The acrosome is a unique membranous organelle located over the anterior part of the sperm nucleus that is highly conserved throughout evolution. This acidic vacuole contains a number of hydrolytic enzymes that, when secreted, help the sperm penetrate the egg’s coats. Although acrosome biogenesis is an important aspect of spermiogenesis, the molecular mechanism(s) that regulates this event remains unknown. Active trafficking from the Golgi apparatus is involved in acrosome formation, but experimental evidence indicates that trafficking of vesicles out of the Golgi also occurs during acrosomogenesis. Unfortunately, this second aspect of acrosome biogenesis remains poorly studied. In this article, we briefly discuss how the biosynthetic and endocytic pathways, assisted by a network of microtubules, tethering factors, motor proteins and small GTPases, relate and connect to give rise to the sperm-specific vacuole, with a particular emphasis placed on the endosomal compartment. It is hoped that this information will be useful to engage more studies on acrosome biogenesis by focusing attention towards suggested directions.

A mammalian spermatozoon is characterized by two morphological and functional parts, i.e., the head and the flagellum, each optimized for a special task. Both units are shaped and assembled during the cytoskeletal and morphogenic phase of spermatogenesis, as described in spermiogenesis. While the flagellum is the motor module that helps to provide the “force” that drives ejaculated sperm to the site of the egg for fertilization, the head encapsulates precisely half of the paternal genome that, once engulfed in the egg’s ooplasm, results in the formation of the zygote and in the restoration of the diploid condition. In order for this to occur, the spermatozoon has to penetrate the protecting barriers of the oocyte, the cumulus cell layer and the zona pellucida (ZP). Before penetrating into the ZP, the fertilizing spermatozoon must undergo a head morphological change involving disruption of the acrosome with the consequent release of stored hydrolytic enzymes, the so-called acrosome reaction (AR). Hereupon, the importance of the acrosome that is thought to be indispensable for fertilization. Acrosomeless spermatozoa are in fact infertile. In early studies in rodents it was reported that the site where the fertilizing spermatozoon begins the AR is in the cumulus, but subsequent studies, carried out with cumulus-free eggs, established over the years that the physiological inducer of the mammalian AR is the ZP. This second view is now widespread and generally accepted. Recently, however, Jin et al. using the technique of in vitro fertilization involving cumulus-enclosed mouse oocytes and fluorescently tagged transgenic spermatozoa to detect the onset of the AR, have shown that spermatozoa, under natural conditions, undergo AR within the cumulus, but subsequent studies, carried out with cumulus-free eggs, established over the years that the physiological inducer of the mammalian AR is the ZP. This second view is now widespread and generally accepted. Recently, however, Jin et al. using the technique of in vitro fertilization involving cumulus-enclosed mouse oocytes and fluorescently tagged transgenic spermatozoa to detect the onset of the AR, have shown that spermatozoa, under natural conditions, undergo AR within the cumulus. So, with this recent finding, it seems that cumulus is crucial to AR as originally conceived.

One would wonder whether a sort of parallel fate involving the studies devoted to the nature of the acrosome exists. Originally, the acrosome was described as a modified lysosome. Successive studies,
however, established that the acrosome is a direct Golgi-derived secretory vesicle. Recent experimental evidence, nevertheless, indicates the need for a revision of the concept regarding ‘acrosome = Golgi-derived organelle’. In line with the original suggestion, Berruti et al. have proposed the acrosome as a novel lysosome-related organelle (LRO). LROs represent a family of membrane-enclosed organelles restricted to certain specialized cell types, which includes melanosomes, lytic granules, platelet dense bodies, exosomes and synaptosomes. LROs have functional and dynamic stages of maturation as indicated by the involvement of Rab family proteins, i.e., small GTPases critical for vesicle fusion and transport. In particular, LRO biogenesis is characterized by the dynamic flow of proteins and vesicles among distinct endosomal compartments; at the hub of the early endosome (EE) the endocytic pathway connects with the exocytic pathway that in turn sorts, through the trans-Golgi network (TGN), newly synthesized proteins from the endoplasmic reticulum (ER) to the endosomal system. From the one hand, vesicular transport systems in LRO biogenesis are quite common among the various cell types, but from the other hand, protein cargo/es that the vesicles carry can vary widely, depending on the tissue- or cell-specific expression of the given cargo. Hu et al. have provided an accurate profiling of LRO proteomes.

In short, we arrived at the conclusion that the acrosome may represent a new member of the LRO family by taking into account, all together, a series of features that characterize the sperm acrosome, some of which have been well established while others have been uncovered only recently. Briefly, (a) the acrosome contains an acidic pH and some lysosomal hydrolases, as well as some unique enzymes/proteins like acrosin and acrosin binding protein (ACRB/OY-TES-1). These processes follow the biosynthetic pathway (anterograde transport) and are packed in electron-dense core vesicles, called pro-acrosomal granules, probably at/after the trans-Golgi network (TGN). Motor proteins as KIFC1 and Rab family members as Rab 27a have been described to function in vesicle trafficking from Golgi to the acrosome; (b) acrosomal acrosin (grouped into four phases: Golgi, Cap, Acrosome and Maturation. By the late Cap-phase, the spermatid Golgi apparatus migrates to the opposite side of the cell, thus ending transport of glycoproteins into the acrosome via the Golgi biosynthetic pathway. Extra-Golgi pathways, contributing to the enlargement and maturation of the developing acrosome, have however been described. The TGN is one of the major traffic hubs of the cell, as it is involved in protein/membrane transport from the biosynthetic pathway, as well as in the receiving of protein cargo by retrograde transport from the endocytic compartments; evidence has shown that components of the endocytic machinery are involved in the biogenesis of the acrosome, providing experimental support to the early suggestion of West and Willison that there are at least two sources of vesicular transport, one derived from the Golgi and one from the plasma membrane, concurrent to the development of the acrosome. Among the discovered components, there are: Afaf (Acrosome formation associated factor), that localizes on EEA1 (early endosome antigen 1)-positive endosomes; SH3P13, a vesicular protein that functions in clathrin-mediated receptor endocytosis; SPE-39, a regulator of lysosomal delivery originally identified in spermatogenic cells; UBPy, a deubiquitinating enzyme originally identified as a protein interacting with the endocytic trafficking protein Hbp and the Ras exchange factor RasGRF1.

In the mouse UBPy, now officially termed Usp8 (ubiquitin-specific protease 8), was molecularly identified as a deubiquitinase containing the typical hallmarks of the UBP family of deubiquitinating enzymes. Although present in more tissues, mUBP8 is highly expressed and restricted to the testis and the central nervous system. Conventionally speaking, deubiquitinases promote the removal and processing of conjugated ubiquitin from proteins, thus playing regulatory roles at the level of both protein turnover and protein degradation. Successively, by exploiting cell transfection technologies, UBPy/Usp8 is emerged to be a key regulator of endosomal sorting and vesicle morphology. However, to establish a physiological role “in vivo”, UBPy/Usp8 has been extensively studied in male germ cells with the following observations: (1) UBPy interacts with spermatid Hbp/STAM2 that, on its own, interacts with its binding partner Hrs to give rise to the spermatid ESCRT-0 complex. ESCRT-0 (Endosomal Sorting Complex Required for Transport-0) is the complex that first assigns directionality to endosomal sorting and is recruited to the EE (early endosome); (2) UBPy/Hbp/Hrs-labelled vesicles develop into the forming acrosome; that is also EEA1 positive; (3) Vps54, a vesicular protein working in the retrograde transport from EE, is involved in acrosomogenesis; (4) UBPy, through its MIT (microtubule interacting and trafficking/transport) domain, directly associates with spermatid microtubules, thus likely mediating the link between the sorted endocytic vesicle and microtubules; acrosomogenesis is a process which is microtubule-dependent analogous to LRO-biogenesis. These findings coupled with other recent studies (the work on EHD1 strongly support evidence that the endocytic pathway also plays a critical role in acrosome biogenesis. Furthermore, very recently it has been shown that mouse sperm expressing the Vps54(L967Q) variant are acrosome-less because UBPy- and Vps54(L967Q)-labelled vesicles are not able to develop into an acrosome. The point mutation Vps54(L967Q) is responsible for the wobbler mouse phenotype, characterized by motor neuron disease and spermiogenesis defect. Wobbler spermatozoa are round-headed, lacking an acrosome, and are infertile. Why the wobbler Vps54 mutation affects in particular motor neurons and spermatids is not yet clear. So far Vps54 has been studied essentially in yeast, where it gives rise together with Vps51, Vps52 and Vps53 to the Golgi Associated Retrograde Protein (GARP) complex; in particular, Vps54 works in the retrograde transport of the EE to the TGN. After the discovery of the GARP complex in the yeast Saccharomyces cerevisiae a decade ago, its study went into a lull and only recently has interest resurfaced with the characterization of the orthologous complex in higher eukaryotes.
However, yeast has no LRO. It might be that—this is only a speculative view to suggest a possible direction for future work—in specialized cell types characterized by the presence of a specific LRO, Vps54, recruited through cell-specific activators/effectors, tethers the EE protein cargo to the forming LRO. Figure 1 illustrates a simplified schematic drawing of the acrosome biogenesis. As animal models are important tools for the investigation on LRO disorders known to characterize some human genetic diseases, the Vps54(L967Q) point-mutation, could be a useful tool to study defective acrosomogenesis.

On a final note, we want to recall attention towards a further, potentially important, direction of research. Sperm are highly polarized cells; sperm achieve not only distinct polarized plasma-membrane domains, but also a strong polarization of cellular organelles like the acrosome at the anterior pole and the flagellum at the posterior pole of the cell. The establishment of such polarization is essential for sperm function as we discuss in the introduction of this commentary. Accumulating evidence now reveals that endocytosis plays an important role not only in establishing/maintaining polarized membrane domains but also in the proper intracellular localization of key polarity proteins. It is known that components of the ESCRT machinery, which control the subsequent sorting of endocytic cargoes from the EE, are required for epithelial polarity whereas, conversely, proteins that act downstream of ESCRT sorting are not required. At the same time, some polarity proteins can also regulate the endocytic machinery. Thus, in other words, this is an emerging concept regarding the reciprocal regulation between polarity proteins and endocytic regulators. Not only, but a further corollary key question reserves attention: how are acrosomal resident proteins sorted among the sperm-specific and conventional anterograde/retrograde trafficking vesicles/organelles? It might be of novel and intriguing significance to undertake research to study the possible ‘endocytosis-polarity-protein sorting signal’ relationship during acrosomogenesis. At last, another open question is how cargo sorting is coupled to vesicle motility during acrosomogenesis, especially in light of the finding that UBPy is able to interact with spermid microtubules. Recent studies have revealed that specific members of the structurally and functionally related AP-1, AP-2, AP-3 and AP-4 complexes, which are components of coated vesicles that mediate intracellular trafficking of integral membrane proteins, together with motor proteins like the kinesin KIF13A, the cytoplasmic dynein and myosinVa, co-ordinately regulate endosomal sorting and positioning to facilitate LRO biogenesis. It would be interesting to verify and clarify if and which clathrin-adaptors and molecular motors are recruited in acrosome biogenesis.

Herein, we have briefly highlighted three main potentially important directions (i.e., the contribution of the endocytic machinery, the cross-talk between endocytic and biosynthetic pathways, and the ‘endocytosis-polarity-sorting signal’ relationship) in the study of acrosome biogenesis. Given that the acrosome has been considered, over the years, essentially as a direct Golgi derivative, the pathway ‘ER-Golgi-acrosome’ has been widely studied and established. The view ‘ER-Golgi-acrosome’ has been so widespread that molecular components of the trafficking machinery (among which the above mentioned motor protein KIFC1 and the vesicle receptor Rab 27a, to cite only two examples), have been ascribed to the biosynthetic (Golgi → developing acrosome) transport. Conversely, proteomic analysis of endocytic vesicles has revealed that KIFC1 is an early endosome-associated protein, while Rab27a, a member of the Rab family of Ras-like proteins.
GTPases, is known to function in LRO maturation and/or trafficking. Finally, we cannot neglect that if key components of the EE machinery like UBPy/ESCRT-0 complex, required for recognition and sorting of selected ubiquitinated transmembrane receptors, are involved in acrosome biogenesis, this suggests the existence of a sperm membrane factor/s that needs to be selectively recruited at the acrosome level. Given that failure in acrosome biogenesis results in male sterility with a particular fallout as to human infertility, it is hoped that future studies are addressed to elucidate the biology of the acrosome in its completeness.

References
1. Yangamichi R. Mammalian fertilization. In: Knobil E, Neil J, Eds. The Physiology of Reproduction. New York: Raven 1994; 189-217.
2. Dan AH, Fensstra I, Westphal JR, Ramos L, van Golde RJ, Kremer JA. Globozoospermia revisited. Hum Reprod Update 2007; 13:63-75.
3. Austin CR, Bishop MW. Role of the rodent acrosome and perforatorium in fertilization. Proc R Soc Lond B Biol Sci 1958; 149:241-8.
4. Floroman HM, Storey BT. Mouse gamete interactions: The zona pellucida is the site of the acrosomal reaction leading to fertilization in vitro. Dev Biol 1982; 91:121-30.
5. Buffone MG, Rodriguez-Miranda E, Storey BT, Gerton GL. Acrosomal exocytosis of mouse sperm progresses in a consistent direction in response to zona pellucida. J Cell Physiol 2009; 220:611-20.
6. Jin M, Fujiwara E, Kukuchi Y, Okabe M, Sanouh Y, Baba SA, et al. Most fertilizing mouse spermatozoa begin their acrosome reaction before contact with the zona pellucida during in vitro fertilization. Proc Natl Acad Sci USA 2011; 108:4892-6.
7. Bedford JM. Site of the mammalian sperm phosphoacrosomal reaction. Proc Natl Acad Sci USA 2011; 108:4793-4.
8. Hartree EF. The acrosome-lysosome relationship. J Reprod Fertil 1975; 44:125-6.
9. Tang XM, Lalli MF, Clermont Y. A cytotoxicchemical study of the Golgi apparatus of the spermatid during spermiogenesis in the rat. Am J Anat 1982; 160:283-94.
10. Martinez MJ, Geuze HJ, Ballesta J. Evidence for a nonlysosomal origin of the acrosome. J Histochem Cytochem 1996; 44:313-20.
11. Moreno RD, Alvarado CP. The mammalian acrosome as a secretory lysosome: new and old evidence. Mol Reprod Dev 2006; 73:1430-4.