Seminars in Cell & Developmental Biology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Actin binding proteins, spermatid transport and spermiation☆

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ARTICLE INFO

Article history:
Available online xxx

Keywords:
Testis
Spermatogenesis
Ectoplasmic specialization
Actin binding proteins
Actin bundling proteins
Branched actin polymerization inducing proteins
Spermiogenesis
Spermiation

ABSTRACT

The transport of germ cells across the seminiferous epithelium is composed of a series of cellular events during the epithelial cycle essential to the completion of spermatogenesis. Without the timely transport of spermatids during spermiogenesis, spermatozoa that are transformed from step 19 spermatids in the rat testis fail to reach the luminal edge of the apical compartment and enter the tubule lumen at spermiation, thereby arriving the epididymis for further maturation. Step 19 spermatids and/or sperms that remain in the epithelium beyond stage VIII of the epithelial cycle will be removed by the Sertoli cell via phagocytosis to form phagosomes and be degraded by lysosomes, leading to subfertility and/or infertility. However, the biology of spermatid transport, in particular the final events that lead to spermiation remain elusive. Based on recent data in the field, we critically evaluate the biology of spermiation herein by focusing on the actin binding proteins (ABPs) that regulate the organization of actin microfilaments at the Sertoli–spermatid interface, which is crucial for spermatid transport during this event. The hypothesis we put forth herein also highlights some specific areas of research that can be pursued by investigators in the years to come.

Contents

☆ This work was supported in part by grants from the National Institutes of Health (NICHD, U54 HD029990 Project 5 to C.Y.C. and R01 HD056034 to C.Y.C.), National Natural Science Foundation of China (NSFC)/Research Grants Council of Hong Kong (RGC) Joint Research Scheme (3126110491 to D.H., N_HKU 717/12 to W.M.L.), The Hong Kong General Research Fund (HKU 771513 to W.M.L.), and Hong Kong University CRCG Seed Funding (to W.M.L.).
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http://dx.doi.org/10.1016/j.semcdb.2014.04.018
1084-9521/

1. Introduction

Spermatogenesis is a complex cellular process [1–4]. It is composed of several discrete cellular events that take place cyclically in the epithelium of the seminiferous tubule in mammalian testes, which include: (i) self-renewal of spermatogonia via mitosis, (ii) meiosis, (iii) spermiogenesis, and (iv) spermiation. In the seminiferous epithelium, spermatogonia that lie on the basement membrane in the basal compartment derived from spermatogonial stem cells located at the stem cell niche [5] undergo rapid expansion via mitosis after puberty, some of which differentiate into type A spermatogonia [6,7]. Some type A spermatogonia differentiate into type B, which are the germ cells that transform into preleptotene spermatocytes. These spermatocytes are connected in "clones" by intercellular bridges (also known as tunneling nanotubes, TNT) so that they can be transported across the blood–testis barrier (BTB) while differentiating into leptotene spermatocytes [8]. Thus spermatocytes undergo meiosis I and II to form haploid spermatids at the adluminal compartment behind the BTB (Fig. 1). Spermatids (step 1) are then transformed into elongated spermatids (step 19) via spermiogenesis which are then lined up at the luminal edge of the tubule lumen. Thus, spermatozoa differentiated from step 19 spermatids are released into the tubular lumen at spermiation that takes place at late stage VIII of the epithelial cycle [9–13] (Fig. 1).

As germ cells differentiate into more advanced stages during the epithelial cycle of spermatogenesis, they are also being transported by the Sertoli cell across the seminiferous epithelium from the basal to the adluminal (apical) compartment, so that spermatozoa transformed from step 19 spermatids at stage VIII of the epithelial cycle can be released to the tubule lumen at spermiation, entering the epididymis for further maturation (Fig. 1). However, germ cells are immotile cells as they lack the apparatus to elicit active locomotion, such as lamellipodia and filopodia found in motile cells like fibroblasts, macrophages and neutrophils. Thus, germ cells are analogous to "cargoes" that must rely on actin microfilaments in the Sertoli cell which serves as the "vehicle" being transported from the basal to the adluminal compartment along the microtubules that act as the "railroad track" [14–16] during the epithelial cycle of spermatogenesis. Studies have shown that the unique anchoring junction in the testis known as ectoplasmic specialization [ES, an actin microfilament-rich anchoring device using F-actin for attachment, also a testis-specific adherens junction (AJ)] indeed serves as the "vehicle" and the microtubule acts as the "railroad track" to transport the cargo (germ cell) along the epithelium during the epithelial cycle [7,8,17–21]. ES is found at the Sertoli cell–cell interface and co-exists with actin-based tight junction (TJ) and gap junction (GJ) known as the basal ES. These junctions together with the intermediate filament-based desmosome constitute the blood–testis barrier (BTB) [7,22,23], one of the tightest blood–tissue barriers in the mammalian body [24–26], which also anatomically divides the seminiferous epithelium into the basal and the apical (adluminal) compartments. On the other hand, ES is also found in the apical compartment at the Sertoli–spermatid interface called the apical ES. Apical ES first appears in step 8 spermatids, however, once it assembles, unlike basal ES which coexists with TJ and GJ, it replaces both desmosome and GJ at the Sertoli–spermatid (steps 1–7) interface [13,19,27], and it remains as the only anchoring device until at late stage VIII when it undergoes complete degeneration to facilitate spermiation [17,21,28,29]. However, both apical and basal ES undergo restructuring at late stages VII–VIII so that proteins at the "old" ES can be endocytosed, transcytosed and recycled to assemble "new" apical and basal ES, in which giant endocytic vesicles are formed at both sites known as apical and basal tubulobulbar complex (TBC) [28,30–32] (Fig. 1). In short, TBC is the ES that undergoes endocytic vesicle-mediated trafficking so that "old" ES proteins can be recycled for the assembly of "new" ES both in the basal (at the basal ES) and the apical (at the apical ES) compartment. Furthermore, TBC is also used to eliminate unwanted cytoplasmic debris in particular from the head region of late spermatids or Sertoli cells at the BTB [31,32]. In this context, it is of interest to note that many proteins at the desmosome and GJ are found at the Sertoli–spermatid (steps 8–19) interface (*i.e.*, apical ES), and also many TJ- and GJ-proteins at the Sertoli cell–cell interface, are being assimilated into the basal ES. Furthermore, proteins that are usually restricted to focal adhesion complex [FAC also known as focal contact, an actin-based anchoring junction at the cell–extracellular matrix (ECM) interface], such as focal adhesion kinase (FAK), c-Src (Rous sarcoma transforming virus or proto-oncogene tyrosine-protein kinase Src) and c-Yes (Yamaguchi sarcoma viral oncogene homolog1), are also found at the apical and/or basal ES, making the ES a hybrid anchoring junction, having the properties of AJ, GJ, TJ, desmosome and also FAC [33–35]. For instance, desmosome proteins desmoglein-2 and plakoglobin, GJ proteins connexin 43 and connexin 33, and TJ proteins JAM-C and CAR are found at the apical and/or basal ES [36–39].

When examined by electron microscopy, ES is constituted by a tripartite ultrastructure composed of: (i) hexagonally arranged actin filament bundles that lie perpendicular to the Sertoli cell plasma membrane, which sandwiched in-between (ii) cisternae of endoplasmic reticulum and (iii) either the apposing Sertoli cell plasma membranes (basal ES) or the apposing Sertoli–spermatid plasma membranes (apical ES) [20,30] (Fig. 1). Morphologically, apical and basal ES are indistinguishable even at the ultrastructural level under electron microscope except that the typical tripartite structure is found in both sides of adjacent Sertoli cells at the basal ES, but only in the Sertoli cell at the apical ES, not at the spermatid [20]. In fact, spermatids do not appear to contribute to the apical ES ultrastructurally even though they express many of the similar ES proteins found in Sertoli cells (*e.g.*, nectin-2, afadin, N-cadherin, β-catenin) as well as unique proteins (*e.g.*, nectin-3, laminin-α3, -β3, -γ3) not found in Sertoli cells [29,40]. Interestingly, constituent proteins at the apical *vs.* the basal ES are quite different [29,40]. Apical ES appears to mechanically grasp the head of spermatids which undergo rapid elongation and maturation via spermiogenesis, and to confer spermatid polarity so that the head of spermatids are pointing toward the basement membrane [20,41]. On the other hand, TBC consists of a cylindrical double-membrane core composed of the plasma membranes of two adjacent cells, cuffed by a network of actin microfilaments [8,30,42] (Fig. 1). Basal TBC develops between adjacent Sertoli cells, which undergoes regressive changes during the epithelial cycle. Most membranous complexes arise during the early stages of the epithelial cycle at II–V and develop into large bulbous endings. At midcycle, namely stages VI–VII, most basal TBC display regressive changes and are eventually resorbed by Sertoli cell lysosomes. Basal TBC resorption is related to the impending breakdown of the "old" BTB above spermatocytes as these cells are being transported upward, crossing the BTB [30] (Fig. 1). It is noted that basal TBC is not clustered at any specific and predictable location at the belt-like junctions, somewhat difficult to distinguish from elements of other junctional complexes [43].

Since both ES and TBC are actin-based ultrastructures, and the regulation of actin dynamics in unique testicular junctions are crucial to spermatogenesis, we focus on actin-binding and actin regulating proteins and their relationship with actin and other proteins at the ES in this review. We also focus our discussion herein on the spermatid transport across the seminiferous epithelium utilizing the apical ES since the biology of preleptotene spermatocyte transport at the basal ES/BTB has recently been reviewed [21,44,45].

Please cite this article in press as: Qian X, et al. Actin binding proteins, spermatid transport and spermiation. Semin Cell Dev Biol (2014), http://dx.doi.org/10.1016/j.semcdb.2014.04.018