thus heavily dependent on the cytoskeletal organization. In light of recent data and references, it has been shown that actin cytoskeleton dynamics play an indispensable role in facilitating these orderly events.

We have known about actin filaments for more than 30 years (Ayscough and Winder, 2004), and that actin cytoskeleton exerts substantial influence on somatic cells, maintaining and regulating the cells' motilities, shaping, intracellular organelle movements, and responses to their environment, etc. (Cappuccinelli, 1987; Alberts *et al*., 1994; Carraway and Carraway, 2000). Nevertheless, the role of actin in male germ cells is understood less than their nonmuscle somatic cell counterparts (Virtanen *et al*., 1984; Howes *et al*., 2001; Breitbart *et al*., 2005). In China, such researches even seem far from content. Fortunately, the inclination has been changing step by step. For instance, some recent advances have shifted

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attention from focusing merely on hormonal events to understanding the dynamics of Sertoli-germ cell adherens junctions and the intercellular junctions of the Sertoli cells under treatment with sex hormones (Cheng *et al*., 2005; Choudhuri *et al*., 2005; Mruk *et al*., 2006; Anahara *et al*., 2006a; 2006b). Such testis-specific junction adherens are all actin-based junctional structures which are important not only in mechanical adhesion of the cells, but in the morphogenesis and differentiation of the germ cells (Cheng and Mruk, 2002; Xia *et al*., 2005; Wong and Cheng, 2005a; 2005b; Yan *et al*., 2007). Moreover, it was found that sperm has a highly specialized cytoskeletal organization, and that the head, neck and tail of mammalian sperm all contain actin. In human sperm, actin has been identified in various regions, specifically the acrosome, subacrosomal area of the head, neck region and principal part of the tail, with this cytoskeletal protein being present mainly in non-polymerized form in many mammalian sperms (Talbot and Kleve, 1978; Virtanen *et al*., 1984; Lora-Lamia *et al*., 1986; Liu *et al*., 1999). It was recently shown that the actin present in sperm cells plays an influential role both in the acquisition of the morphology of the mature sperm during spermatogenesis and in subsequent motility during fertilization. In addition, it was proved that actin is involved in the acrosome reaction (Spungin *et al*., 1995; Howes *et al*., 2001; Liu *et al*., 2002). Yet the significance of these findings remains to be explored. In order to gain an overall view of the advances, some important notions and recent significant studies on actin-based structure and movement like actin-associated protein complexes and adhesion dynamics during spermatogenesis are described in the present review, and we hope that literature listed in this article will contribute to better understanding of this relatively new and developing field.

STRUCTURAL AND FUNCTIONAL OVERVIEW OF ACTIN

In this section, actin cytoskeleton, structural dynamics, and advances in actin research on somatic cells are briefly discussed since these contribute to their cellular function and regulation during spermatogenesis.

Actin cytoskeleton

Cytoskeleton is a highly organized dynamic structure that enables eukaryotic cells to have many specialized and regulated activities in response to extrinsic stimuli or intrinsic demand. Actin cytoskeleton (Alberts *et al*., 1994) is the most abundant one of the three types of cytoskeleton, and actin filaments work in networks or bundles and are in particular responsible for generating and maintaining cell polarity, controlling the shape and surface movements of most animal cells, acquisition of many characteristic morphologies, intracellular transport, ensuring cell mechanical integrity, as well as playing a key role in phagocytosis, exocytosis and cytokinesis.

Dynamic behavior of actin filaments

Actins are broadly categorized as muscle (α) or nonmuscle (β and γ) actins, and β-actin is constitutively expressed as part of the cytoskeleton. Actin filaments (or F-actin) consist of a tight helix of actin molecules (or G-actin). In cells, approximately half of the actin is kept in monomeric form, and the polymerization of actin is a dynamic process. Generally speaking, F-actin networks are continuously reorganized in cells that rapidly change their shape and in fast migrating cells that swiftly change the direction of movement (Diez *et al*., 2005). Continual polymerization and depolymerization of actin molecules in cell-surface protrusions such as lamellipodia and microspikes have been well investigated and defined, and the conversion of these two states of actin existence, which is the foremost point of actin functional performance, is very essential for cell survival.

The local polymerization of actin and the actin-based movement can be better demonstrated by infection of some pathogenic microorganism. For instance, in a recent study highlighted by the journal *Science*, Lehmann *et al*.(2005) observed that the association of virus cell with filopodia and microvilli of the target cell is mediated by the underlying actin cytoskeleton, and F-actin in conjunction with cellular myosin II can promote viral approach to the cell body for successful infection in a surfing type of movement, and it has also been known for years that moving bacterium spreads from cell to cell propelled by comet-like actin tails (Alberts *et al*., 1994; Stevens *et al*., 2006).

Somatic cells: the illumination?

Breakthroughs in the technology of live-cell imaging (Kaksonen *et al*., 2006), electron microscopy, biochemical, pharmacological methods and genetics tools for studying the whole spectrum of cytoskeletal dynamics such as knock-outs for biological function (Costa *et al*., 2004), immunolocalization for cellular localization (Gilk *et al*., 2006) and yeast two-hybrid for specificity of interaction (Campa *et al*., 2006), have allowed the development of new models for understanding the complex mechanisms involved in filament arrays, cell signaling events regulating cell locomotion and intracellular transport (Carraway and Carraway, 2000), and intensive studies have opened up a door for examining the underlying dynamics and structural organization of actin and it has become a leading role of many academic interest, e.g., due to the fact that actin has been especially highly conserved throughout the evolution of eukaryotes, mostly encoded by a multigene family, its sequences have considerable potential for use in phylogenetic studies (Adema, 2002; Fahrni *et al*., 2003; Robalo *et al*., 2007). Actin-related proteins, for instance, actin-binding protein tropomyosin (Stehn *et al*., 2006; Yu and Ono, 2006; Hitchcock-DeGregori *et al*., 2007) and actin-nucleating ARP2/3 complex (Goley *et al*., 2006; Goley and Welch, 2006) have been in the spotlight (Billadeau and Burkhardt, 2006; Malacombe *et al*., 2006; Marston and Goldstein, 2006; Percipalle and Visa, 2006; Weis and Nelson, 2006).

Much less is known about the mechanism of actin dynamics in sperm cells, despite its undoubtedly important role we could benefit from. Nevertheless the findings and advances mentioned above surely shed light not only on the functional significance of actin polymerization and depolymerization in nonmuscle somatic cells, but also suggest that actin could play a similar crucial part in spermatogenesis, though there must be many differences between these two.

In the following sections, the role of actin dynamics during spermatogenesis and acquisition of the morphology of the mature sperm are summarized, with emphasis on its participation in the differentiation process (spermiogenesis) and germ cell movement across the seminiferous epithelium in the testis. We attempted to highlight specific research areas that deserve attention in future studies.

ROLE OF ACTIN DYNAMICS IN SHAPING THE SPERM FORM

Acrosome biogenesis during spermiogenesis

Spermiogenesis is the differentiation of the sperm cell after mitosis and meiosis. It is the last, but not least stage of spermatogenesis and involves significant structural and biochemical changes, including the condensation of the nucleus, the formation of the acrosome and flagellum, the discarding of extra cytoplasm (the cytoplasmic droplet), the redistribution of plasma membrane domains, and at length results in the production of a highly specialized and polarized cell, the mature sperm. Each sperm consists of a compressed haploid nucleus, in front of which lies the acrosome, a sizable and the most visible organelle (Yang and Sperry, 2003) that first appeared early in spermiogenesis. It is a specialized secretory sac deriving from the Golgi apparatus and containing enzymes that digest proteins and complex sugars, and form a cap that covers the sperm nucleus at the conclusion of spermiogenesis and represents one of the defining features of sperm development in the testes. In many species, such as sea urchins (Gilbert, 2000; Hirohashi and Vacquier, 2003), a region of G-actin molecules lies between the nucleus and the acrosome. Intactness and good morphology is of the essence for acrosome to bind to the zona pellucida of the egg and fuse with the egg plasma membrane during fertilization. Despite much advancement of knowledge of spermatogenesis and acrosome formation, yet the fact is we know very little about the underlying molecular basis that regulates the processes (Abou-Haila and Tulsiani, 2000; Yao *et al*., 2002).

Role of actin dynamics during spermatogenesis

Acrosome formation and spermatid nuclear shaping are two central parts of spermiogenesis and the study by Kierszenbaum *et al*.(2003a) has suggested possible contribution of the acrosome to nuclear shaping, for mutations that weaken the formation of this structural framework lead to abnormal sperm head which is one of the causes of male infertility, and spermatid nuclear shaping has already been linked to the perinuclear acrosome and manchette (Fouquet *et al*., 2000; Kierszenbaum *et al*., 2004).

Specific details of the acrosome formation mechanism are not fully understood, but parts of the

mystery are beginning to emerge. Since many aspects of morphogenesis are dependent on the cytoskeleton, with sperm morphology not being an exception. After several decades of study, microscopy, biochemical methods and genetics have given indication of the involvement of cytoskeletal domains such as actin in acrosome formation (Abou-Haila and Tulsiani, 2000).

In one of the studies concerning gametogenesis by Maier *et al*.(2003), arc, an effector molecule that associates with the actin cytoskeleton, is believed to support a role for actin cytoskeleton in the acrosome formation, the sperm acrosome reaction and maintaining the sperm cell motility, as arc was found co-localizing with the developing acrosome in the spermatids and present in the acrosomal region of mature sperm and lost to varying degrees during sperm capacitation and in acrosome-reacted sperm.

The hypothesis that actin filaments in the subacrosomal space may serve as a linking network between the acrosome and nucleus had been put forward by Vogl (1989) for nearly two decades but it remained proofless until not long ago that an actin cytoskeletal plate has been identified. Kierszenbaum *et al*.(2003b) suggested that, in the subacrosomal space of mammalian spermatids, there is an assembly of an F-actin-keratin-containing cytoskeletal plate designated as acroplaxome, which is bordered by a marginal ring. It anchors the developing acrosome to the nuclear envelope during shaping of the spermatid head to secure the acrosome at the corresponding nuclear pole. Probably by virtue of this special cytoskeletal plate, especially the actin polymerization and this dynamic reorganization of the F-actin cytoskeleton in it, the configuration change of developing acrosome could adapt to the nuclear shaping. What is more, there exist exogenous contractile forces exerted by a stack of F-actin-containing hoops, which is the main cytoskeletal element of the Sertoli cell ectoplasmic specializations (to which the spermatogenic germ cells are bound) and the forces help acroplaxome contribute to spermatid nuclear shaping. The studies listed above have provided a novel actin-involved model for the nuclear shaping of the male gamete (Kierszenbaum and Tres, 2004).

In addition to the acroplaxome, F-actin is also contained in the manchette (a structure consisting of a perinuclear ring and parallel cytoplasmic microtubules) presumably, serving as one cytoskeletal track to facilitate the transport (from the Golgi to the acrosome or from the manchette to the centrosome and axoneme) of protein and proacrosomal vesicle cargos during spermatogenesis (Kierszenbaum *et al*., 2003a). Recent research on Rab GTPases (Mruk *et al*., 2005) also suggested the role of actin filaments serving as tracks guiding cargo-containing vesicles in the delivery of newly synthesized proteins.

Actin-binding proteins have provided another perspective to investigate the role of actin in spermatogenesis. Most actin-binding proteins are found in the actin-rich site in living cell and they bind to actin filaments and modulate their properties and functions (Alberts *et al*., 1994), especially actin assemblies and disassemblies. The fact that a large retinue of actin-binding proteins exist in many mammalian testis suggests an important role of actin dynamics in sperm function. Newly-identified proteins of such kind include Profilin IV (Obermann *et al*., 2005), AEP1 (Luk *et al*., 2006) and Dishevelled-1 (Ma *et al*., 2006), and their importance and precise function mechanism in regulating spermatid morphological changes by reorganizing actin cytoskeleton remain to be addressed.

There is one more interesting finding about the important role of actin dynamics during spermatogenesis. In *Drosophila*, individualization is one of the late stages of spermatogenesis, which requires an unusual amount of membrane remodelling using a well-defined actin structure and many other components regulating actin dynamics. And the fruit fly *Drosophila* is an excellent model organism with which to explore the mechanism of actin-involved morphogenesis. Studies showed that an F-actin rich investment cone expels all the cytoplasm and organelles of individual spermatids as a waste bag after elongation. This process requires proper F-actin dynamics with F-actin assembly occurring throughout the cone (Noguchi and Miller, 2003; Ghosh-Roy *et al*., 2005). Similar phenomenon has also been reported by Sahara and Kawamura (2004) in silkworm, *Bombyx mori*, and the dynamics of actin filaments appears to be the main force behind peristaltic squeezing of the sperm bundles as well.

Based on the results of studies conducted in recent years as summarized herein, it is reasonable to assume that actin filaments either serve as tracks for moving vesicles which contain cargo, or generate mechanical forces, or act as linkage to help shaping of

the sperm head. The mechanism underlying these events should be the same, depending on the assembly and disassembly of actin filaments, and the regulating proteins. Remaining to be explored is the exact inducements or signaling to cause the conversion. Since the acrosome formation during spermatogenesis and the succeeding acrosome reaction are so important for sperm-oocyte interaction during fertilization, investigation on the role of actin in these events naturally is of great importance.

ROLE OF ACTIN DYNAMICS IN GERM CELL MOVEMENT IN THE SEMINIFEROUS EPITHELIUM DURING SPERMATOGENESIS

Germ cell movement and Sertoli cell junctions in the testis

In the spermatogenetic process, developing germ cells of different phases migrate from the basal through the intermediate to the adluminal compartment of the testis, via junctional contacts and the cytoskeletal apparatus to from round, elongating, and elongated spermatids. This requests extensive reconstruction of cell junctions in the testis, whose dynamics has always been an elusive task to developmental scientists, largely because of the formidable complexity of the spermatogenesis (Lui *et al*., 2003).

Somatic Sertoli cells reside among the germ cells within the seminiferous tubule, and extend from the base of the tubule to reach its lumen. Sertoli cells perform an impressive range of functions: maintenance of the integrity of the seminiferous epithelium, displacement of germ cells and release of sperm; secretion of proteins; delivery of nutrients to germ cells; and phagocytosis of degenerating germ cells and of germ cell materials (Jégou, 1992). In mammalian testis, the actin-based Sertoli ectoplasmic specialization (ES), which is known as basal ES between Sertoli cells as well as apical ES between Sertoli and developing sperm cells (Yan *et al*., 2007), is one specialized type of the adherens junctions (AJs) in the seminiferous epithelium.

For the past several years, intensive studies have begun to probe the mechanism of germ cell movement in the seminiferous epithelium by investigating the tight junction and AJ (Lui *et al*., 2003; Mruk and Cheng, 2004a; Siu and Cheng, 2004; Wong *et al*., 2005; Yan and Cheng, 2005), and study of the molecular architecture and the regulating mechanism of ES (Mruk and Cheng, 2004b; Yan *et al*., 2007) has become an exciting yet only recently being a studied field.

Current status of research of Sertoli ectoplasmic specialization (ES)

ES is the best-characterized cell-to-cell anchoring junction type using an actin filament attachment site in the testis (Cheng and Mruk, 2002; Lee and Cheng, 2004). As has been described previously, there are two types of ES, designated apical and basal ES, with extensive interactions between Sertoli and germ cells taking place in apical ES to facilitate the movement of germ cells across the epithelium.

The events of ES dynamics and their regulation in the testis are one of the most fascinating phenomena in spermatogenesis, since germ cells must translocate from the basal to the luminal edge of the adluminal compartment of the seminiferous epithelium while they also remain attached to the epithelium. During this process, adherens junctions must be continually de-assembled and then re-assembled (Mruk and Cheng, 2004a). How these events are regulated in the testis is a priority area that needs to be investigated in the near future. A brief review of current status of research on ES is discussed herein with regard its central attributes from two angles of view.

First, following Yan *et al*.(2007), ES acts as a "friend" of spermatogenesis under normal and good physiological conditions. Structurally abnormal or absent Sertoli ESs have been associated with inappropriate release of spermatids or various other abnormalities in spermatids, proven either by testosterone/estradiol (TE) treatment (O'Donnell *et al*., 2000), or by administering agents affecting F-actin and microtubule cytoskeleton enabling normal spermatid and Sertoli cell morphology and function (Wolski *et al*., 2006; Khatchadourian *et al*., 2007). It has also been reported that after treatment with exogenous hormonal chemicals (Anahara *et al*., 2006a; 2006b), abnormal spermiogenesis occurred, as the chemicals act on the actin-binding protein cortactin (Wolski *et al*., 2005), which is one of the ES adhesion proteins as well. Therefore, intact and normal ES function is crucial for cell attachment and spermatid movement and orientation in the epithelium.

Still, there is a point worth additional emphasis that, under the treatment of exogenous hormonal chemicals, there exist simultaneous occurrences of deletion of ES and abnormalities of sperm head shaping (Anahara *et al*., 2004; Toyama *et al*., 2001; 2004). This coincidence may well be attributed to the fact that the ES is responsible for shaping of the sperm head. Moreover, according to Kierszenbaum *et al*.(2003b), actin-based structure in ES does play an important role to help shaping the sperm head, as exogenous contractile forces exerted by a stack of F-actin-containing hoops, which is the main cytoskeletal element of the ES, help acroplaxome contribute to sperm head shaping.

On the other hand, under assault from outside, the testis-specific ES turns into a "foe" of spermatogenesis, making the testis more vulnerable than other organs, as it is most susceptible to toxicants or alternation of the microenvironment in the testis. For instance, Cheng *et al*.(2005) discovered that disruption of Sertoli-germ cell adhesion function by adjudin (AF-2364) in the rat testis can be limited to apical ES without affecting the other junctions, e.g. desmosome-like junctions at the blood-testis barrier site. However, this phenomenon could be a novel approach for male contraceptive development, without the potential side-effects of a drug based on altering the balance of sex hormones, with trials on laboratory animals showing that the contraceptive effect is reversible and that there are no apparent long-term side-effects.

SUMMARY AND PROSPECTS

We have summarized in this review recent advances in the field regarding actin-based structure and movement during spermatogenesis. Although specific role of actin during spermatogenesis may vary among species, the mechanisms underlying it should share many similarities. It is obvious that many problems remain to be solved. Still to be addressed, for example, are the exact molecules and/or mechanisms that regulate the actin-based signaling, and also the opening and closing of the Sertoli cell junctions in the testis.

Because of the fact that some of the major causes of male infertility are due to the abnormal spermiogenesis and sperm maturation, we suspect that herein lies the solution and perhaps our manipulating actin behavior during these processes could be a breakthrough of male infertility or a new way for devising safe and effective contraception. For instance, as mentioned before, the new study of adjudin targeting the testis to help create a male contraceptive without unwanted side effects, has already started on its way to the new drug development strategy. Needless to say, they are at a pretty early stage, and both drug delivery and bioavailability are problems to be solved, but we are optimistic and the research is likely to prove fruitful. We believe such kind of work focusing on the role of actin-based structure and dynamics in sperm cells may provide useful insight into better understanding of the biological events in male reproductive systems and will merit further study and application.

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