thematic review series

Thematic Review Series: Glycosylphosphatidylinositol (GPI) Anchors: Biochemistry and Cell Biology

GPI-AP release in cellular, developmental, and reproductive biology

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Abstract Glycosylphosphatidylinositol-anchored proteins (GPI-APs) contain a covalently linked GPI anchor located on outer cell membranes. GPI-APs are ubiquitously conserved from protozoa to vertebrates and are critical for physiological events such as development, immunity, and neurogenesis in vertebrates. Both membrane-anchored and soluble GPI-APs play a role in regulating their protein conformation and functional properties. Several pathways mediate the release of GPI-APs from the plasma membrane by vesiculation or cleavage. Phospholipases and putative substrate-specific GPI-AP-releasing enzymes, such as NOTUM, glycerophosphodiesterase 2, and angiotensin-converting enzyme, have been characterized in mammals. Here, the protein modifications resulting from the cleavage of the GPI anchor are discussed in the context of its physiological functions.—Fujihara, Y., and M. Ikawa. **GPI-AP release in cellular, developmental, and reproductive biology.** ***J. Lipid Res.*** **2016.** 57: **538–545.**

Supplementary key words genetics • gene expression • membranes • phospholipases • phospholipids/phosphatidylinositol • glypican • GPIase • IZUMO1 receptor/JUNO • reversion-inducing cysteine-rich protein with kazal motifs • testis-expressed gene 101 • glycosylphosphatidylinositol-anchored protein

Eukaryotic organisms produce many membrane proteins that are anchored to the outer cell membrane by glycosylphosphatidylinositol (GPI). GPI-anchored proteins (GPI-APs) have unique structures that contain mannose, glucosamine, ethanolamine, and phosphatidylinositol (1). Although they have similar core backbone structures, GPI-APs are a functionally diverse family of molecules that includes hydrolytic enzymes, adhesion molecules, receptors, protease inhibitors, and complement regulatory proteins (2). GPI-APs are released from the cell surface by bacterial phosphatidylinositol-specific phospholipase C (PI-PLC) (3). In mammals, GPI-APs are released and taken up by both vesiculation-mediated and non-vesiculation-mediated mechanisms (4). Several putative substrate-specific enzymes release GPI-APs from the cell surface (5–8). Thus, GPI-anchor release may be required for different biological functions and regulatory mechanisms. In this review, we will focus on the significance of GPI-AP release in cellular, developmental, and reproductive biology (**Table 1**).

GPI-ANCHOR-RELEASING ENZYME, GPLD1/GPI-PLD, TARGETED FOR VARIOUS SUBSTRATES

A unique feature of GPI-APs is their cleavage by specific phospholipases, resulting in the release of the protein from the cell membrane. Among GPI-specific phospholipases, the only purified and characterized mammalian phospholipase is a GPI-specific phospholipase D (GPI-PLD), GPLD1, which is abundant in serum (9–11). GPLD1 is a soluble protein with two functional domains, an N-terminal catalytic domain and a predicted C-terminal β-propeller domain (12, 13). GPLD1 hydrolyzes GPI anchors that have acylated inositol, unlike GPI anchors cleaved by PI-PLC and GPI-PLC

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Abbreviations: ACE, angiotensin-converting enzyme; ADAM3, a disintegrin and metallopeptidase domain 3; CEA, carcinoembryonic antigen; CEACAM, carcinoembryonic antigen-related cell-adhesion molecule; DALLY, division abnormally delayed; DLL1, Delta-like 1; DLP, division abnormally delayed-like protein; GDE, glycerophosphodiesterase; GDPD, glycerophosphodiester phosphodiesterase; GPC, glypican; GPI, glycosylphosphatidylinositol; GPI-AP, glycosylphosphatidylinositol-anchored protein; GPIase, glycosylphosphatidylinositol-anchored protein-releasing; GPI-PLC, glycosylphosphatidylinositol-specific phospholipase C; GPI-PLD, glycosylphosphatidylinositol-specific phospholipase D; IZUMO1R, IZUMO1 receptor; LY6K, lymphocyte antigen 6 complex locus K; PGAP1, post-GPI attachment to proteins 1; PI-PLC, phosphatidylinositol-specific phospholipase C; PLAU, plasminogen activator urokinase; PLAUR, PLAU receptor; PRSS, protease serine; RECK, reversion-inducing cysteine-rich protein with kazal motifs; SPAM1, sperm-adhesion molecule 1; TDGF1, teratocarcinoma-derived growth factor 1; TEX101, testis-expressed gene 101; ZP, zona pellucida.

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TABLE 1. GPI-APs and GPIase factors involved in cleavage of the GPI anchor from the cell surface

GPI-APs	GPI-AP Releasing Factors	Domain Responsible for GPIase Activity	Phenotype of GPI-AP KO Mice	References
PLUAR/uPAR	GPLD1/GPI-PLD	Catalytic domain	Mild phenotype	(18, 19)
CEACAM5/CEA	GPLD1/GPI-PLD	Catalytic domain	Not determined[a]	(25)
PRSS8/prostasin	GPLD1/GPI-PLD	Catalytic domain	Embryonic lethality	(29, 31)
TDGF1/CRIPTO-1	GPLD1/GPI-PLD	Catalytic domain	Embryonic lethality	(37, 38)
GPC3 (glypicans)	NOTUM	Not determined[a]	Postnatal lethality	(44, 58)
RECK	GDPD5/GDE2	GDPD domain	Embryonic lethality	(5, 67)
TEX101	ACE	Not determined[a]	Male infertility	(6, 7)
IZUMO1R/JUNO	Not determined[a]	Not determined[a]	Female infertility	(104)

[a]Has not been reported.

(3). The catalytic activity of mouse GPLD1 depends on histidines at positions 29, 125, 133, and 158 in the catalytic site (14). Membrane-bound GPI-AP is released from the cell surface by GPLD1 in several important cellular processes, including adhesion, differentiation, proliferation, survival, and oncogenesis. Although the physiological role of GPLD1 is unknown, GPLD1 substrates have been identified, and their roles have been investigated in gene KO mice (Table 1).

PLASMINOGEN ACTIVATOR UROKINASE RECEPTOR

Plasminogen activator urokinase receptor (PLAUR, also known as CD87 and uPAR) is a GPI-AP receptor that binds to plasminogen activator urokinase (PLAU, also known as uPA) (15). PLUAR was identified as a multifunctional cell-surface receptor that regulates cellular differentiation, proliferation, migration, adhesion, and invasion (15, 16). When bound to a PLAU, PLAUR functions as a signaling receptor that converts plasminogen into plasmin, which upon activation triggers an extracellular proteolysis cascade (17). PLAUR also interacts nonproteolytically with vitronectin, an interaction that induces the migration of various cell types. PLAUR activity is regulated by receptor shedding and cleavage. Receptor shedding is mediated by cleavage close to the GPI anchor by GPLD1 and several proteases (18), whereas receptor cleavages release the N-terminal ligand binding domain (D1) from the other domains (D2D3) (15). These modifications are independent, and the resulting forms of PLAUR have different biological activities. Although minor phenotypes were observed in *Pluar* KO mice, PLUAR was dispensable for development and fertility in mice (19).

CARCINOEMBRYONIC ANTIGEN AND RELATED ADHESION MOLECULES

Carcinoembryonic antigen (CEA), first described in 1965, is a highly glycosylated immunoglobulin-related protein whose serum level increases in some types of cancer (20, 21). CEA-related cell-adhesion molecules (CEACAMs) have been implicated in numerous physiological and pathological functions (22). In humans, there are three major CEACAMs on epithelial tissues: one transmembrane type (CEACAM1) and two GPI-anchored types (CEACAM5 and CEACAM6) (23). Because *Ceacam1* KO mice have no gross abnormalities, epithelial CEACAMs seem to contribute to the fine-tuning of cellular events (24). CEACAMs may be more important under pathological conditions, as they are bound by various pathogens (22). CEACAM5 is released by GPLD1-mediated GPI-anchor cleavage (25); it is overexpressed in many cancers and is a widely used tumor biomarker (20, 21). The analysis of *Ceacam5* KO mice has not been reported yet.

PRSS8/PROSTASIN

Protease serine (PRSS)8 (also known as prostasin) is a trypsin-like serine peptidase encoded by the PRSS8 gene. It was purified as a soluble enzyme from human seminal fluid and is highly expressed in kidney, lung, pancreas, and prostate in mice and humans (26, 27). As a GPI-AP, PRSS8 can be removed from the cell membrane by treatment with PI-PLC (28). Under physiological conditions, PRSS8 secretion from the membrane-bound form depends on GPI-anchor cleavage by endogenous GPLD1 in epithelial cells (29). PRSS8 and the type II transmembrane serine protease, matriptase/ST14, are generally recognized to interact with serine protease inhibitors (SPINT1 and SPINT2) and are indispensable for epithelial development and homeostasis (30). PRSS8 is required for terminal epidermal differentiation and postnatal survival in mice (31), but its enzymatic activity is not required for development and postnatal homeostasis (32). Thus, the nonenzymatic activity of PRSS8 is essential for in vivo development. The role of GPLD1-mediated release of PRSS8 in vivo has not been determined.

TERATOCARCINOMA-DERIVED GROWTH FACTOR 1

Teratocarcinoma-derived growth factor 1 (TDGF1) is a GPI-AP and a member of the epidermal growth factor-CFC (CRIPTO-1/FRL-1/cryptic) family. TDGF1 interacts with various components of multiple signal pathways to enhance stem cell renewal, epithelial-mesenchymal transition, proliferation, and oncogenesis (33). TDGF1 acts as both a ligand and coreceptor in the nodal signaling pathway that involves Smad 2, 3, and 4 (34, 35). GPI-anchored TDGF1 is required for paracrine activity as a nodal coreceptor (36). In its soluble form, TDGF1 functions as a ligand in tumor progression. Because the shedding of TDGF1 is mediated by GPLD1 activity at the GPI-anchor site, soluble TDGF1 may stimulate endothelial cell migration and tumor angiogenesis (37). TDGF1 is essential for

embryogenesis and cellular transformation in mice (38), but the physiological functions regulated by the membrane-bound and soluble forms have not been determined (34).

NOTUM-MEDIATED GPI-ANCHOR CLEAVAGE IN DEVELOPMENT

Glypicans (GPCs) are heparin sulfate proteoglycans that are bound to the cell surface of the plasma membrane by a GPI anchor (39). Two GPCs, division abnormally delayed (DALLY) and Dally-like protein (DLP), have been identified in *Drosophila* (40, 41). The six GPC family proteins (GPC1–6) have been conserved in mice and humans (39, 42–44). GPCs regulate signaling pathways triggered by Hedgehogs, Wnts, bone morphogenetic proteins, and fibroblast growth factors, and function in axon guidance and excitatory synapses (45). GPI-anchored GPCs interact directly with Wnt family proteins and can stimulate Wnt signaling by facilitating or stabilizing the interaction between Wnts and their receptors (46–50). GPCs may also inhibit Wnt signaling (47). Moreover, in *Drosophila*, secreted GPCs have been reported to play a role in the transport of Wnts in the imaginal wing discs for regulation of morphogen gradient formation during development (51–54).

NOTUM, a member of the α/β-hydrolase superfamily, regulates Wnt activity by modifying the heparin sulfate chains of GPCs, DALLY, and DLP (55). NOTUM was reported to induce shedding of DLP from the cell surface by cleaving its GPI anchor (8) (Table 1). However, the idea that NOTUM mediates release of GPCs was called into question by a report that recombinant *Drosophila* NOTUM cannot cleave DLP and instead functions as a carboxylesterase that removes an essential palmitoleate moiety from Wnt proteins (56). *Xenopus* NOTUM is also a Wnt deacylase and is required for brain development (57). Mammalian NOTUM was reported to cleave not only GPCs (GPC3, GPC5, and GPC6), but also other GPI-APs (PLAUR and T-cadherin) (58). Further study is needed to resolve questions about the function of NOTUM and its enzymatic activities.

GPI-ANCHOR CLEAVAGE OF RECK BY GLYCEROPHOSPHODIESTERASE 2 IN NEUROGENESIS

Glycerophosphodiester phosphodiesterases (GDPDs) are periplasmic and cytosolic proteins that are critical for the hydrolysis of deacylated glycerophospholipids to glycerol phosphate and alcohol, which are then utilized as a major source of carbon and phosphate (59). Though GDPDs are highly conserved from bacteria to mammals, they differ in their enzymatic activities and biological functions. The mammalian glycerophosphodiesterases (GDEs) have substrate specificities that do not need glycerophosphodiester. Seven homologs (GDE1–7) are conserved in mice and humans. The physiological roles of the GDE family were recently reviewed (60).

GDE2 (also known as GDPD5) has six transmembrane domains and an extracellular GDPD domain (61). GDE2 is an osmoregulated enzyme (62) and a key regulator of motor neuron differentiation (63). GDE2 is expressed in undifferentiated progenitors, but not in mature motor neurons, and its GDPD activity induces non-cell-autonomous motor neuron generation by inhibiting Notch signaling (64). Though the mechanism used by GDE2 to inhibit Notch signaling cannot be explained by conventional GDPD activity, the GPI-AP-releasing (GPIase) activity of GDE2 promotes this process during neural differentiation (5).

Reversion-inducing cysteine-rich protein with kazal motifs (RECK) is a GPI-AP that activates Notch signaling in cortical progenitors by directly inhibiting ADAM10, a disintegrin, and metallopeptidase 10, which removes the Notch ligand Delta-like 1 (DLL1) (65–67). GDE2's GPIase activity is derived from the GDPD activity domain, which sheds the GPI anchor of RECK in a manner different from that of GPI-PLD (5) (Table 1). Thus, GDE2-mediated RECK release controls the timing of Dll1-Notch signal-mediated neurogenesis (**Fig. 1**). GDE3 and GDE6 may have similar GPIase activities (59). Their physiological functions remain to be determined in vivo.

ROLE OF GPI-APS IN FERTILIZATION

Fertilization is a complicated multi-step event. In mammals, sperm ejaculated into the female reproductive tract must migrate through the utero-tubal junction to the ampulla, where fertilization takes place. Oocytes released from the ovary are surrounded by an extracellular matrix, the zona pellucida (ZP), and cumulus cell layers filled with hyaluronic acid. At the ampulla, spermatozoa must cross the cumulus cell layer, penetrate the ZP, interact with the oocyte membrane, and finally fuse with the egg. Experiments in KO mice have been undertaken to classify the various factors that are essential for fertilization (68, 69).

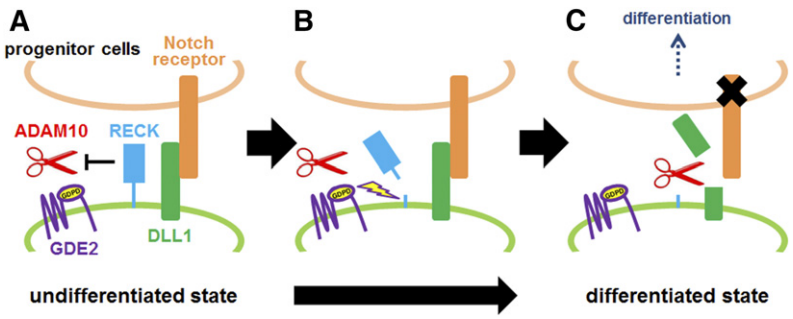

Fig. 1. GDE2 GDPD activity-dependent cleavage of RECK during neurogenesis. A: RECK inhibits ADAM10-mediated DLL1 cleavage (65, 66). Dll1-Notch signaling maintains the undifferentiated state of neuronal progenitor cells. B: GDE2 with its GPIase activity residing in a GDPD domain releases RECK from the cell surface (5). C: The release of RECK enables ADAM10 to access and cleave DLL1 (107). The subsequent discontinuation of Notch signaling non-cell-autonomously induces neural differentiation of progenitor cells.

GPI-APs and their removal play key roles during fertilization. Below, we will discuss the current understanding of the functions of GPI-APs in mammalian fertilization.

GPI-APs and sperm migration in the female reproductive tract

GPI-APs on the surface of spermatozoa originate primarily from de novo synthesis by spermatogenic cells and by transfer from epididymal luminal fluids, likely mediated by exosomes (70). Three surface-bound GPI-APs, CD52, CD55, and CD59, are thought to protect spermatozoa from immunological attack in the female reproductive tract (71). Removal of these GPI-APs was thought to regulate sperm capacitation, which readies sperm for the acrosome reaction (72). However, studies in KO mice showed that the fertilization ability of sperm does not require CD52, CD55, CD59a, and CD59b (73–76). In spermatogenesis, CD59b has a critical role that is independent from complement regulatory function (74). Recently, *Cd55b* was identified in mice, but its physiological function remains to be determined.

During the migration of sperm through the female reproductive tract, the utero-tubal junction is a critical barrier. GPI-APs are directly or indirectly involved in this step. Post-GPI attachment to proteins 1 (PGAP1) is GPI inositol-deacylase that resides in the endoplasmic reticulum and contributes to the maturation of GPI-APs (77). *Pgap1* KO mice have otocephaly and growth retardation, and often die right after birth. Males that survive to sexual maturity are severely subfertile, probably because they have three "footed" GPI anchors that are resistant to cleavage of sperm GPI-APs (78). The mutant spermatozoa are impaired in their ability to migrate from the uterus into the oviduct in vivo and bind to the ZP in vitro. The same phenotype has been reported in KO mice lacking a disintegrin and metallopeptidase domain 3 (ADAM3), a cysteine-rich glycosylated sperm membrane protein (79, 80). However, the link between GPI-APs and ADAM3 has not been elucidated.

More than 10 molecules have been reported to be indispensable for ADAM3 maturation and the fertilizing ability of spermatozoa (68). Male mice lacking angiotensin-converting enzyme (ACE), a zinc peptidase that regulates blood pressure (81), were subfertile and had low blood pressure because of the lack of somatic and testicular ACE isoforms (82, 83). *Ace* KO spermatozoa were deficient in their ability to migrate into the oviduct and bind ZP in vitro, leading to the aberrant distribution of ADAM3 (84). Using sperm-adhesion molecule 1 (SPAM1/PH20) and PRSS21 (PRSS21/TESP5) as substrates, ACE also has GPIase activity in spermatozoa (7); however, the GPIase activity was mild and was not confirmed by other groups (85, 86).

Testis-expressed gene 101 (TEX101) and the TEX101-interacting protein, lymphocyte antigen 6 complex locus K (LY6K), are germ-cell-specific GPI-APs found in the testis, but not on epididymal spermatozoa (87, 88). The GPI-AP complex consisting of TEX101 and LY6K interacts with testicular ADAM3 and is required for sperm-fertilizing ability in mice (6, 89). ADAM3 is absent from the Triton X-114 detergent-enriched phase of *Ace* KO spermatozoa (84). These findings raised the possibility that removal of the TEX101/LY6K complex is mediated by ACE's GPIase activity, which could reasonably explain the link between GPI-APs and ADAM3. In vitro analysis in a HEK293T culture system showed that TEX101, not LY6K, is the specific substrate for ACE and that dipeptidase-defective ACE retains its GPIase activity (6, 7, 89) (Table 1). These findings are consistent with the aberrant persistence of the TEX101/LY6K complex on spermatozoa in *Ace* KO mice. Thus, ACE's GPIase activity in the formation of fertile spermatozoa is independent of its well-known dipeptidase activity (**Fig. 2**).

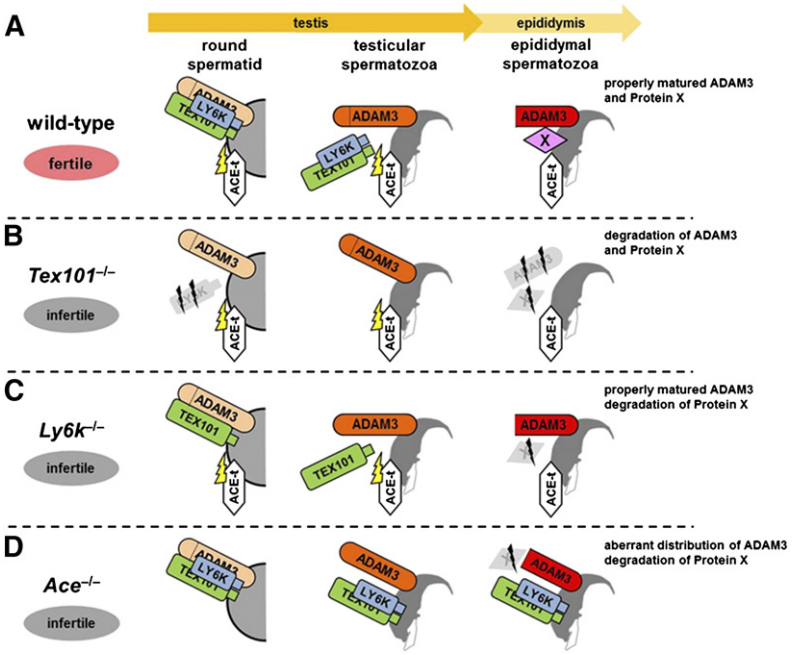

Fig. 2. Fate of GPI-AP complex, TEX101/LY6K, ADAM3, and ACE during spermatogenesis. A: In round spermatids, a GPI-AP complex consisting of TEX101 and LY6K and an immature form of ADAM3 were both localized on the plasma membrane (6, 89). The association with TEX101 is essential for ADAM3 (pale orange to dark orange) to be spliced properly to form mature ADAM3 (red) in spermatozoa. B: Without TEX101, LY6K disappeared from testicular germ cells. ADAM3 (gray) on epididymal spermatozoa was degraded during epididymal maturation, and TEX101 KO male mice were infertile (6). C: Without LY6K, TEX101 and ADAM3 seemed to be processed normally during spermatogenesis. LY6K KO male mice were sterile, even though ADAM3 localized normally in LY6K KO spermatozoa (89). This implies the existence of a key factor (Protein X) in mammalian spermatozoa other than ADAM3. D: The removal of the TEX101/LY6K complex was mediated by ACE. Without ACE, TEX101/LY6K remained on spermatozoa, and the localization of ADAM3 on epididymal spermatozoa became aberrant. ACE KO mice became infertile, possibly because of nonfunctional ADAM3 (6, 84).

GPI-APs and sperm-oocyte extracellular matrix interaction

When spermatozoa arrive in the oviduct, they encounter ovulated eggs surrounded by a layer of cumulus cells. SPAM1 was identified as a sperm GPI-anchored receptor for the ZP in guinea pigs and was later reported to have hyaluronidase activity that enables spermatozoa to pass through the cumulus-cell oocyte complex in mice (90–92). *Spam1* KO mice are fertile, and *Spam1*-deficient spermatozoa bind to the ZP, although they have a reduced ability to disperse cumulus cells via hyaluronidase activity in vitro (93). PRSS21, another surface GPI-AP, helps spermatozoa penetrate the ZP (94). However, *Prss21* KO mice are fertile, and their spermatozoa can penetrate the ZP despite the absence of PRSS21 (95). While they may play a role in fertilization, these GPI-APs did not affect in vivo fertilization in the KO experiments.

GPI-APs and sperm-egg fusion

Sperm-egg fusion is one of the last steps of fertilization. After acrosome reaction and ZP penetration, spermatozoa fuse with egg. Until recently, only two factors were known to be essential for fusion: the transmembrane protein CD9 in the egg and IZUMO1 (named after a Japanese shrine dedicated to marriage) in spermatozoa (96–99). However, recombinant IZUMO1 can bind to *Cd9* KO oocytes, suggesting the existence of IZUMO1-specific ligands rather than CD9 on the egg surface (100).

Besides these transmembrane proteins, there is evidence that GPI-APs on the egg plasma membrane have an essential role in fusion. When mouse oocytes are treated with PI-PLC, GPI-AP clusters are released from the egg surface and spermatozoa fail to fuse with the eggs (101). In addition, oocyte-specific depletion of GPI-AP phosphatidylinositol glycan class A, which is essential for GPI-AP synthesis (102), resulted in female infertility due to an impaired ability to fuse with spermatozoa (103). The GPI-AP responsible for fusion was not known until 2014, when Gavin J. Wright's group, using pentamerized IZUMO1 as bait, identified the GPI-anchored folate receptor 4 as the IZUMO1 receptor (IZUMO1R), which was named JUNO after the Roman goddess of fertility and marriage (104) (Table 1). They also showed that *Izumo1r* KO females are infertile and that *Izumo1r*-deficient eggs cannot fuse with spermatozoa. The interaction between IZUMO1 in spermatozoa and IZUMO1R in eggs is essential for sperm-egg fusion during fertilization (**Fig. 3A**).

Fertilized oocytes need to prevent fusion with other spermatozoa. Although highly expressed on the surface of unfertilized eggs, IZUMIO1R is undetectable on the egg membrane within 40 min after fertilization. IZUMO1R is redistributed into vesicles, presumably derived from the microvillus-rich oolemma, within the perivitelline space (104). This postfertilization disappearance of IZUMO1R prevents polyspermy. These findings demonstrate the importance of IZUMO1R for sperm-egg fusion and shed light on the novel mechanism and physiological function of the removal of GPI-APs from the egg surface (Fig. 3B).

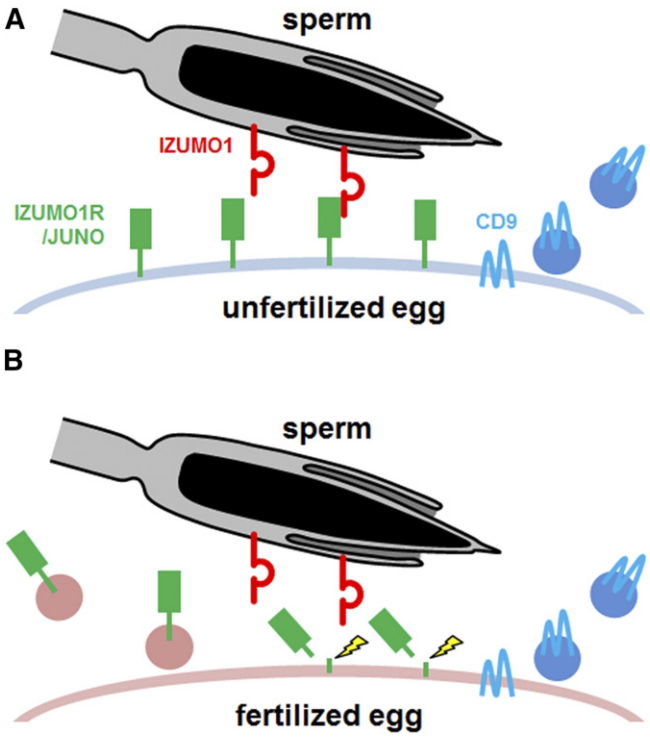

Fig. 3. Molecular mechanism of sperm-egg fusion. A: IZUMO1 and CD9 have been identified as essential factors for sperm-egg fusion (96–99). IZUMO1 is an acrosomal membrane protein that is exposed after the acrosome reaction. Fusion-competent spermatozoa exhibit the equatorial segment pattern of IZUMO1 in acrosome-reacted spermatozoa (108). Except at the metaphase plate, CD9 is localized on the cilia, distributed across the surface of unfertilized eggs. CD9-containing vesicles released from the egg surface interact with the sperm surface (109) and may enhance sperm-egg fusion. However, interaction between the two factors has not been observed. Recently, the egg binding partner for IZUMO1 on the egg surface, IZUMO1R/JUNO, was identified as a GPI-AP (104). B: After fertilization, IZUMO1R is rapidly shed from the egg membrane through an unknown mechanism, possibly mediated by vesiculation or by an unidentified GPIase activity. These events may be a possible mechanism for the polyspermy block at the plasma membrane in eggs.

CONCLUDING REMARKS

Cell-surface GPI-APs are released by GPIase activity in many important biological events, including cellular proliferation, development, neurogenesis, and reproduction. However, it has been challenging to identify the responsible enzymes and mechanisms because GPI-APs can be released from the membrane by many mechanisms, such as vesiculation and cleavages at the peptide or GPI-anchor domains. In this review, we focused on the well-characterized GPIases (GPI-PLD, NOTUM, GDE2, and ACE), but some questions remain to be addressed. Although other groups have questioned the GPIase activity of ACE, we recently showed that ACE has substrate-specific GPIase activity that is independent of its dipeptidase activity. The catalytic domains and the cleaving mechanism involved need to be clarified. On the other hand, surprisingly, NOTUM acts as an extracellular protein deacylase rather than a GPIase (56, 57). Further experiments are required

to establish that NOTUM does not have any GPIase activity.

Most of the GPIases and their substrates have been identified and analyzed in vitro, and their physiological functions have not been investigated in vivo. In vivo studies will be facilitated by using the clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 system, a genome-manipulation technology that will enable us to generate gene-disrupted mice within a few months (105, 106). With this system, enzymes and substrates can be tagged, so their fates can be traced and the proteins they interact with in vivo can be identified. In addition, point mutations can be introduced to identify the catalytic domains and substrate recognition sites.

The release of GPI-APs is also important from the clinical point of view. Like CEACAM5 and TDGF1, some circulating GPI-APs can be used as clinical biomarkers of disease (22, 33). Because GPIase has substrate specificity, it could also be a potential target for drug development. Given their location and function in the extracellular space, GPI-APs are also good therapeutic targets. Small-molecule screening will help decipher the mechanism of release of GPI-APs and will also open new avenues for the development of drugs to treat GPI-associated diseases.

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