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SPOTLIGHT

Mammalian fertilization: Does sperm IZUMO1 mediate fusion as well as adhesion?

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The molecular mechanism of sperm–egg fusion is a long-standing mystery in reproduction. Brukman and colleagues (2022. *J. Cell Biol.* https://doi.org/10.1083/jcb.202207147) now provide evidence that the sperm surface protein IZUMO1, which is essential for mammalian fertilization, can induce membrane fusion in cultured cells.

The basic genetic principles of sexual reproduction are remarkably constant across a wide range of different organisms: diploid cells partition their genetic material into two haploid cells that, at some stage of their lifecycle, are segregated into different mating types or sexes which recognize one another and fuse at fertilization to recreate a single diploid cell (1). In mammals, the haploid cells differ significantly in their size and shape: the female egg is large and spherical, whereas the male sperm is small, elongated, and highly motile. For successful fertilization, these two cells must first recognize and adhere to each other before finally fusing. For many mammals—including humans—fertilization can successfully be performed in vitro, meaning that we have a good cellular description of this process; however, we are only just learning which molecules are involved and what their biological roles are.

IZUMO1 was the first sperm cell surface protein shown to be essential for mammalian fertilization and importantly passed the stringent in vivo test of demonstrating that male mice lacking a functional *Izumo1* gene were infertile (2). Prior to this, targeted knock out of sperm cell surface proteins that had been suggested to be required for fertilization resulted in male mice that remained fertile (3). In the case of IZUMO1 knock outs, the sperm were ostensibly normal: they looked and moved like wild-type sperm but were unable to finally fuse and fertilize the egg.

Since this founding discovery, the molecular mechanism of IZUMO1 function as either mediating adhesion between the sperm and egg membranes or actively driving the fusion of the membranes has been a topic of debate. These two processes are likely to be distinct and ordered: adhesion must first align areas of apposing gamete plasma membranes to within around 10 nm, followed by the action of a fusogen to overcome the thermodynamic barrier that normally prevents inappropriate membrane fusion between neighboring cells (4).

Perhaps surprisingly, experimentally distinguishing between an adhesive and fusogenic role is not trivial: they require specifically designed assays and their interdependence means that even when only adhesion is defective, cellular fusion will not occur. It was conceivable that IZUMO1 could act independently of other factors because some fusogens—such as those used by viruses—are able to act in a unidirectional manner meaning they only need to be present on one of the two fusing cells. By contrast, proteins mediating adhesion usually require a specific binding partner displayed on the apposing cell; however, identifying these extracellular interactions which are typified by very weak binding affinities is technically challenging (5).

Clues that IZUMO1 had an adhesive role came when a receptor displayed on eggs called JUNO was identified (6). Confirmatory studies have shown that both mice and rats lacking IZUMO1 produce normal-looking sperm that are unable to bind eggs (7). There is evidence, however, that IZUMO1 could have additional roles other than just JUNO binding (8). Could IZUMO1 also function as a fusogen?

In this issue, Brukman and colleagues showed that IZUMO1 could act directly as a fusogen in the Baby Hamster Kidney fibroblast (BHK) cell line (9). When IZUMO1 was overexpressed in BHKs, they observed cells with two or more nuclei, an effect that could not be ascribed to a failure in cytokinesis, and they also confirmed that cell fusion occurred using a content-mixing assay. The level of fusion events was low but comparable with that obtained with the established fusogen HAP2.

Using structure-guided mutagenesis, the authors separated the adhesive and fusogenic activities of IZUMO1: the single mutant W148A prevented binding to JUNO but did not affect fusion whereas the triple mutant (F28A, W88A, and W113A) retained the ability to bind JUNO but did not induce fusion. These data indicated that the adhesive and fusogenic roles are performed by two distinct and functionally separable regions of the IZUMO1 protein.

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While the results obtained with the hamster cell line were clear, the picture became more complex when cells and gametes were mixed in fusion assays. The authors found that sperm fused only to JUNO-expressing BHK cells, indicating that the adhesion step driven by JUNO-IZUMO binding was required and that IZUMO1 was not sufficient to induce cell fusion in this assay. Remarkably, while IZUMO1 behaved as a unilateral fusogen in the transfected cells, the authors found that the heterologous expression of the protein was not sufficient to fuse cells and eggs. This is in agreement with previous reports that showed that IZUMO1 ectopically expressed on cell lines adhered to—but did not fuse with—eggs (10). In summary, the mixed cell–gamete assays suggested that JUNO-mediated binding was required for fusion of sperm and cells, but for eggs to fuse with IZUMO1-expressing cells, an additional factor is required. Sperm and eggs are terminally differentiated cells that must fuse with each other, but it is equally important that they do not fuse with other cells in the body. In sperm, this is probably achieved by sequestering IZUMO1 within the cell and making it available at the sperm surface only following the acrosome reaction as the sperm approaches the egg. The extracellular matrix surrounding the egg, the zona pellucida, shields the egg membrane from coming into direct contact with other cells, but it is conceivable that a molecule or perhaps intrinsic feature, such as the thick actin cortex that lines the oolemma, inhibit cell fusion.

The successful manipulation of murine and human gametes in vitro in the 1960s and 1970s (11) brought the promise of quick discoveries, but the dissection of the molecular mechanisms of fertilization has proven more difficult than anticipated (5). While we can biochemically investigate the interactions of cell surface proteins, studying their mechanisms of action in the context of the cell membrane remains challenging. Here, Brukman and colleagues use content mixing and multinucleation as a proxy for measuring cell fusion; consequently, fusion events could be the result of other phenomena such as endocytosis, trogocytosis, nondisjunction of sister cells, and transfer of cargo molecules via special structures like tunneling nanotubes (12, 13). The development of better techniques to monitor cell adhesion, cell fusion, and the dynamics of membrane proteins in real time will shed further light on the function of the sperm proteins during fertilization (14). Crucially, the lack of a suitable cell line for mammalian gametes represents a major limitation within the field meaning that transgenic animals remain necessary to confirm the validity of observations obtained from in vitro assays. For example, the generation of an IZUMO1 transgenic mouse line carrying the triple mutation that retained JUNO binding but lost fusogenic activity would provide important data to support the new role proposed for IZUMO1.

The work from Brukman and colleagues represents a valuable contribution to untangle the molecular mechanisms of fertilization. By unveiling a novel role of the sperm protein IZUMO1, it prompts the deployment of similar approaches to better understand the function of other essential sperm surface proteins such as the structurally similar SPACA6 and TMEM95 (15, 16) and to understand the mechanisms of cell fusion in somatic cells such as skeletal muscle and the placenta.

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