Asian Journal of Andrology (2015) 17, 720–725

www.asiaandro.com; www.ajandrology.com

Open Access

INVITED REVIEW

Sperm Biology

Epididymosomes: transfer of fertility-modulating proteins to the sperm surface

Patricia A Martin-DeLeon

A variety of glycosylphosphatidylinositol (GPI)-linked proteins are acquired on spermatozoa from epididymal luminal fluids (ELF) during sperm maturation. These proteins serve roles in immunoprotection and in key steps of fertilization such as capacitation, acrosomal exocytosis and sperm-egg interactions. Their acquisition on sperm cells is mediated both by membrane vesicles (epididymosomes, EP) which were first reported to dock on the sperm surface, and by lipid carriers which facilitate the transfer of proteins associated with the membrane-free fraction of ELF. While the nonvesicular fraction is more efficient, both pathways are dependent on hydrophobic interactions between the GPI-anchor and the external lipid layer of the sperm surface. More recently proteomic and hypothesis-driven studies have shown that EP from several mammals carry transmembrane (TM) proteins, including plasma membrane Ca^{2+}-ATPase 4 (PMCA4). Synthesized in the testis, PMCA4 is an essential protein and the major Ca^{2+} efflux pump in murine spermatozoa. Delivery of PMCA4 to spermatozoa from bovine and mouse EP during epididymal maturation and *in vitro* suggests that the docking of EP on the sperm surface precedes fusion, and experimental evidence supports a fusogenic mechanism for TM proteins. Fusion is facilitated by CD9, which generates fusion–competent sites on membranes. On the basis of knowledge of PMCA4's interacting partners a number of TM and membrane-associated proteins have been identified or are predicted to be present, in the epididymosomal cargo deliverable to spermatozoa. These Ca^{2+}-dependent proteins, undetected in proteomic studies, play essential roles in sperm motility and fertility, and their detection highlights the usefulness of the hypothesis-driven approach.
Asian Journal of Andrology (2015) **17**, 720–725; doi: 10.4103/1008-682X.155538; published online: 26 June 2015

Keywords: epididymal maturation; epididymal secretory proteins; GPI-linked proteins; lipid carrier; membrane-associated proteins; PMCA4; transmembrane proteins

INTRODUCTION

The epididymis, the major component of the posttesticular pathway, is the organ where spermatozoa mature and gain progressive motility and fertilizing ability. Its role in sperm maturation is known to be mediated via secretory proteins that are delivered to the sperm surface during their transit[1,2] which may be for a protracted period of time such as 5–10 days in mice.[3] These secretory proteins have been identified in the epididymal lumen in the absence of spermatozoa following efferent duct ligation,[4] in the luminal fluid,[5–7] as well as in conditioned media of cultured epididymal epithelial cells.[6] Initially, the secreted epididymal proteins identified were predominantly glycosyl phosphatidylinositol-(GPI)-linked such as CD52, CD59, CD73, which are proteins that play a role in immunoprotection.[8–10] Later, the GPI-linked proteins identified were those that play a role in fertilization such as sperm-egg interaction, e.g., P34H[11,12] and Sperm Adhesion molecule 1 (SPAM1).[13] In the epididymal luminal fluids (ELF) secreted, GPI-linked proteins have been shown to exist partly in the soluble and insoluble fractions,[5,14,15] which consists of extracellular membrane vesicles that are known to play a key role in intercellular cross-talk.[16] These vesicles, termed epididymosomes, have been well-characterized and are known to transfer proteins to the sperm plasma membrane.[16–18] This review will focus on epididymosomal proteins identified to play a role in fertility and the mechanism by which they are acquired by spermatozoa.

TRANSFER OF GPI-LINKED SPERM PROTEINS IN THE EPIDIDYMAL SECRETOME – EPIDIDYMOSOMES AND A LIPID CARRIER COMPRISE DUAL PATHWAYS

GPI-linked proteins uniquely possess acyl chains (**Figure 1**), which when inserted into the outer leaflet of the lipid bilayer of a target membrane anchor the protein and permit its lateral diffusion. Documented uptake of these proteins from the extracellular environment has been reported in red blood cells[19] and sperm cells[13,20,21] and has been demonstrated to occur in the absence of vesicles.[19,20] This suggests that there is a nonvesicular mechanism by which GPI-linked proteins can be transferred to spermatozoa from the ELF. This nonvesicular mechanism was investigated by using SPAM1, known to be present in the ELF of at least five species, including mice and humans,[22] as a model. SPAM1 is a multifunctional protein which is known to perform essential roles in fertilization: (1) cumulus penetration via its neutral hyaluronidase activity, (2) secondary binding to the zona pellucida after the acrosome reaction, (3) penetration of the zona pellucida, and (4) Ca^{2+} signaling- associated acrosomal exocytosis mediated by its hyaluronic acid receptor domain.[22] In murine ELF, SPAM1 has been shown to be present in both nonvesicular (60%) and vesicular (40%) fractions.[5] When cauda epididymidal spermatozoa were co-incubated with each of these fractions, both were able to deliver SPAM1 to the sperm surface, with the nonvesicular fraction doing so more efficiently.[23]

Department of Biological Sciences, University of Delaware, Newark, DE 19716, USA.
Correspondence: Prof. PA Martin-DeLeon (pdeleon@udel.edu)
This article was presented at the 6th International Conference on the Epididymis in Shanghai, Oct 31-Nov 3, 2014.

SPAM1 from the nonvesicular fraction of the ELF was shown to reside in low-molecular weight monomers as well as high-molecular weight oligomeric complexes.[24] The oligomeric complexes were incapable of delivering SPAM1 to the sperm surface, but likely served as a source of monomers (**Figure 2**), which effectively perform the transfer.[24] Monomers are stabilized in an aqueous environment by hydrophobic interactions of the GPI anchors with Apolipoprotein J or clusterin (CLU) that resides in ELF.[24] CLU is a well-known lipid carrier in a variety of biofluids and is abundantly expressed in ELF, where it is involved in facilitating sperm uptake of GPI-linked proteins, as well as their removal during the modification of the membrane, depending on its concentration in the local environment in the epididymal tract.[25] A lipid-exchange model involving CLU or other lipid carriers (**Figure 3**) has been proposed for the delivery of these proteins to the sperm surface.[24] Other fertility-modulating GPI-linked epididymal proteins that are likely to be delivered by this pathway include: GLIPR1L1 (Glioma pathogenesis-related protein 1), which is involved in sperm-zona pellucida binding,[26–27] other hyaluronidase family members, such as HYAL3,[28] HYAL5[23] and HYAL2,[29] membrane-anchored serine protease PRSS21 (testisin)[30] and P34H/P26h/P25b.[18,31]

When the mechanism of delivery of GPI-linked epididymal proteins via the vesicular pathway was studied following co-incubation of murine caudal sperm with dye–labeled epididymosomes, the label was detected over the acrosome and on the midpiece of the flagellum,[23] which are regions where CD9 positive epididymosomes have been shown to bind.[32] Further, these locations coincide with the localization of SPAM1 and other hyaluronidases,[25] and are lipid rafts domain.[23] The data obtained from that study led to the conclusion that vesicular docking on the spermatozoa, followed by hydrophobic interactions between the GPI anchor and the outer leaflet of the lipid bilayer of the membrane, is the mechanism for vesicle-mediated GPI-linked protein transfer.[23] Thus for both the nonvesicular and the vesicular fractions of the ELF, hydrophobic interactions were reported to mediate the delivery of GPI-linked proteins (**Figure 2**). However, it is likely that vesicular docking may precede vesicle fusion since in the image displayed by Griffith's *et al.*[23] there was evidence for membrane fusion (**Figure 4**).

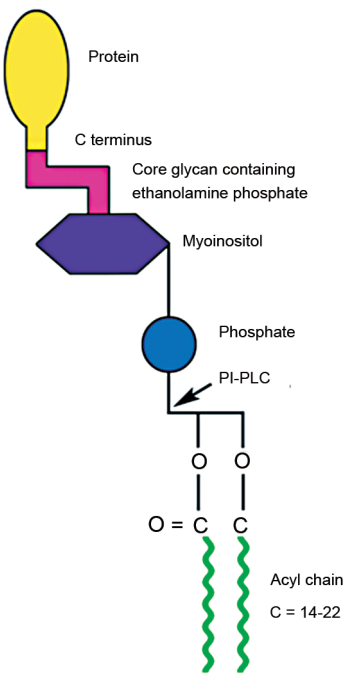

Figure 1: Diagram of glycosyl phosphatidylinositol (GPI)-linked protein showing the acyl chain, which anchors it in the outer leaflet of the lipid bilayer of a target membrane. The arrow points to the position where the phosphatidylinositol link can be enzymatically cleaved with phospholipoase C.

TRANSMEMBRANE AND MEMBRANE-ASSOCIATED PROTEINS IDENTIFIED IN EPIDIDYMOSOMES FROM A PROTEOMIC APPROACH

When the proteome of human epididymosomes was studied, the 146 proteins identified covered a large molecular weight spectrum and were of different functional categories, including enzymes, adhesion molecules, transporters, and signaling competent proteins.[33] In the case of bovine epididymosomes, a comparison of those from the caput (proximal) and cauda (distal) epididymidis showed unique compositions for the lipid and proteome profiles: for the latter, 324 of 555 and 207 of 438 proteins were respectively different in the two regions.[34] The wide variety of protein categories in bovine EP include those involved in sperm-egg interaction or motility, EP genesis/secretion or EP-sperm interaction, remodeling of the epididymal sperm components, and those potentially involved in sperm protection or elimination.[34] Importantly, among these proteins there are transmembrane proteins, which are unlikely to be delivered to the sperm surface via hydrophobic interactions. Sullivan and Saez have proposed, from the complexity of proteins that epididymosomes carry and deliver to spermatozoa, multiple mechanisms of transfer are likely to be involved.[35]

TRANSMEMBRANE AND MEMBRANE-ASSOCIATED PROTEINS IDENTIFIED IN EPIDIDYMOSOMES FROM A HYPOTHESIS – DRIVEN APPROACH

The Plasma membrane calcium ATPase 4 (PMCA4), with variants 4a and 4b, is a 10-pass transmembrane protein. It is the major calcium efflux pump in murine sperm[36] in which deletion of its encoding gene leads to loss of motility and male infertility.[37,38] This essential sperm protein was shown to be synthesized in the testis and epididymal epithelia of rat[39] and bulls[40] where spermatozoa show a progressive shift from splice variant 4b in the upper epididymal (caput) tract to

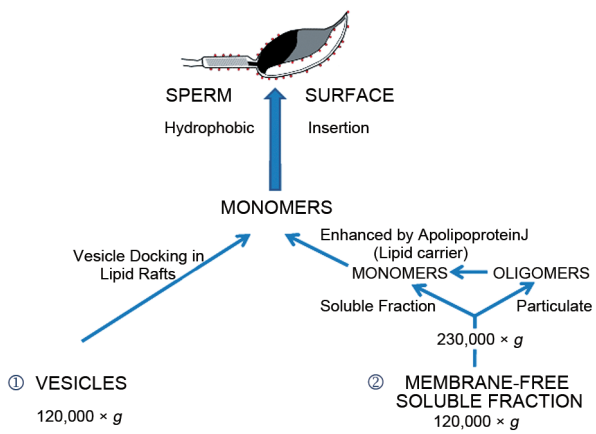

Figure 2: Dual pathways exist for GPI-linked protein delivery to the sperm plasma membrane, via the vesicular and membrane-free soluble fractions of ELF. These fractions are separated by ultracentrifugation at 120 000 × *g* and the supernatant can be fractionated into oligomers and monomers at 230 000 × *g*. The latter are inserted into the sperm plasma membrane in the presence of CLU or Apolipoprotein J via hydrophobic interactions which may also facilitate delivery from vesicles that dock in lipid rafts on the sperm membrane.[23]

mainly 4a in the lower region (caudal).[40] This shift was considered to reflect the acquisition of PMCA4a from the ELF.[40] As the PMCA4a variant is more efficient than 4b in clearing calcium from the cytosol, the presence of increasing amounts of 4a in maturing sperm cells would ensure that they would meet the high demand for calcium efflux after hyperactivated motility, capacitation, and the acrosome reaction, which all require elevated levels of calcium.[41,42] Thus, it was hypothesized that in murine spermatozoa the PMCA4a variant is expressed in the ELF and carried on epididymosomes where it can be delivered to sperm cells during their maturation and transit.[43] Experimental evidence revealed the presence of PMCA4a exclusively in the epididymosomal fraction of the ELF.[43] The findings also demonstrated that the bovine and murine systems differ, since in the latter both PMCA4a and 4b variants are expressed in the testis, the apical surface of the epithelia of all three epididymal regions, as well as in their secreted ELF.[43] It should be noted that while PMCA4a is more efficient than 4b in returning Ca^{2+} to resting levels,[40,44] 4b plays an important role in signal transduction via its C-terminal PDZ ligand.[45,46]

Importantly, murine epididymosomes were able to deliver PMCA4a to caudal spermatozoa following co-incubation *in vitro*, consistent with its transfer *in vivo* which was reflected in a five-fold increase on caudal, compared with caput, sperm cells.[43] This finding, along with the higher Ca^{2+}-ATPase activity in bovine caudal spermatozoa than those in the caput,[47] supports a role of PMCA4a in epididymal sperm maturation. Acquisition of additional PMCA4a in caudal sperm cells parallels their gain in motility and their cytosolic Ca^{2+} concentration, which is 2- to 6-times lower than that in caput spermatozoa.[48] As motility is lost in mature caudal *Pmca4*-null murine spermatozoa where the intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ is significantly elevated,[37,38] physiological immotility of wild-type caput spermatozoa in the presence of increased $[Ca^{2+}]_i$ compared with caudal ones underscores the role of epididymosomal PMCA4 in sperm maturation. It should be noted that both PMCA4a and 4b variants are present on epididymosomes and are co-immunoprecipitated with an interacting partner of PMCA4b, Ca^{2+}/CaM-dependent serine kinase (CASK).[43] This interaction is PDZ domain-mediated, and in murine sperm cells involves the PDZ motif of CASK and PMCA4b's PDZ ligand[49] which is absent from PMCA4a.[50]

Thus, the ability to co-immunoprecipitate both Ca^{2+} pump variants with CASK in the epididymosomal cargo revealed for the first time that the variants work together in a multiprotein complex, to heighten their combined impact in meeting the demands following functional sperm activities that precede fertilization.[43] The inclusion of PMCA4a in a complex with CASK in the absence a PDZ ligand was thought to be facilitated by the formation of a heterodimer between 4a and 4b, with the latter directly interacting with CASK.[43] The co-immunoprecipitation data indicated that CASK, a scaffolding membrane-associated protein that also exists in a soluble form,[51] is a component of the epididymosomal cargo and is likely to be delivered to the sperm surface during epididymal maturation along with PMCA4b when PMCA4a is also transferred. The finding that CASK is an epididymosomal protein is supported by an early study showing its presence in epididymal tissues.[52]

The above demonstrates how knowledge of an interacting partner of a sperm protein that is present in epididymosomes can lead to the identification of other proteins in the epididymosomal cargo. Aravindan *et al.* showed that in murine sperm PMCA4b and junctional adhesion molecule A (JAM-A), which also has a PDZ ligand, are common interacting partners of CASK.[49] Since CASK has a single PDZ domain, PMCA4b and JAM-A must bind sequentially and not simultaneously. As the PMCA4b-CASK interaction was shown to occur preferentially in uncapacitated spermatozoa when the $[Ca^{2+}]_i$ is relatively low,[49] it is likely that $[Ca^{2+}]_i$ also dictates preferential binding of the JAM-A-CASK complex. However, regardless of the condition for binding, the existence of a JAM-A-CASK complex in spermatozoa and the presence of CASK in epididymosomes lead to the prediction that JAM-A is present in murine epididymosomes. Studies to investigate the latter are in progress.

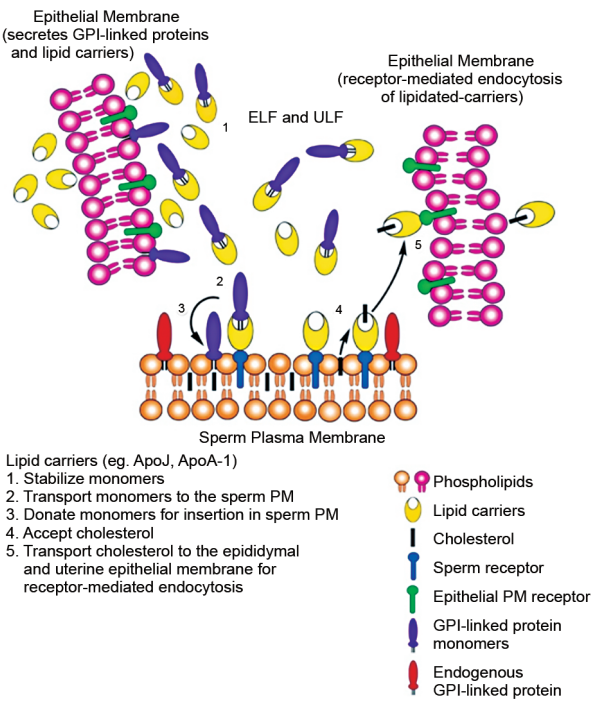

Figure 3: A lipid exchange model for the delivery of GPI-linked proteins from the soluble fraction of ELF is shown. GPI-linked proteins are stabilized by lipid carriers such as CLU or Apolipoprotein J, which transports monomers to the sperm membrane and then acts as an acceptor for cholesterol which it transports to the epididymal epithelial lining for receptor-mediated endocytosis. Taken from *Biol Reprod* 2009; 81: 562–70.[24]

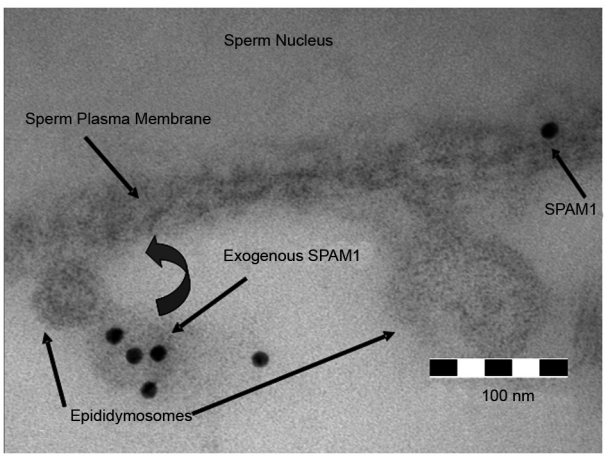

Figure 4: Transmission electron microscopy reveals docking of epididymosomes on the sperm plasma membrane to allow delivery of GPI-linked proteins to the sperm surface via hydrophobic interactions.[23] On the smaller epididymosome SPAM1 which is immunogold- labeled is seen in the process of being transferred to the sperm membrane, while the contact of the larger epididymosomes appears to involve membrane fusion. Taken from *Mol Reprod Dev* 2008; 75: 1627–36.[23]

Since PMCA4b binds to CASK preferentially at low $[Ca^{2+}]_i$,[49] it is useful to ask what is/are PMCA4's interacting partner/s at high $[Ca^{2+}]_i$ in spermatozoa. From what is known of PMCA4's interaction in endothelial and neuronal cells, where PMCA4 has been reported to regulate negatively both endothelial nitric oxide synthase (eNOS)[53] and neuronal nitric oxide synthase (nNOS),[54,55] it can be predicted that these interactions are also present in sperm proteins. Importantly both NOSs, which are rapidly activated by $[Ca^{2+}]_i$,[54,56] are present in spermatozoa where they are responsible for the production of nitric oxide (NO), which is required for a variety of sperm functions.[57] Since excess NO has deleterious effects on spermatozoa,[58] PMCA4's interaction with the NOSs to regulate them negatively would prevent oxidative stress, which is known to affect sperm motility[58,59] as well as the integrity of the sperm genome.[59] In light of this and the finding that in humans eNOS is expressed in the testis, spermatozoa and epididymis,[60] it can be expected that these membrane-associated NOSs, as well as Caveolin-1 (CAV-1), a scaffold protein with which eNOS interacts,[61] are potential epididymosomal proteins that will be transferred to sperm cells along with PMCA4.

From the regulatory relationship between PMCA4 and the NOSs, it would seem advantageous that these proteins be transferred together. This would be similar to the detected PMCA4a-PMCA4b-CASK complex that was co-immunoprecipitated from epididymosomes,[43] indicating that the proteins are likely to be transferred as a complex. A list of transmembrane and membrane-associated proteins identified in EP or potentially present in their cargo, on the basis of a hypothesis-driven approach is seen in **Table 1**. The list includes PMCA1a, and b, which are murine sperm proteins[37] and which have identical partners as PMCA4a, and 4b, and which the Martin-DeLeon Lab has detected in ELF and shown to be delivered to spermatozoa (unpublished data). Interestingly, none of these proteins appears in the list identified from the proteomic approach for human and bovine epididymosomes. This is not surprising for PMCA4, which is very low in abundance, accounting for only 0.01%–0.1% of all membrane proteins.[46] Thus the hypothesis-driven approach, which is based on knowledge of the functional role and the interacting partners of the proteins, might be useful to detect the presence of low-in-abundance membrane or membrane-associated proteins when they exist in the epididymosomal cargo.

How are these transmembrane and membrane-associated epididymosomal protein complexes transferred to the sperm surface? Schwarz *et al.*[62] analyzed the fusogenic properties of bovine epididymosomes and their involvement in the transfer of PMCA4, among other molecules, to bovine spermatozoa. Using labeled epididymosomes in co-incubation experiments, they provided evidence for a fusogenic mechanism for the delivery PMCA4. More recently, studies on oviductal microvesicles/exosomes also provided support for a fusogenic pathway in the delivery PMCA4 and other transmembrane proteins to murine spermatozoa.[63] From the use of a lipophilic dye for the exosomes/microvesicles and three-dimensional super-resolution structured illumination microscopy, sperm-EP fusion was detectable and co-localized with immunolabeled PMCA4a.[63]

Membrane fusion is not only an effective mechanism for the delivery of transmembrane and membrane-associated proteins and their complexes, but should also mediate the delivery of GPI-linked proteins from epididymosomes. Thus the docking of epididymosomes that was detected by the delivery of SPAM1[23] is a step that precedes fusion. As CD9 tetraspanin has been implicated in membrane fusion, EP-sperm fusion appears likely to be mediated via CD9, which is a biochemical marker of exosomes and an adhesion molecule that generates fusion-competent sites.[64–66] Consistent with this is the finding that CD9 has been detected on the murine sperm membrane over the acrosome and on the midpiece,[67] and that CD9-positive microvesicles that fuse to the sperm membrane at these regions have been shown to transfer molecules to maturing live bovine spermatozoa in a tissue-specific manner.[32] Further, with the use of function-blocking antibodies for CD9 there was a significant decrease in protein delivery to sperm cells,[32] providing evidence for CD9-mediated fusion in cargo delivery of epididymosomes.

Table 1: Fertility-modulating proteins identified, or *predicted to be present, in epididymosomal cargo and transferred to sperm in a complex

Number	*Protein*	*Abbreviation*	*Location*	*Role*
1	Plasma membrane Ca^{2+}-ATPase 4a	PMCA4a	Membrane	Motility
2	Plasma membrane Ca^{2+}-ATPase 4b	PMCA4b	Membrane	Motility
3	Plasma membrane Ca^{2+}-ATPase 1a	PMCA1a	Membrane	Motility
4	Plasma membrane Ca^{2+}-ATPase 1b	PMCA1b	Membrane	Motility
5*	Neuronal nitric oxide synthase	nNOS	Membrane-associated	Motility
6*	Endothelial nitric oxide synthase	eNOS	Membrane-associated	Motility
7	Ca^{2+}/CaM-dependent serine kinase	CASK	Membrane-associated	Motility
8*	Junctional adhesion molecules-A	JAM-A	Membrane	Motility
9*	Caveolin-1	CAV-1	Membrane	eNOS regulation

All are associated with Ca^{2+} signaling

CONCLUSION

A variety of sperm proteins that are expressed in the testis are also expressed in the epithelia of the epididymis, where they are secreted into the luminal fluid and delivered to the sperm surface. Fertility-modulating proteins in the secretome may be GPI-linked, transmembrane or membrane-associated. Epididymosomes, membrane vesicles which may be exosomes or microvesicles, serve as the vehicle for the transfer of all three classes of proteins to the sperm surface, while GPI-linked proteins can also be transferred from the soluble membrane-free fraction of the ELF. This fraction exists in both oligomeric and monomeric forms, with protein transfer occurring primarily from the latter while the former serves as a source for monomers. Transfer from monomers is dependent on clusterin (CLU), a lipid carrier which stabilizes GPI monomers and delivers them to the sperm membrane via hydrophobic interactions. Epididymosomes fuse with the sperm membrane in delivering their cargo in a CD9-dependent manner, and transmembrane and membrane-associated proteins in an interactome are likely to be delivered in a complex. Further work is needed to determine the presence of the proteins predicted to reside in the epididymosomal cargo and their transfer to spermatozoa.

ACKNOWLEDGMENTS

The work was supported by NIH-1RO3 HD073523-01.

COMPETING INTEREST

The author declares that there are no competing interests.

REFERENCES

1 Cooper TG. Role of the epididymis in mediating changes in the male gamete during maturation. *Adv Exp Med Biol* 1995; 377: 87–101.
2 Jones R. Membrane remodelling during sperm maturation in the epididymis. *Oxf Rev Reprod Biol* 1989; 11: 285–337.

3 Robaire B, Hinton BT, Orgebin-Crist M. The epididymis. In: Neill J, Plant T, Pfaff D, Challis J, Kretser D, editors. Knobil and Neill's Physiology of Reproduction. New York: Raven Press; 2006. p. 1071–148.
4 Zhang H, Jones R, Martin-DeLeon PA. Expression and secretion of rat SPAM1 (2B1 or PH-20) in the epididymis: role of testicular lumicrine factors. *Matrix Biol* 2004; 22: 653–61.
5 Zhang H, Martin-DeLeon PA. Mouse epididymal Spam1 (pH-20) is released in the luminal fluid with its lipid anchor. *J Androl* 2003; 24: 51–8.
6 Zhang H, Martin-DeLeon PA. Mouse epididymal Spam1 (PH-20) is released *in vivo* and *in vitro*, and Spam1 is differentially regulated in testis and epididymis. *Biol Reprod* 2001; 65: 1586–93.
7 Cooper TG. Interactions between epididymal secretions and spermatozoa. *J Reprod Fertil Suppl* 1998; 53: 119–36.
8 Kirchhoff C, Pera I, Derr P, Yeung CH, Cooper T. The molecular biology of the sperm surface. Post-testicular membrane remodelling. *Adv Exp Med Biol* 1997; 424: 221–32.
9 Kirchhoff C, Hale G. Cell-to-cell transfer of glycosylphosphatidylinositol-anchored membrane proteins during sperm maturation. *Mol Hum Reprod* 1996; 2: 177–84.
10 Yeung CH, Schröter S, Wagenfeld A, Kirchhoff C, Kliesch S, *et al.* Interaction of the human epididymal protein CD52 (HE5) with epididymal spermatozoa from men and cynomolgus monkeys. *Mol Reprod Dev* 1997; 48: 267–75.
11 Sullivan R, Légaré C, Villeneuve M, Foliguet B, Bissonnette F. Levels of P34H, a sperm protein of epididymal origin, as a predictor of conventional *in vitro* fertilization outcome. *Fertil Steril* 2006; 85: 1557–9.
12 Moskovtsev SI, Jarvi K, Légaré C, Sullivan R, Mullen JB. Epididymal P34H protein deficiency in men evaluated for infertility. *Fertil Steril* 2007; 88: 1455–7.
13 Chen H, Griffiths G, Galileo DS, Martin-DeLeon PA. Epididymal SPAM1 is a marker for sperm maturation in the mouse. *Biol Reprod* 2006; 74: 923–30.
14 Fornés WM, Sosa MA, Bertini F, Burgos MH. Vesicles in rat epididymal fluid. Existence of two populations differing in ultrastructure and enzymatic composition. *Andrologia* 1995; 27: 233–7.
15 Cohen DJ, Rochwerger L, Ellerman DA, Morgenfeld MM, Busso D, *et al.* Relationship between the association of rat epididymal protein "DE" with spermatozoa and the behavior and function of the protein. *Mol Reprod Dev* 2000; 56: 180–8.
16 Sullivan R, Saez F, Girouard J, Frenette G. Role of exosomes in sperm maturation during the transit along the male reproductive tract. *Blood Cells Mol Dis* 2005; 35: 1–10.
17 Saez F, Frenette G, Sullivan R. Epididymosomes and prostasomes: their roles in posttesticular maturation of the sperm cells. *J Androl* 2003; 24: 149–54.
18 Frenette G, Sullivan R. Prostasome-like particles are involved in the transfer of P25b from the bovine epididymal fluid to the sperm surface. *Mol Reprod Dev* 2001; 59: 115–21.
19 Sloand EM, Maciejewski JP, Dunn D, Moss J, Brewer B, *et al.* Correction of the PNH defect by GPI-anchored protein transfer. *Blood* 1998; 92: 4439–45.
20 Rooney IA, Heuser JE, Atkinson JP. GPI-anchored complement regulatory proteins in seminal plasma. An analysis of their physical condition and the mechanisms of their binding to exogenous cells. *J Clin Invest* 1996; 97: 1675–86.
21 Griffiths GS, Miller KA, Galileo DS, Martin-DeLeon PA. SPAM1 is secreted by the estrous murine uterus and oviduct in a form which can bind to sperm during capacitation: acquisition enhances hyaluronic acid-binding ability and cumulus penetration efficiency. *Reproduction* 2008; 135: 293–301.
22 Martin-DeLeon PA. Epididymal SPAM1 and its impact on sperm function. *Mol Cell Endocrinol* 2006; 250: 114–21.
23 Griffiths GS, Galileo DS, Reese K, Martin-DeLeon PA. Investigating the role of murine epididymosomes and uterosomes in GPI-linked protein transfer to sperm using SPAM1 as a model. *Mol Reprod Dev* 2008; 75: 1627–36.
24 Griffiths GS, Galileo DS, Aravindan RG, Martin-DeLeon PA. Clusterin facilitates exchange of glycosyl phosphatidylinositol-linked SPAM1 between reproductive luminal fluids and mouse and human sperm membranes. *Biol Reprod* 2009; 81: 562–70.
25 Martin-DeLeon PA. Germ-cell hyaluronidases: their roles in sperm function. *Int J Androl* 2011; 34 (5 Pt 2): e306–18.
26 Gibbs GM, Lo JC, Nixon B, Jamsai D, O'Connor, *et al.* Glioma pathogenesis-related 1-like 1 is testis enriched, dynamically modified, and redistributed during male germ cell maturation and has a potential role in sperm-oocyte binding. *Endocrinology* 2012; 151: 2331–42.
27 Caballero J, Frenette G, D'Amours O, Belleannee C, Lacroix-Pepin N, *et al.* Bovine sperm raft membrane associated glimoa pathogenesis-related 1-like protein 1 (GliPr1L1) is modified during the epididymal transit and is potentially involved in sperm binding to the zona pellucida. *J Cell Physiol* 2012; 227: 3876–86.
28 Reese KL, Aravindan RG, Griffiths GS, Shao M, Wang Y, *et al.* Acidic hyaluronidase activity is present in mouse sperm and is reduced in the absence of SPAM1: evidence for a role for hyaluronidase 3 in mouse and human sperm. *Mol Reprod Dev* 2010; 77: 759–72.
29 Modelski MJ, Menlah G, Wang Y, Dash S, Wu K, *et al.* Hyaluronidase 2: a novel germ cell hyaluronidase with epididymal expression and functional roles in mammalian sperm. *Biol Reprod* 2014; 91: 109–20.
30 Netzel-Arnett S, Bugge TH, Hess RA, Carnes K, Stringer BW, *et al.* The glycosylphosphatidylinositol-anchored serine protease PRSS21 (testisin) imparts murine epididymal sperm cell maturation and fertilizing ability. *Biol Reprod* 2009; 81: 921–32.
31 Légaré C, Bérubé B, Boué F, Lefièvre L, Morales CR, *et al.* Hamster sperm antigen P26h is a phosphatidylinositol-anchored protein. *Mol Reprod Dev* 1999; 52: 225–33.
32 Caballero JN, Frenette G, Belleannée C, Sullivan R. CD9-positive microvesicles mediate the transfer of molecules to Bovine Spermatozoa during epididymal maturation. *PLoS One* 2013; 8: e65364.
33 Thimon V, Frenette G, Saez F, Thabet M, Sullivan R. Protein composition of human epididymosomes collected during surgical vasectomy reversal: a proteomic and genomic approach. *Hum Reprod* 2008; 23: 1698–707.
34 Girouard J, Frenette G, Sullivan R. Comparative proteome and lipid profiles of bovine epididymosomes collected in the intraluminal compartment of the caput and cauda epididymidis. *Int J Androl* 2011; 34: e475–86.
35 Sullivan R, Saez F. Epididymosomes, prostasomes, and liposomes: their roles in mammalian male reproductive physiology. *Reproduction* 2013; 146: R21–35.
36 Wennemuth G, Babcock DF, Hille B. Calcium clearance mechanisms of mouse sperm. *J Gen Physiol* 2003; 122: 115–28.
37 Okunade GW, Miller ML, Pyne GJ, Sutliff RL, O'Connor KT, *et al.* Targeted ablation of plasma membrane Ca^{2+}-ATPase (PMCA) 1 and 4 indicates a major housekeeping function for PMCA1 and a critical role in hyperactivated sperm motility and male fertility for PMCA4. *J Biol Chem* 2004; 279: 33742–50.
38 Schuh K, Cartwright EJ, Jankevics E, Bundschu K, Liebermann J, *et al.* Plasma membrane Ca^{2+} ATPase 4 is required for sperm motility and male fertility. *J Biol Chem* 2004; 279: 28220–6.
39 Wilhelm B, Brandenburger T, Post H, Aumüller G. Expression and localization of PMCA4 in rat testis and epididymis. *Histochem Cell Biol* 2008; 129: 331–43.
40 Brandenburger T, Strehler EE, Filoteo AG, Caride AJ, Aumüller G, *et al.* Switch of PMCA4 splice variants in bovine epididymis results in altered isoform expression during functional sperm maturation. *J Biol Chem* 2011; 286: 7938–46.
41 Xia J, Reigada D, Mitchell CH, Ren D. CATSPER channel-mediated Ca^{2+} entry into mouse sperm triggers a tail-to-head propagation. *Biol Reprod* 2007; 77: 551–9.
42 Fraser LR. Ca+ requirements for capacitation and acrosomal exocytosis in mammalian sperm. *Int Rev Cytol* 1994; 149: 1–46.
43 Patel R, Al-Dossary AA, Stabley DL, Barone C, Galileo DS, *et al.* Plasma membrane Ca^{2+} ATPase 4 in murine epididymis: secretion of splice variants in the luminal fluid and a role in sperm maturation. *Biol Reprod* 2013; 89: 1–11.
44 Caride AJ, Filoteo AG, Penniston JT, Strehler EE. The plasma membrane Ca^{2+} pump isoform 4a differs from isoform 4b in the mechanism of calmodulin binding and activation kinetics: implications for Ca^{2+} signaling. *J Biol Chem* 2007; 282: 25640–8.
45 DeMarco SJ, Strehler EE. Plasma membrane Ca^{2+}-ATPase isoforms 2b and 4b interact promiscuously and selectively with members of the membrane-associated guanylate kinase family of PDZ (PSD95/Dlg/ZO-1) domain-containing proteins. *J Biol Chem* 2001; 276: 21594–600.
46 Di Leva F, Domi T, Fedrizzi L, Lim D, Carafoli E. The plasma membrane Ca^{2+} ATPase of animal cells: structure, function and regulation. *Arch Biochem Biophys* 2008; 476: 65–74.
47 Sánchez-Luengo S, Aumüller G, Albrecht M, Sen PC, Röhm K, *et al.* Interaction of PDC-109, the major secretory protein from bull seminal vesicles, with bovine sperm membrane Ca^{2+}-ATPase. *J Androl* 2004; 25: 234–44.
48 Yeung CH, Cooper TG. Acquisition and development of sperm motility upon maturation in the epididymis. In: Robaire B, Hinton BT, editors. The Epididymis: From Molecules to Clinical Practice. New York: Kluwer Academic/Plenum Publishers; 2002. p. 417–34.
49 Aravindan RG, Fomin VP, Naik UP, Modelski MJ, Naik MU, *et al.* CASK interacts with PMCA4b and JAM-A on the mouse sperm flagellum to regulate Ca^{2+} homeostasis and motility. *J Cell Physiol* 2012; 227: 3138–50.
50 Keeton TP, Shull GE. Primary structure of rat plasma membrane $Ca^{(2+)}$-ATPase isoform 4 and analysis of alternative splicing patterns at splice site A. *Biochem J* 1995; 306 (Pt 3): 779–85.
51 Funke L, Dakoji S, Bredt DS. Membrane-associated guanylate kinases regulate adhesion and plasticity at cell junctions. *Annu Rev Biochem* 2005; 74: 219–45.
52 Burkin HR, Zhao L, Miller DJ. CASK is in the mammalian sperm head and is processed during epididymal maturation. *Mol Reprod Dev* 2004; 68: 500–6.
53 Holton M, Mohamed TM, Oceandy D, Wang W, Lamas S, *et al.* Endothelial nitric oxide synthase activity is inhibited by the plasma membrane calcium ATPase in human endothelial cells. *Cardiovasc Res* 2010; 87: 440–8.
54 Schuh K, Uldrijan A, Telkamp M, Rothlein N, Neyses L. The plasma membrane calmodulin-dependent calcium pump: a major regulator of nitric oxide synthase 1. *J Cell Biol* 2001; 273: 18693–6.
55 Cartwright EJ, Oceandy D, Neyses L. Physiological implications of the interaction between the plasma membrane calcium pump and nNOS. *Pflugers Arch* 2009; 457: 665–71.
56 Sessa WC, Harrison JK, Luthin DR, Pollock JS, Lynch KR. Genomic analysis and expression patterns reveal distinct genes for endothelial and brain nitric oxide synthase. *Hypertension* 1993; 21 (6 Pt 2): 934–8.

57 Herrero MB, Gagnon C. Nitric oxide: a novel mediator of sperm function. *J Androl* 2001; 22: 349–56.
58 Ramya T, Misro MM, Sinha D, Nandan D, Mithal S. Altered levels of seminal nitric oxide, nitric oxide synthase, and enzymatic antioxidants and their association with sperm function in infertile subjects. *Fertil Steril* 2011; 95: 135–40.
59 Aitken RJ, Smith TB, Jobling MS, Baker MA, De Iuliis GN. Oxidative stress and male reproductive health. *Asian J Androl* 2014; 16: 31–8.
60 Zini A, O'Bryan MK, Magid MS, Schlegel PN. Immunohistochemical localization of endothelial nitric oxide synthase in human testis, epididymis, and vas deferens suggests a possible role for nitric oxide in spermatogenesis, sperm maturation, and programmed cell death. *Biol Reprod* 1996; 55: 935–41.
61 Dhillon B, Badiwala MV, Li SH, Li RK, Weisel RD, *et al.* Caveolin: a key target for modulating nitric oxide availability in health and disease. *Mol Cell Biochem* 2003; 247: 101–9.
62 Schwarz A, Wennemuth G, Post H, Brandenburger T, Aumüller G, *et al.* Vesicular transfer of membrane components to bovine epididymal spermatozoa. *Cell Tissue Res* 2013; 353: 549–61.
63 Al-Dossary AA, Caplan J, Martin-DeLeon, PA. The contribution of exosomes/microvesicles to the sperm proteome. *Mol Reprod Dev* 2015; 82: 79.
64 Théry C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. *Nat Rev Immunol* 2002; 2: 569–79.
65 Théry C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. *Nat Rev Immunol* 2009; 9: 581–93.
66 Hemler ME. Tetraspanin proteins mediate cellular penetration, invasion, and fusion events and define a novel type of membrane microdomain. *Annu Rev Cell Dev Biol* 2003; 19: 397–422.
67 Al-Dossary AA, Martin-DeLeon PA. JAM-A-CASK Complex Interacts with CD9 Tetraspanin and αv Integrin to Mediate Ca^{2+} Signaling in Capacitation and the Acrosome-Reaction in Murine Sperm. 39th Annual Meeting of the Am Soc Andrology, Atlanta, Georgia, April 4th-8th, 2014.