COMMENTARY

# A protein bridging the gap between sea urchin generations

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In the second half of the 1800s biologists began to investigate the cellular basis of development. Sea urchins became a favorite material for this endeavor because their gametes can be obtained in vast numbers and fertilization and development are external to the adult body. The first research on fertilization centered on watching sperm interact with eggs. In most invertebrates such as sea urchins the sperm bind to the eggs before the two very different cells fuse. The paper by Wessel et al. (1) focuses on Bindin, the sea urchin sperm protein binding sperm to the egg receptor EBR1 (2–4). CRISPR-Cas9 methods knocked out (KO) Bindin synthesis as demonstrated by Western blots and immunofluorescence. Sperm from KO males could not bind or fuse with eggs. These results prove that sperm Bindin is required for sea urchin fertilization.

## Bindin's Structure

The initial translation product is a PreproBindin of ~460 to 485 amino acids. The Prepro portion is ~244 to 254 amino acids with a Furin site at its C terminus. Furin cleavage creates a mature Bindin of ~218 to 284 amino acids, which is packaged into the acrosomal secretory vesicle. When sperm contact eggs, glycoconjugates trigger acrosomal exocytosis, exposing Bindin on the sperm membrane. The sperm bind to the egg EBR1 receptor and fusion of both cells occurs and the egg draws the sperm into its cytoplasm. The central region of mature Bindin contains the B18 peptide, which does not vary among species. A second Bindin peptide, B55, is a potent membrane-perturbing agent and is used to unload endosomal vesicles containing dextrans, antibodies, RNase, and plasmid DNA into the cytoplasm of cultured cells (5). An unproven hypothesis is that B18 and B55 fuse sperm and egg membranes. All of B18 and most of B55 are present in starfish Bindin with one amino acid difference in 35 positions compared to sea urchin Bindin. This sequence has been preserved in sea urchins and starfish for at least 450 million y (6). B18 and B55 could become commercial reagents to fuse cells and also get large molecules through the plasma membrane.

## Expression of Bindin and EBR-1

Wessel et al. (1) required 1.5 y to culture KO urchins from zygote to adult. To continue studying Bindin, expression systems using cultured cells must be developed as was done for mammalian sperm proteins (7–11). Cryo-electron microscopy (CryoEM) structures of PreproBindin with the Furin site deleted should be compared to mature Bindin to see if the long proregion covers B18 and B55 to protect spermatocyte membranes from the membrane-perturbing properties of these peptides. Expression of Bindins with substitutions in B18 and B55 in cultured cells and lipid vesicles should be attempted. Green fluorescent protein–Bindins could be produced that quantitatively and species-specifically bind the egg surface. Immobilized expressed Bindin could be used to isolate ERB1. Heterologous expression of EBR1 domains as smaller repeat units should be an experimental goal. The structural regions of Bindin and EBR1 having affinity for each other should be determined by cryoEM. Portions of Bindin sequences from different species should be exchanged to create recombinant Bindins to define regions governing species-specific binding. The Bindin- and EBR1-expressing cultured cells might bind each other and allow for detailed structural studies (7, 8). One hypothesis is that Bindin uses its species-specific regions to bind to ERB1 and then mediates a nonspecies-specific fusion of the two cells using B18 and B55.

## Indispensable Proteins Mediating Fertilization

Bindin was the first protein shown to mediate sperm binding to eggs (2). Mouse genetics, rapid sequencing, gene editing, and heterologous expression were used to discover mammalian sperm proteins mediating fertilization. Izumo1 is a vertebrate sperm transmembrane protein that binds to egg Juno (7, 8). Izumo1 is indispensable for fertilization in mammals and probably all vertebrates. The interacting binding sites between these two proteins have been determined (7, 8). Such detailed information is important to have for Bindin and EBR1. Knowing the interacting domains between Izumo1 and Juno could lead to design and synthesis of

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low-molecular-weight compounds with high affinity to either protein, which should prevent the binding of the two proteins and result in blocking fertilization. Such nonhormonal, nonimmunologic fertilization inhibitors would be ideal contraceptives if they were nontoxic and their inhibition reversible. In addition to Izumo1, other sperm proteins required for fertilization are DCST1, DCST2, SPACA6, FIMP, SOF1, and TMEM95 (9–11). Sperm–egg interaction in sea urchins with external fertilization might not be as complicated as in mammals with internal fertilization and thus be mediated by fewer proteins. Mammalian sperm fuse to the egg membrane by their equatorial segment, whereas sea urchin sperm fuse with eggs with the tip of the acrosome process. The six mammalian sperm proteins required for fertilization compared to only one for sea urchins may reflect the mammalian genome's being four times larger than the sea urchin genome.

## Bindin and Speciation

Could changes in Bindin sequences establish reproductive isolation within populations and be a mechanism for speciation of sea urchins? If correct, this hypothesis could explain the strong barriers to cross-fertilization that have evolved between closely related sympatric, congeneric species. Space constraints prohibit comprehensive acknowledgment of all contributors and the reader is referred to three papers for most past work (12–14). Pairwise comparisons of Bindins from sympatric, congeneric species may show many codon differences that change the amino acid. Such "nonsynonymous" nucleotide substitution is termed "positive selection" and shows there is selective benefit (increased fitness) to alter the amino acid sequence. Conversely, if pairwise codon comparisons show mainly "synonymous" nucleotide substitutions that do not change the amino acid then the Bindin is said to be evolving "neutrally." Closely related species can show blockage of sperm binding and strong positive selection in their Bindins (15). However, Bindin sequences can also evolve slowly between two sympatric congeneric species and show no species-specific sequences and no specificity in fertilization (16). Slow, neutral Bindin evolution has also been found in eight species of *Diadema* sea urchins, with only one species showing positive selection (14). Prezygotic blocks to sperm binding could occur as a result of positive selection in Bindin when speciation is beginning (15). In cases where cross-fertilization between species readily occurs postzygotic blocks such as reproductive sterility could maintain species purity. This is shown to be the case in mass spawning of reef coral species where many F1 interspecies hybrids show high sterility (17). It would be important, but impractical, to grow sea urchin F1 hybrids to maturity to determine if they are sterile. Because sea urchin generations are long it probably will not be able to answer the question of whether Bindin plays a critical role in speciation. In hybrids between two species, which Bindin allele is expressed, the one from the egg or the one from the sperm? If both alleles are expressed, do back-crosses to either parent at controlled sperm concentrations show a decrease in percent fertilization? Unfortunately, such experiments take too long to be practical. When we analyze Bindin and EBR1 we can only infer how they have changed during millions of years of evolution.

## Continuing Studies of Gamete Recognition Proteins

Although our knowledge is increasing, we can still report that animal fertilization is one of the least understood fundamental biological processes. Progress in the discovery and function of mammalian fertilization proteins has made enormous gains using crystallography, gene editing, and heterologous expression. The complexity of mammalian gamete interaction compared to sperm and egg interaction occurring in seawater could be one reason why fewer proteins are required for fertilization in sea urchins. In the case of Bindin, a three-dimensional (3D) structure is needed to inform us whether it belongs to a known protein family. The paper by Wessel et al. (1) proves definitively that sea urchin sperm Bindin mediates sperm binding to EBR1 on the egg envelope. If these are the only two proteins mediating sperm–egg interaction then they must also mediate sperm–egg fusion, but that has yet to be proven. Expressed B55 bound to fluorescent beads or B55 incorporated into fluorescent multilamellar vesicles could be a way to test the hypothesis.

## Looking Deeper

Until recently we could not detect ancient evolutionary relationships among gamete recognition proteins and concentrated solely on amino acid sequences. Mammalian sperm bind to the ZP3 glycoprotein, one of the four glycoproteins comprising the zona pellucida envelope of the egg. Crystallography shows that the N-terminal half of ZP3, named ZP-N, is a novel immunoglobulin (Ig) fold (18). This same 3D fold is found in abalone egg VERL, the egg envelope receptor for sperm lysin, and the yeast mating protein VEZP14. These three organisms span most of the tree of eukaryotic life (18). Mammalian Izumo1 also has an Ig fold (7, 8), as does *Caenorhabditis elegans* sperm protein SPE-45 (19). Mouse sperm DCST1 is considered to be an ortholog of *C. elegans* SPE-49 and *Drosophila* SNEAKY and mouse DCST2 an ortholog of *C. elegans* SPE42 and *Drosophila* DCST2, all these proteins being involved in reproduction (11). Structural data on gamete recognition systems in a variety of animals would be welcomed (20). Future discovery may show that most, if not all, gamete recognition proteins evolved from the same protein folds that arose once and then structurally and functionally differentiated through time.


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