Evolution of Gamete Recognition Proteins

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REVIEW

Although fertilization has been studied for more than a century, the cell surface proteins mediating the process are only now becoming known. Gamete interaction in animals appears to be molecularly complex. Although it is difficult to generalize at present, diversity of structure may be a recurring theme in the evolution of fertilization proteins. Examples of rapid evolution of fertilization proteins by positive selection are known, and concerted evolution can influence the differentiation of gamete recognition proteins between closely related species.

Sexual reproduction, defined here as the fusion of two haploid cells during fertilization to form a diploid zygote, occurs in almost all eukaryotes. Fertilization is the bridge between generations. Although studied for more than a century, it remains one of the least understood fundamental biological processes.

The interactions of sex cells (gametes) are mediated by cell surface fertilization proteins. What little we presently know about them indicates that they are structurally diverse. When biochemical processes are reduced to their component molecules, one expects to find conservation in structure and function across eukaryotic phyla. For example, yeast and human cells use nearly identical proteins to regulate the cell division cycle and the intracellular trafficking of secretory vesicles. However, many different proteins have evolved in animal phyla to mediate fertilization. The apparent diversity of fertilization proteins suggests that they may have evolved independently in different phyla (convergent evolution). Structural diversity of fertilization proteins among closely related species also suggests that their rate of evolutionary change may have been rapid.

This article describes molecular diversity in five steps of sperm-egg interaction in animals and gives examples of the rapid evolution of fertilization molecules. Discussion is limited to molecules of known structure or proteins of known sequence. One example is then presented to illustrate how the species specificity of sperm-egg interaction may evolve. See (*1*) for detailed reviews describing recent progress in fertilization research.

Five Steps of Sperm-Egg Interaction

A generalized scheme of animal fertilization depicts five steps in sperm-egg interaction (Fig. 1). Most animal sperm have an acrosomal vesicle anterior to the nucleus. The opening (exocytosis) of the vesicle is required for fertilization. Animal eggs are contained within an egg envelope. A gelatinous matrix is external to the envelope. Spermatozoa may be chemotactically attracted to swim toward the egg by egg-released molecules (step A). Depending on the species, sperm bind to the egg envelope either before or after the opening of the acrosomal vesicle (step B or C). Soon after exocytosis of the acrosomal vesicle occurs [the acrosome reaction (AR)], a hole is created in the egg envelope through which the sperm passes (step D). Once the sperm is through the envelope, the two cells fuse (step E) and the sperm nucleus is incorporated into the egg cytoplasm. One or more of the five steps can exhibit species specificity, meaning that if spermatozoa and eggs are from the same species, their interaction leading to fusion is more efficient than if the two gametes are from different species. Cross-species fertilizations between closely related species usually yield lower percentages of fertilized eggs, indicating that species-specific differences exist in gamete recognition proteins.

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Chemoattraction

How far do sperm have to swim to reach eggs? Many marine invertebrates and algae broadcast gametes into seawater, where fertilization and embryogenesis occur external to the adult. Although sperm chemoattraction to egg-derived factors has been phenomenologically demonstrated in most invertebrate and some vertebrate groups, the chemical nature of the attractants is known in but a few species. The structures of known sperm chemoattractants are chemically unrelated, indicating that they evolved independently in different phyla.

For example, in brown algae, female gametes release species-specific, 11–carbon atom, cyclo-olefinic hydrocarbons, which in picomolar concentrations attract male gametes (*2*). In the ciliate *Euplotes*, small mating-type–specific proteins control the cellular activities of chemotaxis, conjugation, and growth (*3*). In marine invertebrates, sperm swim up gradients of an egg-derived peptide. The chemoattraction can be species-, genus-, or family-specific (*4*). Sea urchin egg jelly contains small peptides that induce species-specific stimulation of respiration, motility, and chemoattraction of sperm (*5*). In one species, the chemoattracting peptide binds to a receptor guanylate cyclase in the sperm flagellar membrane, activating cyclase to make cyclic guanosine 5′-phosphate, which probably controls ion channels regulating flagellar motility (*6*). Although human sperm are attracted to the follicular fluid surrounding the human egg, the chemoattractant molecules remain unknown (*7*).

Induction of the Sperm AR

The sperm acrosomal vesicle must open and release its contents before sperm are capable of fertilizing eggs. What egg surface molecules induce the sperm AR? Once again it appears to be a diverse collection of molecules, all of which involve carbohydrates (chains of various sugar residues). In starfish, three very different types of molecules in egg jelly, named ARIS, Co-ARIS, and asterosap, are involved in AR induction, presumably by binding to sperm membrane receptors. ARIS is a polysaccharide with a mass of 10 million daltons, made of a sulfated pentasaccharide repeating unit. Co-ARIS is a steroid saponin, and asterosap is a collection of peptides 34 amino acids long of differing sequences. Eleven asterosaps have been sequenced from one starfish species (*8*). All three types of molecules are necessary for optimal induction of the starfish sperm AR.

When sea urchin sperm contact the egg jelly coat, a polymer of sulfated fucose (FSP) binds to a sperm receptor, activating calcium ion channels that induce the AR. FSP can be species-specific in its structure and activity to trigger the AR (*9*). The sperm receptor protein for FSP has the structure of a carbohydrate binding protein. Sixty percent of the receptor sequence (>900 amino acids) is evolutionarily related (homologous) to 20% of human polycystin-1, a protein of unknown function that is mutated in polycystic kidney disease, the most frequent human genetic disease (*10*). Further study of this sea urchin sperm protein could help elucidate the function of polycystin-1 in humans.

The mammalian egg envelope, the zona pellucida, is composed of three glycoproteins: ZP1, ZP2, and ZP3. In the mouse, chains of sugar residues (oligosaccharides) on specific amino acids of ZP3 bind receptor proteins on the sperm plasma membrane (*11*). The sperm receptor for the ZP3 sugars is currently being debated (*1*). Binding of

sperm to ZP3 activates calcium ion channels, which trigger the exocytosis of the acrosomal vesicle (*1*, *12*). In mammals, the steroid hormone progesterone will also induce the sperm AR in vitro at concentrations found in the follicular fluid surrounding the egg. Progesterone presumably acts by binding to sperm receptor proteins that control the chloride ion channel activity of sperm (*13*).

Although the opening of calcium ion channels in the sperm cell membrane is probably required for the induction of the AR in all animal sperm (*12*), the AR inducers from the egg surface are diverse molecules, lacking structural similarity and detectable evolutionary homology across phyla.

Binding of Sperm to Egg Envelopes

At some step in sperm-egg interaction, sperm of most species bind tightly to the egg envelope. In ascidians, amphibia, and most mammals, the sperm bind to the egg envelope before undergoing the AR. Binding is mediated by proteins on the sperm that recognize sugar residues on egg glycoproteins. Binding is necessary for AR induction. In mammals, the acrosome-reacted sperm binds presumably by its now exposed inner acrosomal membrane to the ZP2 glycoprotein of the egg envelope (*1*). About one dozen sperm proteins have been implicated in the adhesion of sperm to mammalian eggs (*1*). It is not known if one or more of them are indispensable to fertilization.

In clams, horseshoe crabs, annelids, echiurids, starfish, and sea urchins, sperm bind to the egg envelope after undergoing the AR. In these invertebrates, the AR also involves the generation of an actin-cored acrosomal process that drills through the egg envelope (*8*). In sea urchins, the acrosomal protein bindin mediates species-specific binding between the gametes. Bindin sequences are species-specific (*14*). All bindins have a central domain of approximately 60 amino acids, which has been conserved for at least 150 million years of evolution. The most species-specific structural attributes of bindins are in the number and location of short repeating amino acid sequence elements flanking the central conserved domain (*14*). Sea urchin bindins are not related to any other proteins.

Passage of Sperm Through the Egg Envelope

Ascidian sperm have a surface-exposed chymotrypsinlike protease that appears to digest a passage for the sperm through the egg envelope (*15*). The zona pellucida is a thick fibrous barrier that the mammalian sperm must traverse before reaching the egg cell membrane. Acrosin is a proteolytic enzyme found in acrosomal vesicles of vertebrate sperm. For decades it was thought that acrosin digested a path for the sperm through the zona. However, male mice in which the acrosin gene was made nonfunctional produced fertile sperm, showing that acrosin is not essential for fertilization (*16*). Experiments indicate that the role of acrosin is probably to increase the rate of dispersal of the acrosomal contents after AR induction (*16*). Two recently discovered acrosomal proteases may be the ones involved in penetration of the mouse egg envelope by sperm (*17*).

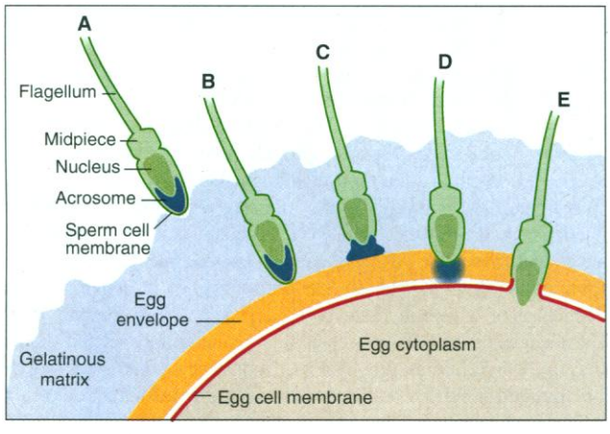

Fig. 1. A general scheme of animal fertilization combining invertebrate and mammalian attributes. A: The sperm is chemotactically attracted to the egg. B: The sperm binds to the egg envelope. C: The AR occurs, externalizing the contents of the acrosomal vesicle. D: The sperm creates a hole in the egg envelope and reaches the egg cell membrane. E: The membranes of the two cells fuse to restore the diploid genome and activate development.

Not all animal spermatozoa use proteolytic enzymes to traverse egg envelopes. For example, abalone eggs (a marine mollusk), like mammalian eggs, are enclosed in an envelope elevated from the egg surface and made of glycoprotein fibers. Abalone spermatozoa release 16-kD lysin from their acrosomes during the AR. Lysin dissolves a hole in the egg envelope by a nonenzymatic reaction exhibiting species specificity (*18*).

Lysin was the first fertilization protein whose structure was solved by x-ray crystallography (*19*). The crystal structure of monomeric lysin (Fig. 2) revealed a protein with high α-helical content and three interesting structural features. (i) The NH_2-terminal domain projects away from the helical bundle composing the rest of the molecule; the sequence of this domain is always species-specific. (ii) One face of lysin contains two nearly parallel tracks of basic amino acids running the entire length of the molecule. These basic tracks give lysin a high net positive charge and are consistent with lysin being a surface-active protein. The basic tracks are conserved among species. (iii) The surface opposite the basic tracks has 11 water-exposed hydrophobic residues that form a hydrophobic patch. The patches are involved in lysin dimerization. When isolated egg envelopes are added to lysin dimers, the dimers rapidly dissociate, which suggests that it is the monomer that dissolves the egg envelope (*19*).

Gamete Fusion

After a sperm passes through the egg envelope, fusion of its plasma membrane with the egg plasma membrane must occur to unite the two cells. In many species it is difficult to distinguish between binding of sperm to the egg membrane and fusion of the two cells; in a given species the same proteins could mediate both steps. It is difficult to assay sperm-egg fusion directly. Indirect assays using fusion-blocking antibodies made against suspected fusion proteins, or addition of synthetic peptides representing specific domains of suspected fusion proteins, are the usual experimental approaches.

Microorganisms offer a direct genetic approach for studying cell fusion. Fusion between + and − mating types of the green alga *Chlamydomonas* is controlled by the *fus1* gene, which encodes a plasma membrane glycoprotein. *fus1* mutants are fusion-defective. Transformation of a normal *fus1* gene into fusion-defective mutant cells rescues fusion and zygote formation (*20*). Mutations in the *spe-9* gene of the nematode *Caenorhabditis elegans* block gamete fusion, even though the amoeboid spermatozoa are in contact with the egg surface. *spe-9* does not seem to code for a fusion protein. It resembles ligands of a family of signal-transducing receptors, which suggests that it either indirectly sets the stage for fusion or directly participates in fusion (*21*).

An 18-kD protein, released with lysin during the AR of abalone sperm (*22*), and sea urchin sperm bindin (*14*) both coat the sperm plasma membrane destined to fuse with the egg. Both proteins are potent mediators of fusion of phospholipid vesicles (an assay used to quantitate membrane fusion), indirectly implicating them as mediators of gamete membrane fusion. An 18–amino acid peptide sequence of bindin, found in the middle of the highly conserved region of 60 amino acids, possesses the membrane-fusing activity. This short peptide behaves identically to full-length bindin of 246 amino acids in its ability to aggregate and fuse

phospholipid vesicles (*23*).

Mammalian and amphibian sperm possess two plasma membrane proteins, α- and β-fertilin, that are thought to mediate sperm-egg fusion. Fertilins are members of the ADAM (a disintegrin and metalloprotease domain) family of membrane proteins (*24*). After synthesis, the metalloprotease domain is removed. In one model, the two fertilins are a dimer couple embedded in the sperm membrane. The disintegrin domain of β-fertilin binds to an integrin receptor protein in the egg plasma membrane. Binding induces a conformational change in α-fertilin, exposing a 23–amino acid hydrophobic region that inserts into the egg membrane, fusing the two cells (*1*, *24*). Cyritestin is another ADAM family member implicated in mammalian sperm-egg plasma membrane binding and fusion. A synthetic peptide representing the disintegrin domain of cyritestin and antibodies specific to this domain are potent inhibitors of mouse egg fertilization (*25*).

In summary, sea urchin sperm bindin, abalone sperm 18-kD protein, and vertebrate sperm fertilin/cyritestin are implicated as mediators of plasma membrane binding and fusion between sperm and egg. With the exception of the homology between fertilins and cyritestin, there is no detectable sequence homology among these three types of proteins, which suggests that proteins mediating sperm-egg fusion are diverse in their origin and evolutionary history.

What Fertilization Proteins Have Been Conserved During Evolution?

The biochemical reactions inside the sperm, such as the activation of sperm ion channels underlying induction of the AR, the fusion of the acrosomal vesicle membrane with the sperm plasma membrane, and the regulation of sperm flagellar motility, appear to be evolutionarily conserved across invertebrate and vertebrate phyla (*12*). Within the acrosomal vesicle, the protease acrosin is probably found in all vertebrate sperm. On the outside surfaces of the sperm membrane, the fertilins and cyritestins appear to be phylogenetically conserved among vertebrate classes. However, across invertebrate and vertebrate phyla, what are generally diversified among animals are the surface proteins sperm use to transmit signals to their interiors, attach to eggs, penetrate egg envelopes, and fuse with eggs.

Arguing conservation versus diversification in protein evolution is indeed problematic. For example, the class I major histocompatibility glycoproteins are highly conserved in all regions except the small antigen binding site, which varies greatly in sequence (*26*). Extracellular proteins frequently contain ancient protein modules found in all eukaryotes. When comparing phylogenetic conservation, the relative times of divergence of animal taxa must be considered. Each of the glycoproteins of the vertebrate egg envelope (in mammals, ZP1, ZP2, and ZP3) has homologs in all vertebrate species. For example, carp and human ZP3 homologs are 37% identical and 56% similar in amino acid sequence, and human and toad ZP3 homologs are 41% identical and 59% similar in amino acid sequence (*27*). However, these are all examples from classes of Phylum Vertebrata. Real conservation would be to find homologs of ZP proteins in the egg envelopes of invertebrate taxa.

Rapid Divergence and Selection in the Evolution of Fertilization Genes

In many organisms, mate choice involves complex mating rituals culminating in species-specific fertilization. However, in many invertebrate groups, which release gametes into seawater, species-specific interaction of proteins on the two gametes is the most important aspect of mate recognition. In such broadcast-spawning organisms, the divergence of only those few proteins mediating sperm-egg interaction could be enough to establish barriers to fertilization and hence new species. For example, in two species of Hawaiian sea urchins, strong barriers to fertilization have arisen whereas mitochondrial and nuclear genes have diverged little (*28*). The block to cross-species fertilization is in the failure of sperm bindin to attach the sperm to the egg envelope. Bindins of these species show high sequence variation and may be subjected to positive Darwinian selection, which suggests that there is some adaptive value in altering the bindin amino acid sequence (*28*).

Seven species of California abalone are broadcast spawners with overlapping habitats and breeding seasons. Mitochondrial DNA analysis suggests that four of these species diverged as recently as 1 to 2 million years ago (*29*). Analysis of nucleotide substitutions of the full-length sequences of lysins and 18-kD proteins shows that the evolution of both proteins has been promoted by positive Darwinian selection, meaning that there is adaptive value in altering the amino acid sequences of both proteins (*18*). The most likely hypothesis to explain the strong positive selection is that these sperm proteins are adapting to maintain proper interaction with their changing cognate egg surface receptors. The rates of evolution of abalone sperm lysin and 18-kD protein are 2 to 50 times faster than the rate of evolution of mammalian gamma-interferon and relaxin, two of the fastest evolving mammalian proteins yet discovered (*29*, *30*). In some com-

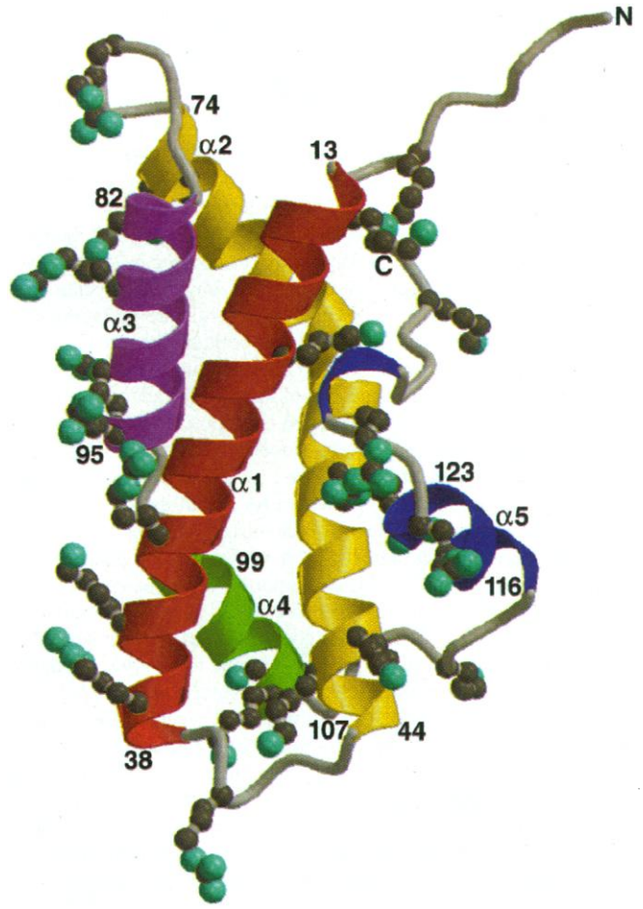

Fig. 2. Abalone sperm lysin: the crystal structure of a fertilization protein. The species-specific NH_2-terminus (N) projects away from the bundle of five α helices. The COOH-terminus (C) and NH_2-terminus lie on the same face of the molecule. The five α helices are numbered α1 to α5; numbers refer to amino acid position. The side chains of 23 arginine and lysin residues making up the two basic tracks are shown. Carbon atoms are gray; nitrogen atoms are cyan. Lysin has no binding cleft or pocket characteristic of an enzyme; it has the structure of a surface-active protein (*19*).

parisons, the genes for these fertilization proteins evolve faster than mitochondrial DNA (*29*). These data support the idea that differentiation of the gamete recognition genes can occur rapidly, creating barriers to cross-species fertilization.

Are there other fertilization proteins showing such dramatic evolutionary change as abalone lysin and 18-kD protein? The seven protein pheromones of the marine ciliate *Euplotes raikovi*, which control conjugation (mating), are remarkably divergent. The only conservation among them is in the positions of 7 amino acids out of 45 (*3*). In the unicellular green alga *Chlamydomonas reinhardtii*, the *mid* gene determines whether a cell is to be + or − mating type, and the *fus1* gene codes for a cell surface protein needed in fusion of + and − cells. Homologs of both genes were searched for in 12 other *Chlamydomonas* species. No genes with sequence homology to *fus1* were found, and only one species had a *mid* gene homolog to that of *C. reinhardtii* (*31*). These data indicate that extensive divergence of these two fertilization genes has occurred since speciation of *Chlamydomonas*.

The Evolution of Species-Specific Fertilization

The first biologists to observe fertilization were impressed by the degree of species specificity it exhibited. How might the species specificity of fertilization evolve? The study of abalone sperm lysin and its egg envelope receptor provides a possible molecular mechanism. As discussed above, lysin evolves rapidly by positive Darwinian selection (*18*, *29*). The reason for this remarkable rate of evolution might be found by studying lysin's receptor in the egg envelope. That receptor is a large fibrous glycoprotein composing 30% of the egg envelope (*32*). Each receptor molecule contains ~28 tandem repeats of a 153–amino acid sequence motif, and about two lysin molecules bind each repeat. Such tandem repeat sequences can be subjected to concerted evolution, a process by which unequal crossing over and gene conversion propagate and homogenize the repeat sequences within a species (*33*). Thus, repeat sequences become more similar to each other within a species than between any two species. The redundant construction of the receptor could tolerate mutations that disfavor lysin binding to some repeats, because the other repeats would be initially unchanged. If concerted evolution propagated the mutant repeat, selective pressure would increase for adaptive changes to occur in lysin. Eventually, concerted evolution would homogenize the receptor with this new repeat sequence. Put more simply, the female gamete component changes first by concerted evolution, and the cognate male component is selected for its ability to bind to new female repeat sequences. Such a mechanism would be continuous, require no external forces, and could operate within a population. The ultimate result would be the differentiation of the gamete recognition system by concerted evolution of a receptor and adaptive evolution by positive selection on its ligand. This coevolution of sperm lysin and its egg receptor would operate as species continue to diverge, ultimately creating molecular barriers to cross-species fertilizations (*33*).

Abalone sperm lysin and its egg envelope receptor are the only sperm-egg dyad for which species-specific binding and affinity constants have been determined in vitro. Knowledge of other sperm-egg dyad fertilization proteins is sorely needed. Whether concerted evolution operates on the divergence of other fertilization proteins is unknown. For concerted evolution to operate, genes must have tandem repetitive sequence elements. Whether tandem repeats will be found in one member of each sperm-egg molecular dyad remains unknown. However, like the abalone lysin receptor, other fertilization proteins in worms (*21*) and mammals (*34*) contain tandem repeat sequences that could be subjected to concerted evolution.

Conclusions

The study of fertilization has a rich history and a large literature that cannot be adequately covered in this sort of synthesis. Fertilization is more fascinating today than at any time in the past, because today we can isolate proteins, clone genes, and determine the three-dimensional structures of proteins. We are only now beginning to identify and study the structure of fertilization molecules. Gamete recognition is much more complicated than we imagined a decade ago. The discovery of new fertilization proteins and their genes will continue in model organisms, and solution of the three-dimensional structures of sperm and egg receptor-ligand complexes should be possible. Increased knowledge of fertilization molecules will be important in developing novel methods of nonhormonal contraception in higher species.

Most animal species exhibit complex mate recognition phenotypes that mediate species-specific reproduction. Finding the genes responsible for such phenotypes is a daunting task. However, in some cases, mate recognition phenotypes are determined by the interaction of the protein products of only a few genes found on the surfaces of the gametes. Continued study of the genes of fertilization systems will yield new information on the evolution of reproductive specificity.

References and Notes

1. G. S. Kopf, in *Encyclopedia of Reproduction*, E. Knobil and J. D. Neill, Eds. (Academic Press, San Diego, CA, 1998), vol. 1, pp. 17–26; S. B. McLeskey, C. Dowds, R. Carballada, R. R. White, P. M. Saling, *Intern. Rev. Cytol.* **177**, 57 (1998); K. R. Foltz, *ibid.* **163**, 249 (1995).
2. W. Boland, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 37 (1995).
3. P. Luporini, A. Vallesi, C. Miceli, R. A. Bradshaw, *J. Eucary. Microbiol.* **42**, 208 (1995); H.-W. Kuhlmann, C. Brunen-Nieweler, K. Heckmann, *J. Exp. Zool.* **277**, 38 (1997).
4. R. L. Miller and R. Vogt, *J. Exp. Biol.* **199**, 311 (1996); R. L. Miller, *J. Exp. Zool.* **279**, 189 (1997).
5. N. Suzuki, *Zool. Sci.* **12**, 13 (1995).
6. D. M. Hardy, T. Harumi, D. L. Garbers, *Semin. Dev. Biol.* **5**, 217 (1994).
7. M. Eisenbach, *Curr. Opin. Endocrinol. Diabetes* **2**, 468 (1995).
8. T. Nishigaki, K. Chiba, W. Miki, M. Hoshi, *Zygote* **4**, 237 (1996); S. Koyota, K. M. S. Wimalasir, M. Hoshi, *J. Biol. Chem.* **272**, 10372 (1997).
9. A.-P. Alves, B. Mulloy, J. A. Diniz, P. A. S. Mourao, *J. Biol. Chem.* **272**, 6965 (1997); V. D. Vacquier and G. W. Moy, *Dev. Biol.* **192**, 125 (1997).
10. G. W. Moy, L. M. Mendoza, J. R. Schulz, C. G. Glabe, V. D. Vacquier, *J. Cell Biol.* **133**, 809 (1996); R. Sandford *et al.*, *Hum. Mol. Gen.* **6**, 1483 (1997).
11. J. Chen, E. S. Litscher, P. M. Wassarman, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 6193 (1998).
12. A. Darszon, A. Lievano, C. Beltran, *Curr. Top. Dev. Biol.* **34**, 117 (1996).
13. S. Meizel, *Biol. Reprod.* **56**, 569 (1997).
14. V. D. Vacquier, W. J. Swanson, M. E. Hellberg, *Dev. Growth Differ.* **37**, 1 (1995); A. Hoffman and C. G. Glabe, *Semin. Dev. Biol.* **5**, 233 (1994).
15. M. Hoshi, S. Takizawa, N. Hirohashi, *Semin. Dev. Biol.* **5**, 201 (1994).
16. K. Yamagata *et al.*, *J. Biol. Chem.* **273**, 10470 (1998).
17. N. Kohno *et al.*, *Biochem. Biophys. Res. Commun.* **245**, 658 (1998).
18. V. D. Vacquier, W. J. Swanson, E. C. Metz, C. D. Stout, *Adv. Dev. Biochem.*, in press; V. D. Vacquier, W. J. Swanson, Y.-H. Lee, *J. Mol. Evol.* **44** (Suppl. 1), S15 (1997).
19. A. Shaw, D. E. McRee, V. D. Vacquier, C. D. Stout, *Science* **262**, 1864 (1993); A. Shaw, P. A. G. Fortes, C. D. Stout, V. D. Vacquier, *J. Cell Biol.* **130**, 1117 (1995).
20. P. J. Ferris, J. P. Woessner, U. W. Goodenough, *Mol. Biol. Cell* **7**, 1235 (1996).
21. A. Singson, K. B. Mercer, S. W. L'Hernault, *Cell* **93**, 71 (1998).
22. W. J. Swanson and V. D. Vacquier, *Biochemistry* **34**, 14202 (1995).
23. A. S. Ulrich, M. Otter, C. G. Glabe, D. Hoekstra, *J. Biol. Chem.* **273**, 16748 (1998).
24. E. A. C. Almeida *et al.*, *Cell* **81**, 1095 (1995); J. P. Evans, G. S. Kopf, R. M. Schultz, *Dev. Biol.* **187**, 79 (1997); R. Hooft van Huijsduijnen, *Gene* **206**, 273 (1998); L. Lum and C. P. Blobel, *Dev. Biol.* **191**, 131 (1997); F. M. Shilling *et al.*, *ibid.* **186**, 155 (1997); T. G. Wolfsberg and J. M. White, *ibid.* **180**, 389 (1996).
25. R. Yuan, P. Primakoff, D. G. Myles, *J. Cell Biol.* **137**, 105 (1997).
26. S. V. Edwards and P. W. Hedrick, *Trends Ecol. Evol.* **13**, 305 (1998).
27. H. Kubo *et al.*, *Dev. Growth Differ.* **39**, 405 (1997); J. C. Yang and J. L. Hedrick, *ibid.*, p. 457.
28. S. R. Palumbi and E. C. Metz, *Mol. Biol. Evol.* **8**, 227 (1991); E. C. Metz, R. E. Kane, H. Yanagimachi, S. R. Palumbi, *Biol. Bull.* **187**, 23 (1994); E. C. Metz and S. R. Palumbi, *Mol. Biol. Evol.* **13**, 397 (1996).
29. E. C. Metz, R. Robles-Sikisaka, V. D. Vacquier, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
30. W.-H. Li and D. Graur, *Fundamentals of Molecular Evolution* (Sinauer, Sunderland, MA, 1991); H. F. Rosenberg, K. D. Dyer, H. L. Tiffany, M. Gonzales, *Nature Genet.* **10**, 219 (1995).
31. P. J. Ferris, C. Pavlovic, S. Fabry, U. W. Goodenough, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 8634 (1997).
32. W. J. Swanson and V. D. Vacquier, *ibid.*, p. 6724.
33. _____, *Science* **281**, 710 (1998).
34. Z. Gao and D. L. Garbers, *J. Biol. Chem.* **273**, 3415 (1998); L. H. Bookbinder, A. Cheng, J. D. Bleil, *Science* **269**, 86 (1995).

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