Cell Tissue Res (2006) 323: 495–503
DOI 10.1007/s00441-005-0097-9

REGULAR ARTICLE

Wei-Pang Huang · Han-Chen Ho

Role of microtubule-dependent membrane trafficking in acrosomal biogenesis

Received: 2 September 2005 / Accepted: 5 October 2005 / Published online: 9 December 2005

Abstract The role of microtubule-based trafficking in acrosomal biogenesis was examined by studying the effects of colchicine on spermiogenesis. In electron micrographs of untreated cap-phase mouse spermatids, coated vesicles were always seen on the apex and caudal margins of the developing acrosomal cap. The increase in volume and the accumulation of materials in the acrosome during the Golgi and cap phases were observed to occur via fusion of vesicles at various sites on the growing acrosome. By studying the acid phosphatase localization pattern and colchicine-treated spermatids, the role of clathrin-coated vesicles became clear. Coated vesicle formation at the caudal margin of the acrosome appeared to be responsible for the spreading and shaping of the acrosome over the surface of the nucleus and also established distinct regional differences in the acrosome. In colchicine-treated spermatids, the Golgi apparatus lost its typical membranous stack conformation and disintegrated into many small vesicles. Acrosome formation was retarded, and there was discordance of the spread of the acrosomal cap with that of the modified nuclear envelope. Many symplasts were also found because of the breakdown of intercellular bridges. Colchicine treatment thus indicated that microtubule-dependent trafficking of transport vesicles between the Golgi apparatus and the acrosome plays a vital role in acrosomal biogenesis. In addition, both anterograde and retrograde vesicle trafficking are extensively involved and seem to be equally important in acrosome formation.

Keywords Coated vesicles · Microtubules · Spermatogenesis · Acrosome formation · Colchicine · Mouse (ICR)

This work was supported by grants 83-0211-B-002-184 and 93-2320-B-320-012 from the National Science Council, Taiwan, Republic of China.

W.-P. Huang
Department of Life Science, Institute of Zoology,
National Taiwan University,
Taipei, 10617, Taiwan

H.-C. Ho (✉)
Department of Anatomy, Institute of Integrative Physiology
and Clinical Sciences, Tzu Chi University,
Hualien, 97004, Taiwan
e-mail: hcho@mail.tcu.edu.tw
Fax: +886-3-8564641

Introduction

The development of mammalian sperm in the testis from spermatids to spermatozoa is called spermiogenesis and can be divided, according to significant cytological features, into four major phases: Golgi phase, cap phase, acrosome phase, and maturation phase (Leblond and Clermont 1952; Clermont and Leblond 1955). From the Golgi phase to the cap phase, the most important event is the formation of the acrosome. The acrosome has a distinct shape and size depending on the species. Although many studies have focused on the characteristics of the acrosome in various species, including development (Griffiths et al. 1981; Sinowatz and Wrobel 1981; Peterson et al. 1992), structure and membrane composition (Friend and Fawcett 1974; Stackpole and Devorkin 1974; Pelletier and Friend 1983), distribution of enzymes (Holt 1979; Tang et al. 1982; Bozzola et al. 1991), effects of drug treatment (Handel 1979; Russell et al. 1981; Russell et al. 1983), and abnormalities in sterile mutants (Sotomayor and Handel 1986; Fouquet et al. 1992; Russell et al. 1994), little is known about the mechanisms that regulate the attachment, shaping, and spreading of the acrosome over the nucleus.

During acrosome biogenesis, the Golgi apparatus is actively engaged in sorting and delivering proteins and membranes to the developing acrosomal cap (Tanii et al. 1998; Ventela et al. 2000; Ramalho-Santos et al. 2001). Microtubules are well known to play a key role in organelle and membrane-bound vesicle transportation in somatic cells (Schnapp et al. 1985; Sheetz et al. 1987; Jordens et al. 2001; Dell 2003) and have been suggested to have an important function during early stages of acrosomal differentiation

(Ventela et al. 2000). In mammalian spermatids, microtubule configurations change according to developmental stage (Moreno and Schatten 2000). Since nuclear shaping is dependent on manchette microtubules (Russell et al. 1991; Yamaguchi et al. 2004), the shaping and spreading of the acrosome over the nucleus might also depend on microtubules. Therefore, we have used colchicine, which binds to free tubulins and causes microtubule depolymerization, to evaluate the effects of microtubule disruption on acrosome formation in mouse spermatids.

Normal and colchicine-treated mouse seminiferous tubules were investigated by thin sectioning, cytochemistry, and osmium-dimethyl sulfoxide (DMSO)-osmium (O-D-O) cryofracturing methods. The maintenance of acrosomal shape, the mechanism of the acrosome spreading over the nucleus, and the relationship between microtubules and acrosome formation were examined.

Materials and methods

Animals and tissue collection

The experimental protocol complied with NIH (USA) recommended procedures for animal use and care. Mature male ICR mice were sacrificed by cervical dislocation, and their testes were prepared as follows for ultrastructural observations. Testes were cut open, and the seminiferous tu-

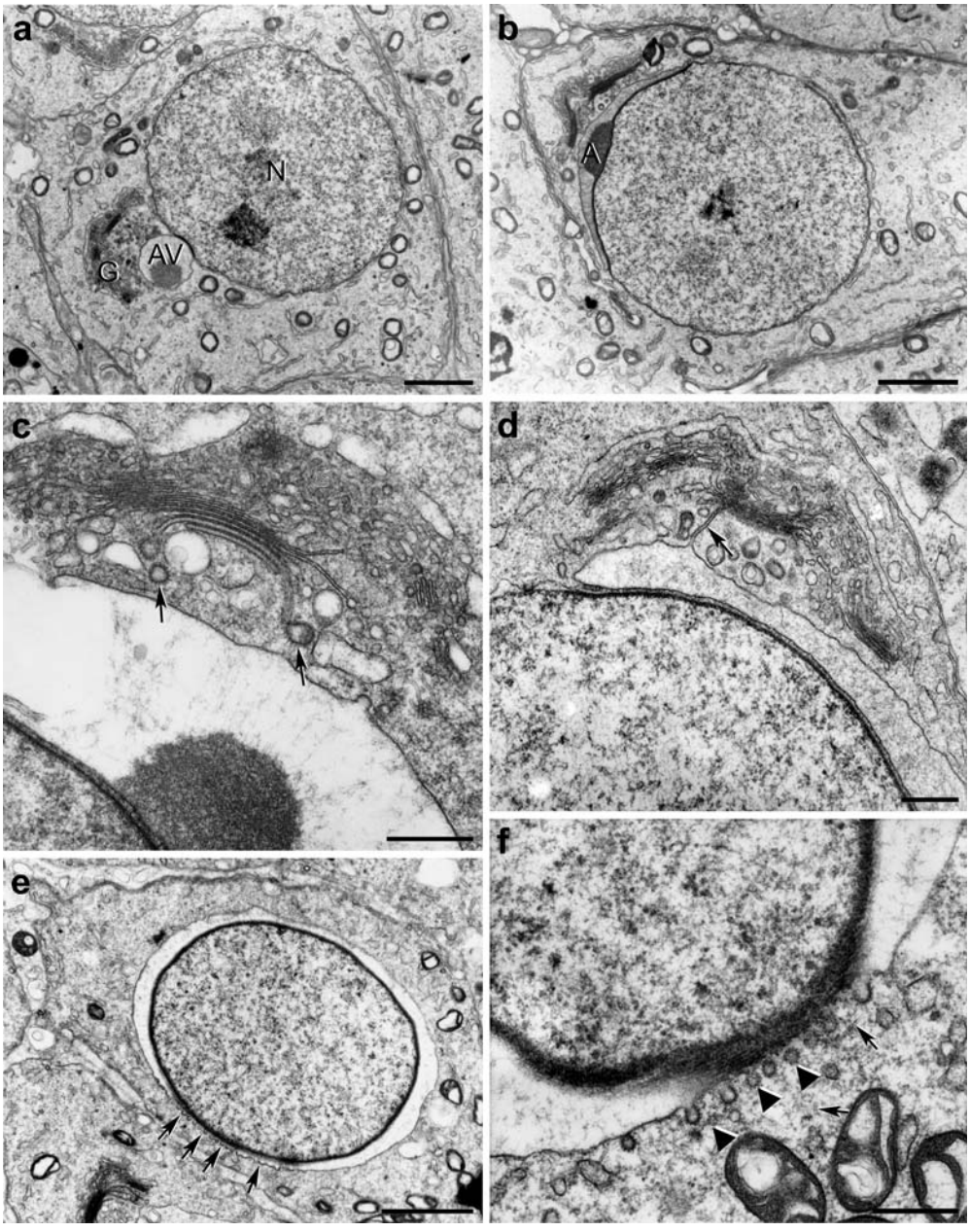

Fig. 1 Acrosome formation during Golgi and cap phases of normal mouse spermatids. **a** Round spermatid at the Golgi phase consisting of a single large Golgi apparatus (*G*) and an acrosomal vesicle (*AV*) attached to the nucleus (*N*). **b** Cap-phase spermatid with an acrosomal cap (*A*) spreading over the nucleus. **c** Several coated vesicles (*arrows*) on the *trans* face of the Golgi apparatus. **d** *Trans* Golgi saccule exhibiting direct fusion with the developing acrosomal cap (*arrow*). **e** Section through the caudal margin of the developing acrosome showing a row of coated vesicles (*arrows*). **f** Higher magnification; hexagonal structures on the coated vesicle (*arrowheads*). Scattered microtubules are also found near this region (*arrows*). *Bars* 2 μm (**a**, **b**, **e**), 0.5 μm (**c**, **d**, **f**)

bules were placed in Petri dishes containing culture medium (a 1:1 mixture of Ham's F12 and Dulbecco's modified medium with L-glutamine and 15 mM HEPES) with the addition of 0.006% colchicine or saline (control) alone. Media and chemicals were from Sigma. Seminiferous tubules were incubated at 32°C with 5% CO_2 for 1–3 days (Parvinen et al. 1983).

Transmission electron microscopy

Normal saline-treated (control) and colchicine-treated mouse seminiferous tubules were removed, pre-fixed in 2.5% glutaraldehyde/0.1 M cacodylate buffer (pH 7.2) containing 2% tannic acid at 0–4°C for 1 h, and post-fixed in 1% osmium tetroxide (OsO_4), 0.1 M cacodylate buffer for 1 h at room temperature. After fixation, specimens were dehydrated through a graded series of ethanol and then embedded in Spurr's resin. Ultrathin sections (approximately 80 nm thick) were cut on a Reichert-Jung Ultracut S ultramicrotome, stained with uranyl acetate and lead citrate, and examined with Hitachi H-7100 or H-7500 transmission electron microscope (Hitachi, Tokyo, Japan) at 75 kV.

Enzyme cytochemistry

For cytochemical studies, testes were fixed in 1.25% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer at 0–4°C for 1 h, immersed in 2 M sucrose solution for cryoprotection, and frozen with liquid propane. Semithin cryosections of about 10–30 μm were cut on an ultramicrotome in a cryosystem (Reichert FC S) under -80°C. Specimens were incubated with β-glycerophosphate at 37°C for 1 h. After post-fixation with 1% OsO_4 in cacodylate buffer for 30 min, the specimens were embedded in Spurr's resin. Ultrathin sections were taken and examined by the methods described above.

Solutions for detecting acid phosphatase activity were prepared according to Griffiths (1979). First, 25 ml acetate buffer (0.05 M) and 0.25 ml lead nitrate (12%) were mixed, and then 2.5 ml 3% aqueous β-glycerophosphate were added drop by drop. The solution was filtered and heated in a 60°C oven for 1 h. Control groups were incubated with the same solution except distilled water was used instead of β-glycerophosphate as a substrate.

Scanning electron microscopy

Specimens were prepared by the O-D-O method according to Tanaka (1989) with some modifications (Ho et al. 1999). Small blocks of tissues were fixed with 1% OsO_4 in 0.1 M cacodylate buffer at 0–4°C for 1 h, rinsed with cacodylate buffer, and then immersed in 25% and 50% DMSO for cryoprotection. The specimens were frozen in liquid propane and cracked into small pieces in liquid nitrogen. Tissue fragments were thawed in 50% DMSO and post-fixed in 1% OsO_4 in cacodylate buffer. Maceration was carried out by immersion of the specimens in 0.1% OsO_4 at 20°C for 1–2 days. The specimens were dehydrated through a graded series of ethanol solutions and critical-point dried with a Hitachi HCP-2 critical-point dryer. After being coated with

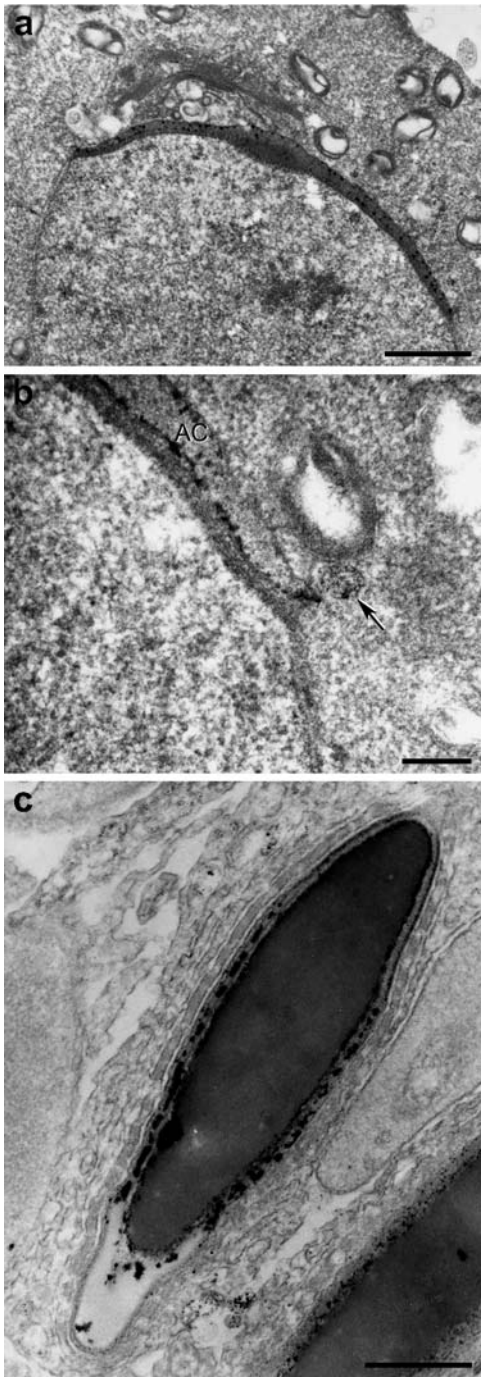

Fig. 2 Electron micrographs showing the localization of acid phosphatase activity (electron-dense particles) in round and elongated spermatids. **a** Acid phosphatase activity appears on both the outer and inner acrosomal membranes in a cap phase spermatid. **b** A vesicle (*arrow*) near the extension edge of the acrosomal cap (AC) contains some enzyme. **c** Accumulation of acid phosphatase in the central dense portion of the equatorial segment of the acrosome at maturation phase. *Bars* 1 μm (**a**), 0.2 μm (**b**), 0.5 μm (**c**)

Au-Pd, the specimens were examined with a Hitachi S-800 field emission scanning electron microscope.

Results

Morphological changes of normal spermatids during spermiogenesis

In the beginning of spermiogenesis (the Golgi phase), a single large Golgi apparatus bordered by a single saccule of endoplasmic reticulum on its *cis* face appeared near the nucleus in spermatids. Numerous coated vesicles accumulated on the *trans* face of the Golgi apparatus and fused together to form a proacrosomal vesicle attached to the nucleus (Fig. 1a). During the next phase (the cap phase), the acrosomal vesicle gradually increased in volume to form an acrosomal cap. The cap spread over the nucleus on the modified nuclear envelope, where the inner and outer nuclear membranes lay extremely close to each other, until it covered one third of the nuclear surface (Fig. 1b). During these phases of rapid acrosomal formation, coated vesicles budding from the edge of the *trans* Golgi network were commonly seen in sections (Fig. 1c). Occasionally, *trans* Golgi saccules were seen directly fused with the developing acrosomal cap (Fig. 1d). A row of coated vesicles aligned on the caudal margin of the acrosomal cap could be found if the section passed through the extending edge of the acrosome (Fig. 1e). At higher magnification, the presence of hexagonal patterns indicated that these coated vesicles were clathrin-coated. Some scattered microtubules were also observed in this region (Fig. 1f).

Localization of acid phosphatase in the acrosome

In cytochemical studies, electron-dense spots showing acid phosphatase activity were localized on the acrosomal membrane inside the acrosomal cap from the Golgi phase through the acrosome phase. The numbers of these electron-dense

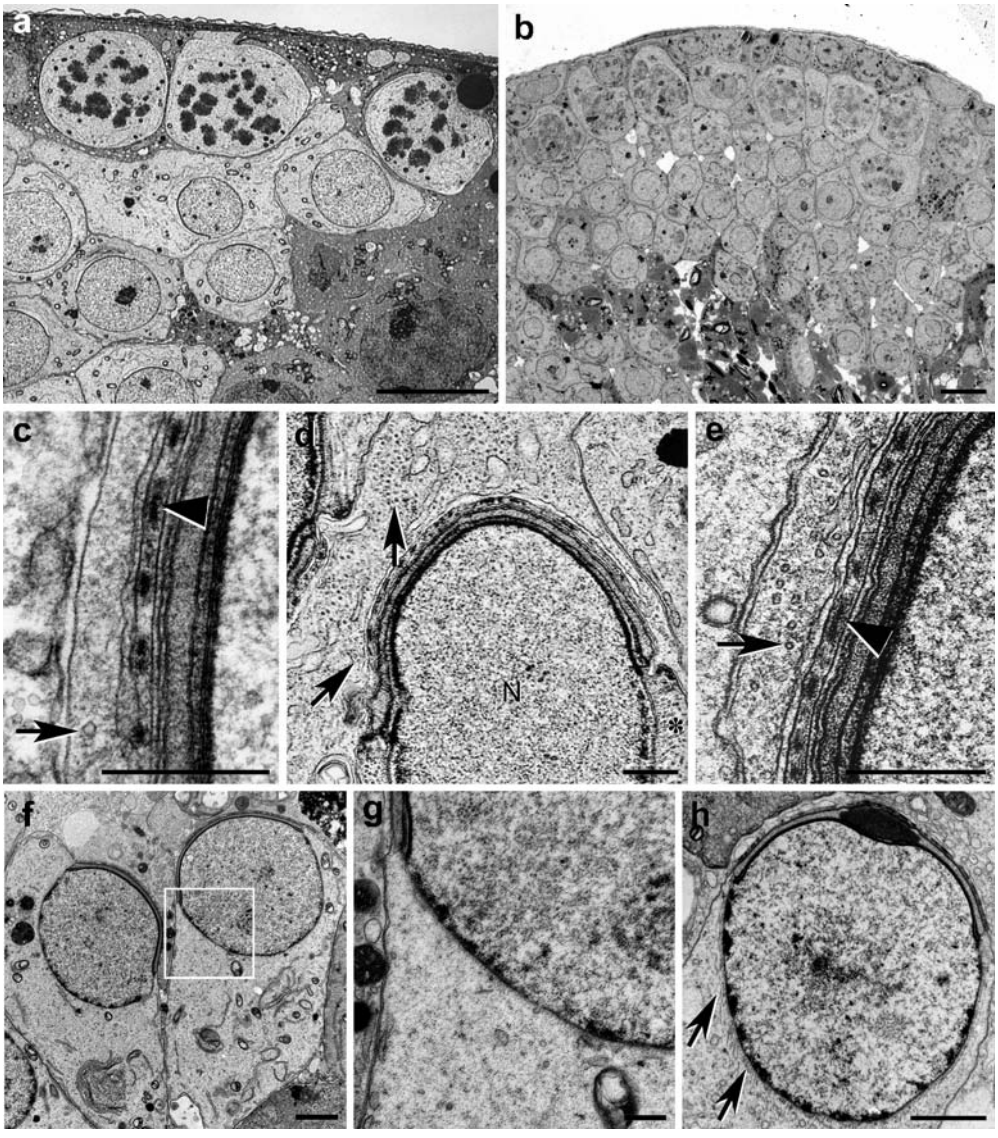

Fig. 3 Comparison of colchicine-treated (**a**, **c**, **f**, **g**) and control (**b**, **d**, **e**, **h**) seminiferous tubules. **a** Spermatogonia in the basal compartment of the seminiferous tubules are arrested at metaphase after colchicine treatment. **b** In controls, spermatogonia are mostly found in interphase, with chromatin lying within an intact nuclear envelope. **c** At the ectoplasmic specialization between Sertoli cell and spermatid, actin bundles (*arrowheads*) aggregate as seen in controls (**d**, **e**); however, fewer microtubules (*arrows*) are found in the colchicine-treated specimen (**c**). **f** No manchette can be detected in step 8–9 spermatids after colchicine treatment, (*rectangle* shown at higher magnification in **g**). **h** In controls, the manchette (*arrows*; see also *asterisk* in **d**) is easily seen around elongating nuclei of control spermatids. *Bars* 10 μm (**a**, **b**), 0.5 μm (**c**–**e**, **g**), 2 μm (**f**, **h**)

spots gradually increased as the acrosomal cap spread over the nucleus, and most of them appeared to be localized on both the outer and inner acrosomal membranes (Fig. 2a). Occasionally, some vesicles near the caudal edge of the acrosomal cap, corresponding to transport vesicles found in most cap-phase spermatids, could also be detected containing enzyme activity (Fig. 2b). During the maturation phase, the electron-dense spots were seen to disperse into the interior of the acrosome instead of remaining restricted to the acrosomal membranes, and most of them accumulated in the equatorial segment (Fig. 2c). These densities were not seen on control sections.

Ultrastructural alterations in colchicine-treated seminiferous tubules

Under our experimental conditions, colchicine affected not only spermatids, but also Sertoli cells and early spermatogenic cells, such as spermatogonia. The effect of colchicine was apparent when comparing colchicine-treated with control seminiferous tubules. Many spermatogonia were found arrested at metaphase (Fig. 3a) because microtubules were disrupted after colchicine treatment, whereas control spermatogonia were in the longer interphase (Fig. 3b). Moreover, at the ectoplasmic specialization (a junctional complex formed between Sertoli cells and spermatids) of controls (Fig. 3d,e), bundles of actin filaments were aggregated between a single saccule of the endoplasmic reticulum and the plasma membrane of Sertoli cells, whereas fewer microtubules were observed around the ectoplasmic specialization in colchicine-treated samples (Fig. 3c), indicating the decreased numbers of microtubules in Sertoli cells. In spermatids, one of the most evident effects caused by colchicine was the inhibition of manchette formation around elongating spermatid nuclei, where no or few microtubules were detected (Fig. 3f,g). A collection of microtubule bundles was a prominent feature found in control elongating spermatids (Fig. 3d,h). All these results suggested that the colchicine was effective in disrupting the polymerization of microtubules.

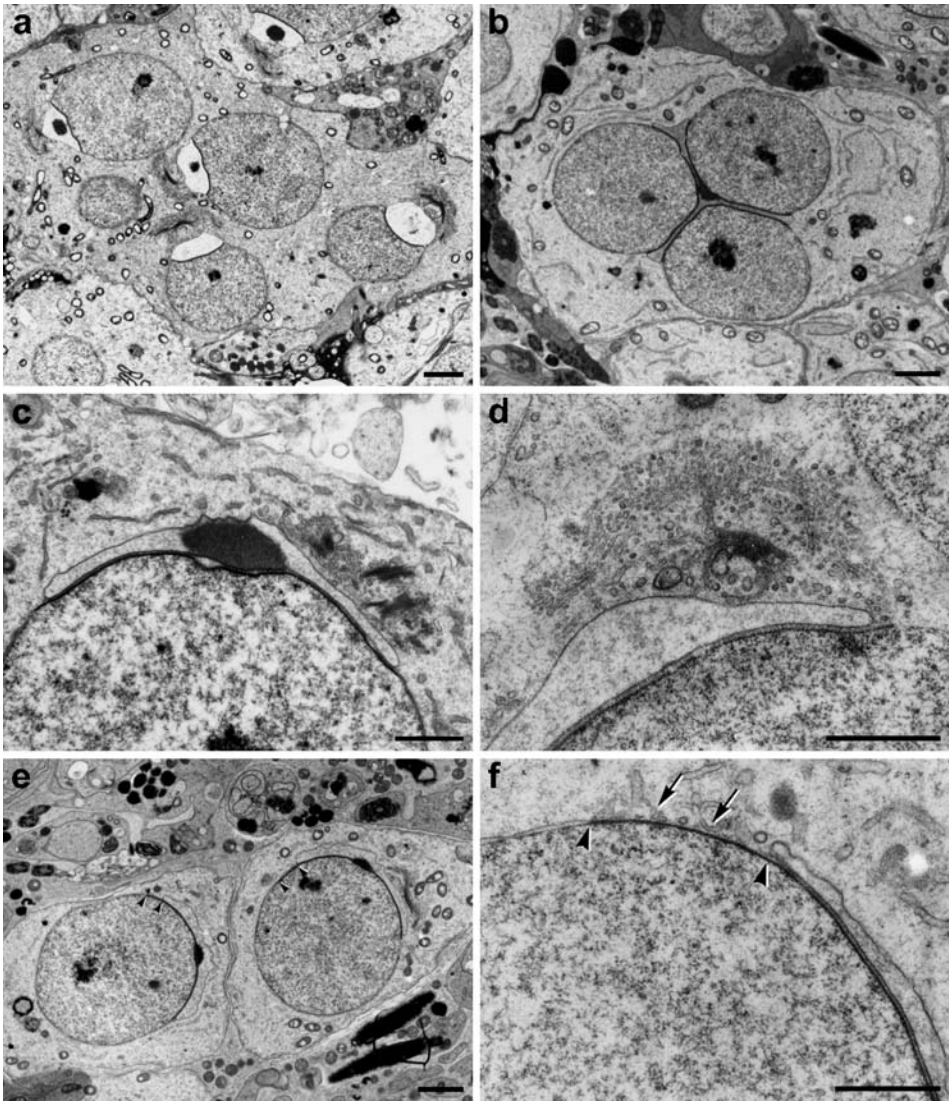

Fig. 4 Colchicine-induced alterations in spermatids. **a** A symplast consisting of several cap-phase spermatids. **b** Three spermatids share a fused acrosome in a symplast. **c**, **d** Different degrees of disorganization of the Golgi apparatus after colchicine treatment. **e** Colchicine-treated spermatids show partial development of the acrosome. The modified nuclear envelope extends further than the acrosomal cap (*between arrowheads*). **f** Vesicular elements (*arrows*) lying over the extended modified nuclear envelope (*between arrowheads*). *Bars* 2 μm (**a**, **b**, **e**), 1 μm (**c**, **d**, **f**)

Effects of colchicine on acrosome formation

After the second meiotic division of the spermatocytes, the spermatids are connected to each other by the intercellular bridges. This incomplete cytokinesis allows the interchange of materials between spermatids and synchronizes their development.

In colchicine-treated seminiferous tubules, many giant symplasts with all the daughter cells at the same stage of development were observed (Fig. 4a). Sometimes, several nuclei shared one large acrosome following fusion of their acrosomal caps, if the symplast was at the late cap phase (Fig. 4b). The appearance of these symplasts was probably induced by colchicine because of the rupture of the intercellular bridges.

In addition, different levels of disorganization of the Golgi apparatus were found in colchicine-treated spermatids. Some of them dissociated into several short, partially dilated cisternae (Fig. 4c). Moreover, some Golgi apparatus were completely ruptured into an aggregation of transport vesicles (Fig. 4d). In addition to the effects on the intercellular bridges and the Golgi apparatus, some influences of colchicine were also noticed on the spread of the acrosome over the nucleus. In normally developing spermatids, the formation of the modified nuclear envelope and the extension of the acrosomal cap was coordinated. After colchicine treatment, the modified nuclear envelope was frequently observed extending beyond the margins of the acrosome (Fig. 4e). Although no acrosome spreading occurred over these areas, some fuzzy materials similar to that in the subacrosomal space could be seen, and some vesicular elements were observed attaching over the nucleus in the position normally occupied by the acrosomal cap (Fig. 4f).

Under the scanning electron microscope, the three-dimensional structures of the acrosomal cap and the relationship between the organelles inside the spermatid could be clearly seen in controls. In a mid-cap phase spermatid, a complete acrosomal cap, surrounded at its caudal edge by a ring of coated vesicles, was most obvious when the spermatid was viewed from the nucleus side toward the acrosomal cap (Fig. 5a). On the other side of the acrosome, a crenated structure could be seen on the caudal edge arising from the formation of coated vesicles, and numerous vesicles were present on the outer surface of the acrosomal cap near the apex (Fig. 5b). In colchicine-treated spermatids, instead of regular Golgi stacks, numerous vesicles of various sizes accumulated in the *trans* Golgi network (Fig. 5c). Most of the cap-phase spermatids showed abnormally swollen acrosomal caps and greatly reduced numbers of coated vesicles on their caudal edges (Fig. 5d).

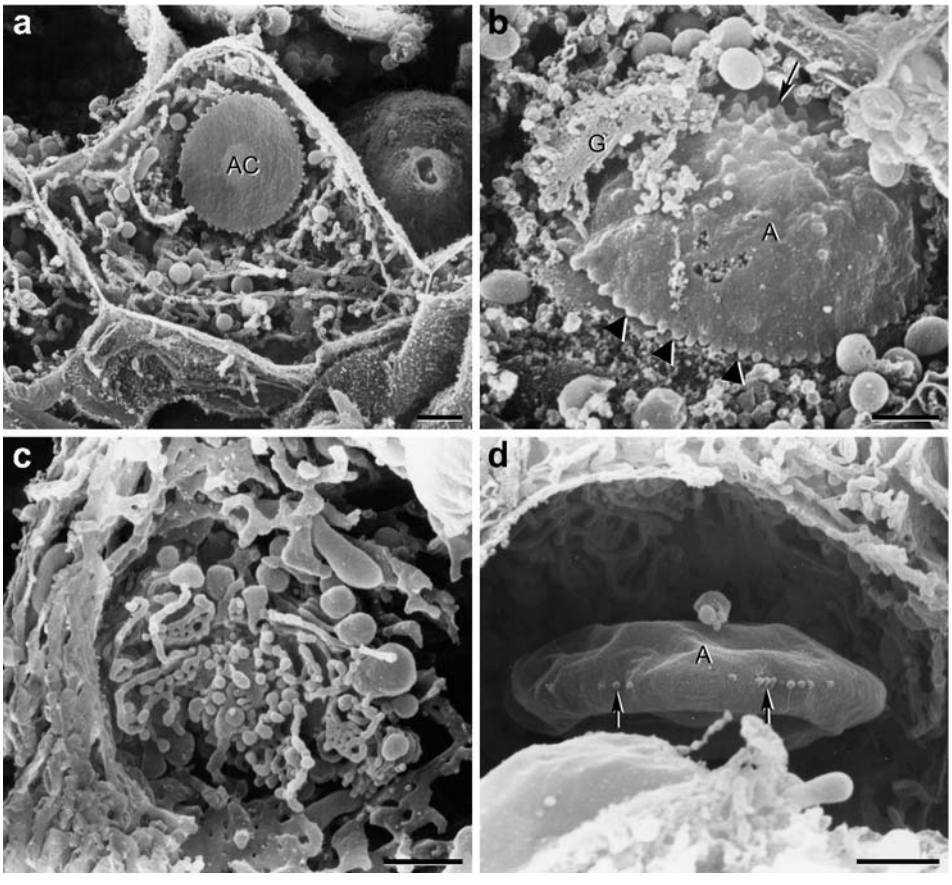

Fig. 5 Scanning electron micrographs showing internal structures of control (**a**, **b**) and colchicine-treated spermatids (**c**, **d**). **a** Caudal view of an entire acrosomal cap (*AC*) at mid-cap phase. **b** A rostral view of the acrosome of a late cap-phase (step 7) spermatid shows numerous vesicles on the apical (*arrow*) and caudal margin (*arrowheads*) of the acrosome (*A*). A Golgi apparatus (*G*) lies on one side of the acrosome. **c** *Trans* face view of the Golgi apparatus in a colchicine-treated spermatid shows several vesicles accumulated in this area. **d** Fewer vesicles (*arrows*) are seen scattered on the caudal edge of an abnormally shaped acrosome (*A*) after colchicine treatment. *Bars* 1 μm

Discussion

Distribution and transport of acrosomal membranes and enzymes

Active vesicle trafficking is involved in acrosome biogenesis, and many components of the machinery involved in intracellular membrane fusion have been found to participate in this event (Ramalho-Santos et al. 2001; Yang and Sperry 2003). Several Golgi proteins (e.g., Golgin-97, Golgin-95) have been found to be "missorted" to the acrosome during acrosome formation; however, those proteins are eventually retrieved via retrograde vesicular transport pathways and are not present in mature sperm (Moreno et al. 2000; Ramalho-Santos et al. 2001). Moreno and colleagues (2000) have proposed that acrosome flattening and spreading over the nucleus is the result of vesicle fusion at the edges of the acrosome coupled with membrane retrieval occurring at the apex. However, clathrin-coated pits and vesicles are only observed during vesicle formation; coated vesicles shed their clathrin coats immediately after vesicle scission (Bonifacino and Glick 2004; Galli and Haucke 2004). Based on our observations, most vesicles found at the caudal margin of the acrosome are clathrin-coated and therefore are presumably leaving the acrosome, instead of being added to it, which is in agreement with Pelletier and Friend (1983). There is no doubt that the enlargement of the developing acrosome is the result of fusion of vesicles originating from the *trans* Golgi network with the acrosome. The vesicles are speculated to fuse with the developing acrosomal cap at various sites, resulting in an undulated outer acrosomal membrane. Large amounts of acrosomal proteins and membranes might also derive from the fusion of a whole Golgi saccule during rapid acrosomal growth. Missorted Golgi proteins and components of the vesicle fusion machinery, such as v-SNARE, have to be recycled back to the Golgi apparatus to ensure continuing vesicle transport (Bonifacino and Glick 2004; Galli and Haucke 2004). Clathrin has been localized over the whole acrosomal cap (Moreno et al. 2000), indicating that retrograde vesicle trafficking is involved during acrosome formation.

Our finding of coated vesicles appearing on the apex and the caudal margin of the developing acrosomal cap is intriguing. In order to determine whether the distribution of coated vesicles plays a role in acrosomal enzyme distribution, we chose to study the localization of acid phosphatase. In our cytochemical studies with β-glycerophosphate as the substrate, acid phosphatase activity was found on both the outer and inner acrosomal membranes of cap-phase spermatids in addition to small transport vesicles nearby. However, in elongated spermatids, acid phosphatase activity appeared only in the equatorial segment. These results suggest that the extensive acrosomal shaping and spreading process leads to the redistribution of acrosomal enzymes, which could be one of the mechanisms for localizing each enzyme to a specific site.

Bozzola and colleagues (1991) have proposed that acrosin, a major protease of mammalian sperm, associates with the acrosomal granule only during the cap and acrosomal phases, but disperses throughout the acrosome, except for the equatorial segment, during the maturation phase. The outer acrosomal membrane is known to fuse with the plasma membrane at specific sites during the acrosome reaction, but membrane at the equatorial segment does not participate in this fusion. The equatorial region remains unchanged and is the site at which sperm-egg fusion eventually takes place (Yanagimachi 1994). Various systems have been suggested to be involved in the transport of specific proteins from the Golgi apparatus to the apical and basolateral surfaces in polarized epithelial cells (Rindler et al. 1987). Our observations indicate that many vesicles fuse with the acrosomal cap at various sites during the cap phase. A possible explanation is that the specific fusion of transport vesicles to various areas of the acrosome facilitates the specific distribution of certain acrosomal enzymes within the acrosome. In other words, specific mechanisms may exist to regulate the transport of particular membrane domains and enzymes to the appropriate place of the cap at the right time. The specific distribution of acrosomal membranes and enzymes in the acrosome may be related to the sequence of membrane fusion and enzyme release during fertilization.

Effects of colchicine on developing spermatids

In seminiferous tubules, developing germ cells remain connected to each other by intercellular bridges after mitotic and meiotic divisions. These intercellular bridges are not static structures but show size variation and considerable structure-related diversity during spermatogenesis (Weber and Russell 1987). In our study, colchicine-treated seminiferous tubules show many symplasts during the cap phase, indicating that microtubules are important for maintaining the structure of intercellular bridges, at least during certain developmental stages.

Two decades ago, microtubules were shown to play a vital role in maintaining the ultrastructural organization of the Golgi apparatus in somatic cells (for a review, see Thyberg and Moskalewski 1985). Morphological changes of the Golgi apparatus, such as the dissociation of the Golgi stacks and the accumulation of transport vesicles, are commonly seen in cells treated with microtubule-inhibiting agents. Functional microtubules are required for vesicle trafficking, including that of Golgi-derived secretory granules (Kraemer et al. 1999). Recently, specific molecular motor proteins have been demonstrated to play an important role in vesicle transport and acrosome biogenesis in rat spermatids (Yang and Sperry 2003). Similar disintegrations of Golgi bodies have been observed in our studies of colchicine-treated spermatids (Fig. 4c,d), indicating that microtubules are necessary to maintain the structural integrity of the Golgi apparatus in mouse spermatids. With the O-D-O cryofracture method, which enables the visualization of the three-dimensional organization of the Golgi apparatus, organized Golgi stacks (Ho et al. 1999) were harder to find in colchicine-treated samples than in controls. However, since

the O-D-O method involves the washing away of most cytoplasmic proteins and even small vesicles during sample preparation, disintegrated Golgi apparatus may have been partially washed away.

In normally developing spermatids, the spreading of the acrosomal cap over the nucleus is correlated with the spreading of the modification of the nuclear envelope (Russell et al. 1983; Fouguet et al. 1992). However, the modified nuclear envelope underlying the acrosome grows beyond acrosomal margins in OVE 219 transgenic mice (Russell et al. 1994). The modifications in the nuclear envelope have been suggested to signal the acrosomal spread. In our study, inconsistent growth between the acrosomal cap and the modified nuclear envelope has been found in most of the colchicine-treated spermatids during the cap phase. This inconsistency might be attributable to the retarded growth of the acrosome instead of the overgrowth of the modified nuclear envelope. Two reasons are proposed to account for the retarded acrosomal growth. The first is the lack of anterograde vesicle trafficking, as the transport of small vesicles from the Golgi apparatus to the growing acrosomal cap is interrupted because of the rupture of the cytoplasmic microtubular system by colchicine. The second is the lack of retrograde vesicle trafficking, because colchicine-treated cap-phase spermatids have swollen acrosomes and greatly reduced numbers of coated vesicles at the caudal margins of the acrosomes. Therefore, coated vesicles leaving the caudal margin of the acrosomal cap might act as a pulling force to facilitate acrosomal shaping and spreading over the nucleus.

Recently, the subacrosomal structure linking the developing acrosome to the spermatid nucleus has been found to be an F-actin/keratin-containing plate, termed the acroplaxome (Kierszenbaum et al. 2003; Kierszenbaum and Tres 2004). During acrosomal biogenesis, the acroplaxome behaves as the nucleation site to which Golgi-derived proacrosomal vesicles attach and fuse. Both microtubule-based and actin-based molecular motors are proposed to be involved in transporting proacrosomal vesicles from the Golgi apparatus to the acroplaxome (Kierszenbaum and Tres 2004). Although no apparent disruption of the actin cytoskeleton has been found in our study (Fig. 3c), microtubule depolymerization might affect actin integrity at or around the acroplaxome, thereby resulting in retarded acrosomal growth. Therefore, details of the interactions between microtubules and actin filaments should be determined.

Concluding remarks

In conclusion, the enlargement of the acrosome is achieved by the fusion of vesicles secreted by the Golgi apparatus and transported via microtubules to various sites of the growing acrosome. Our observations indicate that the shaping, spreading, and maintaining of the acrosomal cap is regulated, at least partially, by clathrin-coated vesicle-mediated retrograde trafficking. Our results further show that, in mouse spermatids, microtubules not only play an important role in determining the organization of the Golgi apparatus, but also participate in the mechanism regulating the spreading and shaping of the acrosome over the nucleus.

Acknowledgements We express our gratitude to our mentors Jia-Chyuan Chen and Hsiao-Hsu Jen for introducing us to the world of electron microscopy. We also thank Dr. Susan S. Suarez for critical review of the manuscript.

References

Bonifacino JS, Glick BS (2004) The mechanisms of vesicle budding and fusion. Cell 116:153–166

Bozzola JJ, Polakoski K, Haas N, Russell LD, Campbell P, Peterson RN (1991) Localization of boar sperm proacrosin during spermatogenesis and during sperm maturation in the epididymis. Am J Anat 192:129–141

Clermont Y, Leblond CP (1955) Spermiogenesis of man, monkey, ram and other mammals as shown by the periodic acid-Schiff technique. Am J Anat 96:229–253

Dell KR (2003) Dynactin polices two-way organelle traffic. J Cell Biol 160:291–293

Fouquet JP, Valentin A, Kann ML (1992) Perinuclear cytoskeleton of acrosome-less spermatids in the blind sterile mutant mouse. Tissue Cell 24:655–665

Friend DS, Fawcett DW (1974) Membrane differentiations in freeze-fractured mammalian sperm. J Cell Biol 63:641–664

Galli T, Haucke V (2004) Cycling of synaptic vesicles: how far? How fast! Sci STKE 264:1–12

Griffiths G, Warren G, Stuhlfauth I, Jockusch BM (1981) The role of clathrin-coated vesicles in acrosome formation. Eur J Cell Biol 26:52–60

Griffiths GW (1979) Transport of glial cell acid phosphatase by endoplasmic reticulum into damaged axons. J Cell Sci 36:361–389

Handel MA (1979) Effects of colchicine on spermiogenesis in the mouse. J Embryol Exp Morphol 51:73–83

Ho HC, Tang CY, Suarez SS (1999) Three-dimensional structure of the Golgi apparatus in mouse spermatids: a scanning electron microscopic study. Anat Rec 256:189–194

Holt WV (1979) Development and maturation of the mammalian acrosome. A cytochemical study using phosphotungstic acid staining. J Ultrastruct Res 68:58–71

Jordens I, Fernandez-Borja M, Marsman M, Dusseljee S, Janssen L, Calafat J, Janssen H, Wubbolts R, Neefjes J (2001) The Rab7 effector protein RILP controls lysosomal transport by inducing the recruitment of dynein-dynactin motors. Curr Biol 11:1680–1685

Kierszenbaum AL, Rivkin E, Tres LL (2003) Acroplaxome, an F-actin-keratin-containing plate, anchors the acrosome to the nucleus during shaping of the spermatid head. Mol Biol Cell 14:4628–4640

Kierszenbaum AL, Tres LL (2004) The acrosome-acroplaxome-manchette complex and the shaping of the spermatid head. Arch Histol Cytol 67:271–284

Kraemer J, Schmitz F, Drenckhahn D (1999) Cytoplasmic dynein and dynactin as likely candidates for microtubule-dependent apical targeting of pancreatic zymogen granules. Eur J Cell Biol 78:265–277

Leblond CP, Clermont Y (1952) Spermiogenesis of rat, mouse, hamster and guinea pig as revealed by the "periodic acid-fuchsin sulfurous acid" technique. Am J Anat 90:167–215

Moreno RD, Ramalho-Santos J, Sutovsky P, Chan EK, Schatten G (2000) Vesicular traffic and Golgi apparatus dynamics during mammalian spermatogenesis: implications for acrosome architecture. Biol Reprod 63:89–98

Moreno RD, Schatten G (2000) Microtubule configurations and post-translational alpha-tubulin modifications during mammalian spermatogenesis. Cell Motil Cytoskeleton 46:235–246

Parvinen M, Wright WW, Phillips DM, Mather JP, Musto NA, Bardin CW (1983) Spermatogenesis in vitro: completion of meiosis and early spermiogenesis. Endocrinology 112:1150–1152

Pelletier RM, Friend DS (1983) Development of membrane differentiations in the guinea pig spermatid during spermiogenesis. Am J Anat 167:119–141

Peterson RN, Bozzola J, Polakoski K (1992) Protein transport and organization of the developing mammalian sperm acrosome. Tissue Cell 24:1–15

Ramalho-Santos J, Moreno RD, Wessel GM, Chan EK, Schatten G (2001) Membrane trafficking machinery components associated with the mammalian acrosome during spermiogenesis. Exp Cell Res 267:45–60

Rindler MJ, Ivanov IE, Sabatini DD (1987) Microtubule-acting drugs lead to the nonpolarized delivery of the influenza hemagglutinin to the cell surface of polarized Madin-Darby canine kidney cells. J Cell Biol 104:231–241

Russell LD, Lee IP, Ettlin R, Peterson RN (1983) Development of the acrosome and alignment, elongation and entrenchment of spermatids in procarbazine-treated rats. Tissue Cell 15:615–626

Russell LD, Malone JP, MacCurdy DS (1981) Effect of the microtubule disrupting agents, colchicine and vinblastine, on seminiferous tubule structure in the rat. Tissue Cell 13:349–367

Russell LD, Russell JA, MacGregor GR, Meistrich ML (1991) Linkage of manchette microtubules to the nuclear envelope and observations of the role of the manchette in nuclear shaping during spermiogenesis in rodents. Am J Anat 192:97–120

Russell LD, Ying L, Overbeek PA (1994) Insertional mutation that causes acrosomal hypo-development: its relationship to sperm head shaping. Anat Rec 238:437–453

Schnapp BJ, Vale RD, Sheetz MP, Reese TS (1985) Single microtubules from squid axoplasm support bidirectional movement of organelles. Cell 40:455–462

Sheetz MP, Vale R, Schnapp B, Schroer T, Reese T (1987) Movements of vesicles on microtubules. Ann N Y Acad Sci 493:409–416

Sinowatz F, Wrobel KH (1981) Development of the bovine acrosome. An ultrastructural and cytochemical study. Cell Tissue Res 219: 511–524

Sotomayor RE, Handel MA (1986) Failure of acrosome assembly in a male sterile mouse mutant. Biol Reprod 34:171–182

Stackpole CW, Devorkin D (1974) Membrane organization in mouse spermatozoa revealed by freeze-etching. J Ultrastruct Res 49:167–187

Tanaka K (1989) High resolution scanning electron microscopy of the cell. Biol Cell 65:89–98

Tang XM, Lalli MF, Clermont Y (1982) A cytochemical study of the Golgi apparatus of the spermatid during spermiogenesis in the rat. Am J Anat 163:283–294

Tanii I, Yoshinaga K, Toshimori K (1998) The effects of brefeldin A on acrosome formation and protein transport to the acrosome in organ cultures of rat seminiferous tubules. J Electron Microsc 47:161–167

Thyberg J, Moskalewski S (1985) Microtubules and the organization of the Golgi complex. Exp Cell Res 159:1–16

Ventela S, Mulari M, Okabe M, Tanaka H, Nishimune Y, Toppari J, Parvinen M (2000) Regulation of acrosome formation in mice expressing green fluorescent protein as a marker. Tissue Cell 32:501–507

Weber JE, Russell LD (1987) A study of intercellular bridges during spermatogenesis in the rat. Am J Anat 180:1–24

Yamaguchi N, Takanezawa Y, Koizumi H, Umezu-Goto M, Aoki J, Arai H (2004) Expression of NUDEL in manchette and its implication in spermatogenesis. FEBS Lett 566:71–76

Yanagimachi R (1994) Mammalian fertilization. In: Knobil E, Neill J (eds) The physiology of reproduction. Raven, New York, pp 189–317

Yang WX, Sperry AO (2003) C-terminal kinesin motor KIFC1 participates in acrosome biogenesis and vesicle transport. Biol Reprod 69:1719–1729