Fertilin, a heterodimeric protein complex composed of \( \alpha \) (ADAM1) and \( \beta \) (ADAM2) subunits on the sperm surface, is believed to mediate adhesion and fusion between the sperm and egg plasma membranes. Here we have shown that mutant male mice lacking ADAM1b are fertile and that the loss of ADAM1b results in no significant defect in sperm functions such as migration from the uterus into oviduct, binding to egg zona pellucida, and fusion with zona pellucida-free eggs. ADAM1b-deficient epididymal sperm showed a severe reduction of ADAM2 on the cell surface, despite the normal presence of ADAM2 in testicular germ cells. The appearance of ADAM1b and ADAM2 on the sperm surface depended on formation and abundance of ADAM1b/ADAM2 fertilin in testicular germ cells. These results suggest that mouse ADAM1b/ADAM2 fertilin may play a crucial role not in the sperm/egg fusion but in the appearance of these two ADAMs on the sperm surface.

Members of the ADAM (a disintegrin and metalloprotease) family are multifunctional, transmembrane proteins consisting of pro-, metalloprotease, disintegrin, cysteine-rich, epidermal growth factor-like, transmembrane, and cytoplasmic tail domains (1–3). The ADAM proteins are involved in the regulation of membrane fusion, cell-to-cell adhesion, cell migration, and shedding of cytokines, growth factors, and their receptors in the processes such as fertilization, neurogenesis, myogenesis, cancer, and inflammation (3). Although at least 40 ADAM genes have been identified in a variety of species, only the roles of some ADAMs have been elucidated. In particular, there are many members of the ADAM family that are exclusively or predominantly expressed in the testis but are functionally uncharacterized yet.

Mammalian fertilization requires sperm to adhere/bind to and penetrate through the zona pellucida (ZP), an extracellular glycoprotein matrix surrounding the egg, and then to fuse with the egg membrane (4, 5). Fertilin, a heterodimeric protein complex composed of \( \alpha \) (ADAM1) and \( \beta \) (ADAM2) subunits present on the sperm surface, has been believed to mediate adhesion and fusion between the sperm and egg plasma membranes, because the cysteine-rich and disintegrin domains of ADAM1 and ADAM2 contain the putative sequences that resemble the fusogenic peptide of viral fusion proteins and the integrin binding Arg-Gly-Asp tripeptide of snake venom toxins, respectively (6–10). In mouse, two different isoforms of ADAM1, ADAM1a and ADAM1b, are synthesized in the testis, and only ADAM1b is present on the plasma membrane of epididymal sperm (11, 12). The functional roles of ADAM1a (13), a protein resident within the endoplasmic reticulum (ER) of testicular germ cells (TGC), ADAM2 (14, 15), and ADAM3 known as cyritestin (15, 16) have been evaluated by analysis of null mutant mice. The loss of ADAM1a or ADAM2 resulted in male infertility; sperm of ADAM1a- or ADAM2-deficient mice were incapable of ascending from the uterus into the oviduct through the uterotubal junction and of binding to the egg ZP (13, 14). The inability of these mutant sperm to bind the ZP may be explained by a negligibly low level of ADAM3 on the sperm surface (13, 15). Indeed, ADAM3-deficient male mice were infertile, and the mutant sperm showed the defect in the ZP binding (15, 16). Interestingly, the loss of ADAM2 in TGC resulted in the lack of both ADAM1b and ADAM3, in addition to ADAM2, on the sperm surface (15, 17). Because ADAM2-deficient mouse sperm were also defective in adhesion to and fusion with the egg membrane (14, 15), the functional roles of ADAM1b, ADAM2, and ADAM1b/ADAM2 fertilin complex in fertilization are still controversial.

In this study, we have produced mice carrying a null mutation in Adamb1 using homologous recombination. Unexpectedly, the male mice lacking ADAM1b are fertile, and ADAM1b-deficient epididymal sperm are functionally normal in the migration into the oviduct, binding to the ZP, and fusion with the egg. Despite the normal presence of ADAM2 in TGC, only ADAM2 among membranous proteins tested is severely reduced on epididymal sperm of the ADAM1b-deficient mice. On the basis of the experimental results on four lines of mutant mice lacking ADAM1a, ADAM1b, ADAM2, or ADAM3, we conclude that, at least in the mouse, ADAM1a/ADAM2 fertilin is responsible for the appearance of ADAM3 on the sperm surface, whereas ADAM1b/ADAM2 fertilin is responsible for the appearance of ADAM1b and ADAM2 on the sperm surface.

**EXPERIMENTAL PROCEDURES**

Generation of Mutant Mice Lacking Adamb1—A targeting vector containing an expression cassette of the neomycin-resistance gene, neo, flanked by 7.5- and 1.45-kbp genomic regions of Adamb1 was constructed by using a mouse genomic clone, mFAG1 (11), encoding ADAM1b (Fig. 1A). For negative selection, the MC1 promoter-driven herpes simplex virus thymidine kinase gene was inserted at the 3′-end of the targeting vector. The vector was linearized by digestion with Sall and electroporated into mouse D3 embryonic stem cells, and homologous recombinants were selected by using G418 and ganciclovir as described previously (18). Seven embryonic stem cell clones carrying the targeted mutation were identified from 450 clones resistant to G418.

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1 Supported by the JSPS Postdoctoral Fellowship Program for Foreign Researchers.

2 To whom correspondence should be addressed. Tel./Fax: 81-29-853-7196; E-mail: acroman@sakura.cc.tsukuba.ac.jp.

3 The abbreviations used are: ADAM, a disintegrin and metalloprotease; ZP, zona pellucida; ER, endoplasmic reticulum; TGC, testicular germ cells.

4 www.people.virginia.edu/~jw7g/Table_of_the_ADAMs.html.
and ganciclovir and injected into C57BL/6 mouse blastocysts. Chimeric male mice were crossed to C57BL/6 female mice (Japan SLC Inc.) to establish heterozygous mutant lines. Homozygous mice were obtained by mating of heterozygous males and females. All animal experiments were carried out according to the Guide for the Care and Use of Laboratory Animals at the University of Tsukuba.

**Blot Hybridization**—Genomic DNA was prepared from mouse tail, digested by EcoRI, separated by agarose gel electrophoresis, and transferred onto Hybond-N+ nylon membranes (Amersham Biosciences). Total cellular RNA was prepared from testicular tissues using Isogen (Nippon Gene, Toyama, Japan) as described previously (19). The RNA samples were glyoxylated, separated by agarose gel electrophoresis, and transferred onto the nylon membranes. The blots were probed by 32P-labeled DNA fragments and analyzed by a BAS-1800II Bio-Image analyzer (Fuji Photo Film, Tokyo, Japan) as described (20).

**Antibodies**—Affinity-purified polyclonal antibodies against ADAM1a (12), ADAM1b (12), ADAM3 (21), and PH-20 (22) were prepared as described. Mouse monoclonal antibody against ADAM2 (9D2.2) and horseradish peroxidase-conjugated goat antibodies against rabbit or mouse IgG (H + L) were purchased from Chemicon and Jackson Immunoresearch Laboratories, respectively. Anti-ADAM2 (23), anti-calmegin (24), anti-angiotensin-converting enzyme (25), and anti-Izumo (26) polyclonal antibodies were kind gifts from Dr. M. Okabe.

**Immunohistochemical Analysis**—Testicular tissues from 3- to 4-month-old mice were minced with a razor blade in 4 mM Heps-NaOH, pH 7.4, containing 140 mM NaCl, 4 mM KCl, 10 mM glucose, and 2 mM MgCl2, filtered through a nylon mesh, and centrifuged at 1,200 × g for 10 min at 4 °C as described (15). The cell pellet was suspended in the same buffer, and the suspension was put on a 52% Percoll gradient (Amersham Biosciences) in the above buffer and centrifuged at 11,000 g for 10 min at 4 °C. TGC was then recovered from a white band near the top of the gradient and washed three times with phosphate-buffered saline. TGC and cauda epididymal sperm were suspended in a lysis buffer consisting of 20 mM Tris-HCl, pH 7.4, 1% Triton X-100, 150 mM NaCl, and 1% SDS and 1% 2-mercaptoethanol, separated by SDS-PAGE, and transferred onto Immobilon-P membranes (Millipore). After blocking with 1% skim milk, the blots were incubated with primary antibodies for 2 h and then with horseradish peroxidase-conjugated secondary antibodies for 1 h. Immunoreactive proteins were detected by an ECL Western blotting detection kit (Amersham Biosciences), and the intensities of the immunoreactive protein bands were quantified by using a Fuji Film Science Lab Image Gauge software (version 3.4). Protein concentration was determined using a Coomassie protein assay reagent kit (Pierce). Immunoprecipitation analysis was carried out according to the previously published method (13).

**Sperm Migration into Oviduct**—Female B6C3F1 mice were caged with male mice 12 h after intraperitoneal injection of human chorionic gonadotropin (Teikoku Zoki Co.), and formation of vaginal plug was observed every 30 min as described previously (13, 27). The oviducts were excised with a connective part of the uterus ~2 h after copulation, fixed in phosphate-buffered saline containing 4% paraformaldehyde, washed with phosphate-buffered saline, and frozen in OCT compound (Sakura Finetechical Co., Tokyo, Japan). Sections were stained with hematoxylin and observed under an Olympus BX50 microscope (Tokyo, Japan) equipped with an HC-2500 camera (Fuji Film, Tokyo, Japan).

In Vitro Fertilization—Eggs tightly packed with cumulus cells were collected from the oviductal ampulla of superovulated BDF1 mice 14 to 15 h after human chorionic gonadotropin injection and placed in a 0.1-mL drop of a modified Krebs-Ringer bicarbonate solution (TYH medium) (28) covered with mineral oil. Fresh cauda epididymal sperm from 3-month-old mice were capacitated by incubation for 2 h in a 0.2-mL drop of TYH medium at 37 °C under 5% CO2 in air. An aliquot (1.5 × 104 sperm/10 μL) of the capacitated sperm suspension was mixed with the eggs in a 90-μL drop of TYH medium. After incubation at 37 °C under 5% CO2 in air, the eggs were treated with bovine testicular hyaluronidase (3 units/ml, Sigma-Aldrich) for 10 min to remove cumulus cells. The female and male pronuclei in the eggs were fixed in 4% paraformaldehyde for 15 min, stained with Hoechst 33342 (2 μg/ml) for 30 min, and then viewed under an Olympus BX70 epifluorescence microscope as described (13).

**Sperm-ZP Binding**—Superovulated eggs were briefly treated with bovine hyaluronidase (3 units/ml), washed, and placed in a 0.1-mL drop of TYH medium covered with mineral oil. An aliquot (1.5 × 104 sperm/10 μL) of capacitated sperm suspension was added to a 90-μL drop containing cumulus-free eggs and two-cell embryos in TYH medium, and the mixture was incubated for 30 min at 37 °C under 5% CO2 in air. The eggs were transferred to 100 μl of fresh TYH medium, washed by pipetting, and fixed in 0.25% glutaraldehyde. The number of sperm tightly bound to the egg ZP was counted under an Olympus BX71 microscope equipped with a DP-12 camera as described (29). The two-cell embryos were used as an internal negative control for nonspecific binding, and the average number of bound sperm/two-cell embryo was <1.0 under the above conditions.

**Sperm-Egg Fusion**—ZP-free eggs were mechanically prepared by using a piezo-driven micromanipulator (Prime Tech Ltd., Ibaraki, Japan) without the use of α-chymotrypsin as described previously (30). The ZP-free eggs previously treated with Hoechst 33342 (2 μg/ml) were inseminated with capacitated sperm (1.5 × 105 cells/ml) followed by incubation for 30 min at 37 °C under 5% CO2 in air. After fixation in 0.25% glutaraldehyde, the eggs were observed under the above fluoromicroscope.

**RESULTS**

To explore the functional role of ADAM1b in fertilization, we produced mutant mice lacking ADAM1b by homologous recombination in embryonic stem cells. The targeting construct was designed to replace the 455-residue protein-coding region containing the pro-, metalloprotease, disintegrin, and cysteine-rich domains of ADAM1b at positions 79–533 with neo (Fig. 1A). The genotypes of wild-type (Adam1b+/+), heterozygous (Adam1b+/−), and homozygous (Adam1b−/−) mice for the targeted mutation of Adam1b were identified by Southern blot analysis of genomic DNA (Fig. 1B). Northern blot analysis indicated the absence of ADAM1b mRNA in Adam1b−/− testis. In addition, protein extracts of Adam1b−/− TGC completely lacked a 120-kDa protein corresponding to the precursor form of ADAM1b. These data demonstrate the absence of ADAM1b in the Adam1b−/− testis.

Mating of Adam1b+/− male and female mice yielded the expected Mendelian frequency of Adam1b+/−/− mice (Adam1b+/−/+:Adam1b+/−/−:Adam1b+−/− = 20 (27%):35 (47%):19 (26%) for 76 offspring from 9 litters). Both Adam1b+−/− males and females were apparently normal in behavior, body size, and health condition. Morphological analysis demonstrated no significant difference in the shapes, numbers, and sizes of TGC and epididymal sperm among Adam1b+−/−, Adam1b+/−, and Adam1b−/− mice. The motility of cauda epididymal sperm was also normal in Adam1b−/− mice. Most importantly, Adam1b−/− male mice...
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exhibited normal fertility and produced an average litter size (means ± S.E. = 9.9 ± 0.7 and 9.3 ± 0.3 offspring for 10 litters in Adam1b+/+ and Adam1b−/− mice, respectively). Thus, we conclude that ADAM1b is not essential for fertilization in the mouse.

To examine the interaction of Adam1b−/− mouse sperm with eggs, in vitro fertilization assays were carried out using capacitated cauda epididymal sperm. When cumulus-intact eggs were used, the fertilization rate 2 (data not shown) and 6 h after insemination was normal in Adam1b−/− mouse sperm, respectively (25 two-cell embryos for each group, n = 4); sperm/egg fusion. ZP-free eggs previously loaded with Hoechst 33342 were incubated with capacitated epididymal sperm from Adam1b+/+ and Adam1b−/− mice for 30 min. Numbers in the columns represent those of the eggs examined (n = 4).

FIGURE 1. Production of mice lacking ADAM1b and characterization of ADAM1b-deficient mouse sperm. A, targeting strategy of mouse Adam1b. A part of the protein-coding region (closed box) in Adam1b was replaced by neo (darkly shaded box). The herpes simplex virus thymidine kinase gene (tk, lightly shaded box) was included in the targeting construct for negative selection. Open boxes represent the 5′- and 3′-untranslated regions. Restriction enzyme sites: E, EcoRI; G, BglII; X, KpnI; S, SacI; Sa, SalI; X, XhoI. B, characterization of ADAM1b-deficient mice. Genomic DNA, total cellular RNA, and protein extracts were prepared from mouse testis, testicular tissues, and testicular germ cell (TGC) of Adam1b+/+(+/+), Adam1b−/− (+/−), and Adam1b−/− (−/−) mice. Probes S and N (panel A) were used as probes for Southern and Northern blot analysis, respectively. Immunoblot analysis was carried out using anti-ADAM1b antibody. C, fertilization in vitro. Cauda epididymal sperm of Adam1b+/− (open column) and Adam1b−/− (shaded column) mice were capacitated and incubated with metaphase II-arrested, cumulus-intact eggs for 6 h. The eggs containing female and male pronuclei were defined as “fertilized eggs.” Numbers in the columns represent those of the eggs examined (n = 3). D, binding of sperm to egg ZP. Cumulus-free, ZP-intact eggs and two-cell embryos were incubated with capacitated epididymal sperm from Adam1b−/− and Adam1b−/− mice, and the number of sperm bound to the ZP was counted. Numbers in the columns represent those of the eggs examined. Data are expressed as the means ± S.E., where n = 4. The numbers of bound sperm/two-cell embryo as a negative control were 0.2 ± 0.1 and 0.4 ± 0.2 in Adam1b+/− and Adam1b−/− mice, respectively (25 two-cell embryos for each group, n = 4). E, sperm/egg fusion. ZP-free eggs previously loaded with Hoechst 33342 were incubated with capacitated epididymal sperm from Adam1b+/+ and Adam1b−/− mice for 30 min. Numbers in the columns represent those of the eggs examined (n = 4).

FIGURE 2. Normal migration of ADAM1b-deficient mouse sperm from uterus into oviduct. Sections were stained with hematoxylin and observed under a microscope (magnification, ×100). Arrows indicate sperm present in the uterine lumen (U) and colliculus tubarius (CT).

ADAM1a, ADAM1b, and ADAM2 verified the loss of ADAM1a/ADAM2 and ADAM1b/ADAM2 fertilins in Adam1a−/− and Adam1b−/− TGC, respectively (Fig. 3C).

Although the level of ADAM2 in TGC was similar between Adam1b+/+ and Adam1b−/− mice (Fig. 3A), epididymal sperm of Adam1b−/− mice showed the loss of ADAM2 as well as of ADAM1b (Fig. 3D). To verify the loss of ADAM2 on Adam1b−/− mouse sperm, we carried out immunoblot analysis of sperm extracts from Adam1b+/+ and Adam1b−/− mice using two different antibodies (Fig. 4A). As described above, anti-ADAM2 polyclonal antibody raised against the C-terminal 22-residue peptide (23) was not immunoreactive with the 45-kDa mature form of ADAM2 in the Adam1b−/− sperm extracts. However, the mature protein in the Adam1b−/− extracts was slightly but significantly recognized by anti-ADAM2 monoclonal antibody 9D2.2. Moreover, the Adam1b−/− and Adam1b−/− sperm extracts con-
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FIGURE 3. Presence of testis (sperm)-specific proteins in Adam1b-deficient mice. Proteins from TGC and cauda epididymal sperm of Adam1b+/+ (+/+) or WT, and Adam1b−/− (−/−) mice were extracted with 1% Triton X-100, separated by SDS-PAGE under reducing (A, C, and D) or non-reducing (B) conditions, and subjected to immunoblot analysis using antibodies against testis (sperm)-specific proteins indicated. Arrow, testis-specific isoform of angiotensin-converting enzyme. A, immunoblot analysis of TGC proteins. Seven testis-specific proteins were normally present in immunoblot analysis using antibodies against testis (sperm)-specific proteins indicated. tACE, a testis-specific isoform of angiotensin-converting enzyme.

FIGURE 4. ADAM1b-deficient mouse sperm contain a severely reduced level of ADAM2. A, immunoblot analysis. Sperm protein extracts (3 µg) from a wild-type mouse (lane 1) and three Adam1b-deficient mice (lanes 2, 3, and 4) were separated by SDS-PAGE under reducing conditions and subjected to immunoblot analysis using anti-ADAM2 polyclonal (pAb) and monoclonal (mAb) antibodies. An arrow indicates the location of 100-kDa ADAM2 precursor. B, quantitative analysis. The intensities of immunoreactive protein bands corresponding to the 45-kDa mature form of ADAM2 were quantified.

FIGURE 5. Appearance of ADAM1b and ADAM2 on the surface of Adam1b+/+ and Adam1b−/− mouse sperm. Immunoblot analysis of sperm proteins was carried out using polyclonal antibodies against ADAM1b, ADAM3, and PH-20 and anti-ADAM2 monoclonal antibody. The intensities of immunoreactive protein bands were quantified. Both levels of ADAM1b and ADAM2 on Adam1b+/+ (+/+) mouse sperm were reduced to 52 and 61% of those on Adam1b+/+ (+/+)-/− mouse sperm, respectively. The levels of Adam1b+/+ (+/+) mouse sperm were similar to those on Adam1b−/− (−/−)-/− mouse sperm. Data are expressed as the means ± S.E., where n = 3 (three different sperm samples from each group of Adam1b+/+, Adam1b−/−, and Adam1b−/− mice). ND, not detected.

Adam1b+/+ mouse sperm, see Fig. 3D). These data suggest that the appearance of ADAM1b and ADAM2 on the sperm surface may depend on the formation and abundance of Adam1b/ADAM2 fertilin in TGC.

DISCUSSION

This study shows that ADAM1b and ADAM1b/ADAM2 fertilin are not essential for the fertilization processes of sperm migration into the oviduct, sperm/ZP binding, and fusion between the sperm and egg membranes (Fig. 1). We have also demonstrated the importance of...
ADAM1b/ADAM2 fertilin formation in TGC for the appearance of these two ADAMs on the sperm surface (Figs. 3 and 5). Table 1 summarizes the correlation between the abundance of ADAM proteins and sperm function in four mouse lines lacking ADAM1a, ADAM1b, ADAM2, or ADAM3. Our data on Adam1b/ADAM2 mouse sperm strongly support the previous finding that ADAM3 is crucial in the sperm binding to egg ZP (15, 16).

We have previously demonstrated that the loss of ER-resident ADAM1a results in the lack of ADAM1a/ADAM2 fertilin in TGC and ADAM3 on epididymal sperm, despite the normal presence of ADAM1b/ADAM2 fertilin and ADAM3 in TGC (13). The levels of ADAM1b and ADAM2 on the sperm surface are also normal in Adam1a/ADAM2 null mice (13). In the present study, Adam1b/ADAM2 null mice lack ADAM1b/ADAM2 fertilin in TGC and contain a severely reduced level of ADAM2 on epididymal sperm, whereas the ADAM3 level is normal on Adam1b/ADAM2 mouse sperm (Figs. 3 and 5). Thus, the formation of the ADAM1a/ADAM2 and ADAM1b/ADAM2 fertilins in TGC is presumably essential for the appearance of ADAM3 and both ADAM1b and ADAM2 on the sperm surface, respectively. This probability is consistent with the fact that the loss of ADAM1a/ADAM2 in TGC leads to the lack of ADAM1b and ADAM3, in addition to ADAM2, on the sperm surface (13, 15). The defective transport of ADAM3 onto the sperm surface in Adam1a/ADAM2 null mice, and of both ADAM1b and ADAM2 in Adam1b/ADAM2 null mice, may simultaneously occur in Adam2/ADAM2 mice. It is also possible that Adam1a/ADAM2 mouse sperm are functionally identical to Adam2/ADAM2 mouse sperm, because Adam1b/ADAM2 mouse sperm show no functional defect (Figs. 1 and 2). Indeed, the Adam2/ADAM2 mouse sperm show the same phenotype as Adam1a/ADAM2 mouse sperm except for a reduced rate of sperm/egg fusion in Adam2/ADAM2 mouse sperm (Table 1).

Very small amounts of the precursor and mature forms of ADAM2 are still present on Adam1b/ADAM2 mouse sperm (Figs. 4 and 5). There are several possibilities that may explain the presence of ADAM2 on the surface of Adam1b/ADAM2 mouse sperm. ADAM2 is possibly transported onto the Adam1b/ADAM2 sperm surface by a protein complex with ER-resident ADAM1a or a truncated form of ADAM1b or by ADAM2 itself. Immunoblot analysis of Adam1b/ADAM2 sperm extracts indicated the absence of the precursor, processing intermediate, and mature forms of Adam1a (Fig. 3D). No signal for ADAM1b mRNA was found when total testicular RNA of Adam1b/ADAM2 mice on blots was probed by 140- and 815-bp DNA fragments encoding the 5’-untranslated region and 18-nucleotide protein-coding region (data not shown) and the protein-coding region corresponding to the cysteine-rich, epidermal growth factor-like transmembrane and cytoplasmic tail domains of Adam1b (Fig. 1, Probe N), respectively. Thus, it is conceivable that some ADAM2 in TGC may be capable of appearing on the sperm surface without the complex formation with ADAM1a or ADAM1b. Moreover, ADAM2 on Adam1b/ADAM2 mouse sperm may compensate for the loss of ADAM1b and ADAM1b/ADAM2 fertilin, because Adam1b/ADAM2 mouse sperm are functionally normal (Figs. 1 and 2). This possibility seems unlikely but cannot be ruled out completely at the present time.

Male mice lacking an ER-resident molecular chaperone, calmegin (Clgn(8)), share the sterile phenotype with Adam1a/ADAM2 and Adam3-deficient mice: the defects of sperm both in binding egg ZP and in migrating from the uterus into oviduct (23, 24, 27). Importantly, calmegin is required for heterodimerization between ADAM1a and ADAM2 and/or between ADAM1b and ADAM2 in TGC (23). The defect of Clgn/Clgn mouse sperm in the ZP binding may be explained by the lack of ADAM3 on the sperm surface because of a possible absence of ADAM1a/ADAM2 fertilin in TGC. Clgn/Clgn mouse sperm, indeed, lack ADAM3 on the cell surface. At any rate, the mechanism of the ADAM3 transport onto the sperm surface probably regulated by ADAM1a/ADAM2 fertilin and/or calmegin remains to be clarified. Recently, Stein et al. (17) reported that the loss of ADAM3 in Adam2 null mice may result, at least in part, from a disruption of protein trafficking.

### Table 1: Role of Mouse Fertilin in Fertilization

| Null mutant mouse | Fertilin formation in TGC | Level of sperm protein | Sperm function |
|-------------------|---------------------------|------------------------|---------------|
|                   | ADAM1a/ADAM2 | ADAM1b/ADAM2 | ADAM1b | ADAM2 | ADAM3 | UTI | ZP | Fusion |
| ADAM1a null       | Loss          | Normal       | 100 ± 8 | 84 ± 2 | 11 ± 5 | Loss | SI | Normal |
| ADAM1b null       | Normal        | Loss         | ND     | 4 ± 0.6 | 97 ± 3 | Normal | Normal | Normal |
| ADAM2 null        | Loss          | Loss         | 0      | 11     | SI     | Loss | ~50% | Normal |
| ADAM3 null        | Normal        | Normal       | 70     | 75     | ND     | Normal | Normal | Normal |

Data of ADAM1a- and ADAM1b-deficient mice from this study (means ± S.E., n = 3), and of ADAM2- and ADAM3-deficient mice from Stein et al. (17). The level of each protein on wild-type sperm is assumed as 100%.

![Image](372x26 to 400x38)

* M. Okabe, personal communication.
whether the mouse sperm fertilin is indeed a non-functional protein complex or still contributes to an unknown sperm function(s).

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Addendum—During the revision process of this paper, it has come to our attention that the level of ADAM5 is severely reduced on epididymal sperm of Adam2<sup>−/−</sup> mice (32). Because the ADAM5 level in the Adam2<sup>−/−</sup>TGC is normal, ADAM1a/ADAM2 or ADAM1b/ADAM2 fertilin may also be required for the appearance of ADAM5 on the sperm surface.

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