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Egg's ZP3 Structure Speaks Volumes

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Binding of mammalian sperm to eggs depends in part on ZP3, a glycoprotein in the egg's extracellular coat, the zona pellucida. In this issue, Han et al. (2010) describe the structure of an avian ZP3 homolog, providing insights into ZP3 processing and polymerization and the roles of the ZP3 polypeptide and its carbohydrate in sperm binding.

The plasma membrane of mammalian eggs is surrounded by a relatively thick extracellular coat called the zona pellucida (ZP). It is composed of long interconnected fibrils that consist of only a few proteins held together by noncovalent interactions. For example, the mouse egg's ZP consists of three glycosylated proteins, called ZP1–3, that are synthesized, secreted, and assembled by growing oocytes (Wassarman, 2008). ZP proteins have been conserved for more than 600 million years, and proteins closely related to ZP1–3 are found in the ZP of all mammalian eggs, including humans, as well as in the extracellular coat (vitelline envelope) of nonmammalian eggs. During fertilization, sperm must bind to and then penetrate the ZP in order to reach and fuse with the egg's plasma membrane to produce a zygote. It has been known for some time that sperm bind to the ZP of unfertilized eggs but do not bind to the ZP of fertilized eggs (Figure 1A) (Florman and Ducibella, 2006). In this context, a wide variety of evidence suggests that ZP3 functions as a receptor during binding of sperm to eggs (Wassarman and Litscher, 2008). Both ZP3 polypeptide and its attached carbohydrate groups have been implicated in binding of sperm to the ZP, but it has not been possible to reconcile the results of three decades of experiments on ZP3 with a three-dimensional structure for the protein. Now Han et al. (2010) overcome the many problems associated with crystallization of ZP3 and determine the structure of full-length chicken ZP3 (cZP3) at 2.0 Å resolution by X-ray crystallographic methods.

All ZP proteins are synthesized as precursor polypeptides possessing an N-terminal signal sequence and a C-terminal propeptide that contains a transmembrane domain, a protease cleavage site, and a hydrophobic patch (external hydrophobic patch, EHP) (Figure 1B). The latter is thought to interact with another hydrophobic patch (internal hydrophobic patch, IHP) along the nascent polypeptide to prevent premature polymerization of ZP proteins. During secretion of ZP proteins the propeptide, including the EHP, is excised from nascent polypeptides, thereby enabling them to polymerize. Furthermore, ZP proteins are founding members of a very large class of proteins that have diverse functions and are found in a variety of tissues in all multicellular eukaryotes (Jovine et al., 2005). All of these proteins possess a ZP domain that consists of ~260 amino acids and 8–12 conserved Cys residues present as disulfides. Each ZP domain has an N-terminal (ZP-N) and C-terminal (ZP-C) subdomain separated by a short linker region (Figure 1B). The structure of ZP-N represents a new subtype of the immunoglobulin (Ig)-like fold (Monné et al., 2008) and is thought to be responsible for generating polymers of ZP proteins. In this context, it has been shown that mutations in ZP-N can result in severe pathologies, such as infertility, deafness, and cancer. It is likely that polymers assembled by different types of ZP domain proteins share a similar structure.

The structure of cZP3 provides a wealth of information about ZP proteins (Figure 1C). Han et al. (2010) show that ZP-C adopts an Ig-like fold with the same topology as ZP-N, suggesting that ZP proteins may have arisen by duplication of a common Ig-like domain. Within crystals, cZP3 forms antiparallel dimers held together by interactions between ZP-N and ZP-C of opposing molecules, and Han et al. (2010) show that dimer formation is essential for cZP3 secretion from cells. These findings are consistent with the propensity of purified ZP proteins to polymerize in vitro and with the inability of mouse oocytes lacking either ZP2 or ZP3 to assemble a ZP in vivo (Wassarman, 2008). The latter has been attributed to the failure to form intracellular ZP2-ZP3 dimers that can then polymerize in the extracellular space into long fibrils. From the structure of cZP3 it appears likely that disulfides of the ZP-C subdomain determine whether ZP proteins form homo- or heteropolymers. However, additional experiments that address this issue, including the generation of mutant ZP proteins, will be required to confirm such a role for ZP-C disulfides.

The structure of cZP3 reveals that, as previously proposed (Jovine et al., 2005), the EHP present in the propeptide acts as a "molecular glue" that maintains the dimer in a conformation required for secretion but that is incompatible with polymerization of the dimer into higher-order structures. The structure of the cZP3 dimer also suggests that the transmembrane region of the propeptide may specifically orient the precursor molecule during proteolytic processing at the oocyte membrane, thus enabling it to be incorporated into the ZP. Indeed, this conclusion is consistent with previous findings (Jovine et al., 2005).

It has been proposed that the C-terminal region of ZP3 lying just downstream of its ZP domain is, at least in part, the binding site for sperm (Figure 1B) (Wassarman and Litscher, 2008). Several studies have concluded that this particular region of the polypeptide

exhibits considerable interspecific sequence diversity due to positive Darwinian selection (Turner and Hoekstra, 2008) and could form the basis of species-restricted fertilization. On the other hand, whether sperm binding to ZP3 depends on the protein's polypeptide, carbohydrate, or both is unclear. Although a role for carbohydrate in many other types of cell-cell adhesion is well established (Varki et al., 2009), its role in sperm-egg interaction remains controversial (Clark and Dell, 2006).

Han et al. (2010) address the role of ZP3's carbohydrate in sperm binding directly because their engineered cZP3 possesses a single O-glycan, probably Galβ1-3GalNAc, linked to threonine 168. The glycan is located on the surface of cZP3 in a flexible region of the polypeptide and should be readily accessible to sperm (Figure 1C). Thus, the glycan, together with the nearby cZP3 hypervariable C-terminal polypeptide, could form a docking platform for sperm. Han et al. (2010) analyze the binding of chicken sperm to wild-type cZP3 and to a mutant cZP3 in which threonine 168 was converted to alanine. They find that elimination of the O-glycan causes a large decrease (~80%) in sperm binding to cZP3. This result provides convincing evidence for a role of this carbohydrate in sperm binding to cZP3. It is of interest that this O-glycan site, called site 1, is retained from cZP3 to human ZP3. Another O-glycan site, called site 2, lies very close to site 1 and may also be involved in sperm binding.

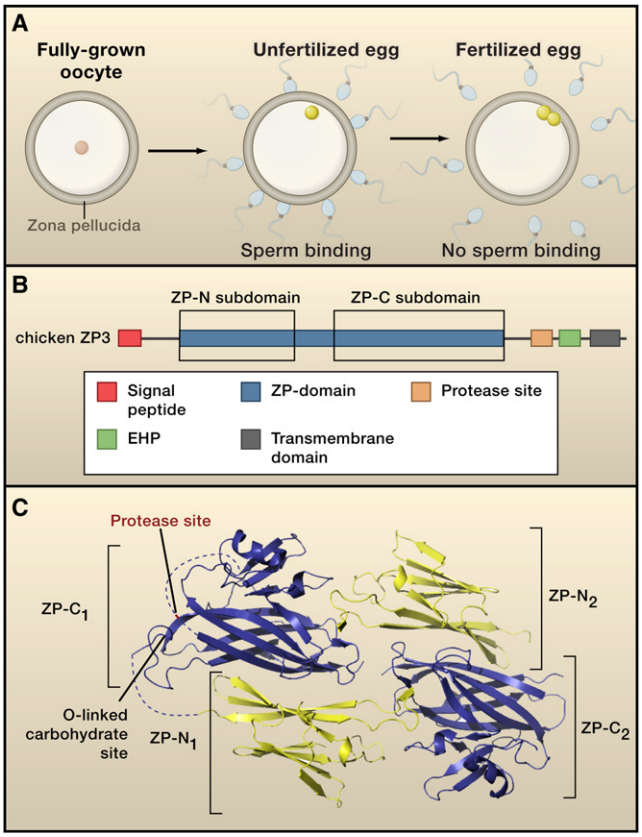

Figure 1. Clues to Sperm-Egg Binding

(A) A fully grown oocyte is surrounded by a thick extracellular coat, the zona pellucida (ZP), that is composed of glycoproteins. Sperm bind tightly to the ZP of unfertilized eggs, but they are unable to bind to the ZP of fertilized eggs because the ZP glycoproteins are modified following fertilization.

(B) The glycoprotein ZP3 is a key component of the ZP of all mammalian eggs and apparently serves as a receptor for sperm binding. The mature ZP3 polypeptide has an N-terminal signal sequence (red), a ZP domain that consists of two subdomains, ZP-N and ZP-C (blue), and a C-terminal region that has a protease cleavage site (yellow), an external hydrophobic patch (EHP, green), and a transmembrane domain (gray).

(C) In the X-ray crystallographic structure of an avian ZP3 homolog, the glycoprotein forms a dimer in which the ZP-N subdomain of one molecule interacts with the ZP-C subdomain of another molecule to hold the dimer together (Han et al., 2010).

In a recent report, Gahlay et al. (2010) concluded that sperm fail to bind to the ZP of fertilized eggs due to limited proteolysis of ZP2 shortly after fertilization (Figure 1A). Han et al. (2010) suggest that these findings may be due to structural rearrangements within the extracellular coat following fertilization that result in shielding of the ZP3-binding surface (i.e., its polypeptide and O-glycan). However, this explanation does not account for several observations. First, ZP3 purified from unfertilized egg ZP inhibits binding of sperm to eggs, but ZP3 purified from fertilized egg ZP does not. Second, solubilized ZP and purified ZP3 from unfertilized eggs induce sperm to undergo cellular exocytosis (i.e., the acrosome reaction), but solubilized ZP and purified ZP3 from fertilized eggs do not. Rather, these observations indicate that ZP3 is somehow modified shortly after fertilization, possibly by cortical granule enzymes, and thereby inactivated as a receptor for sperm. Further structural studies are needed to resolve this thorny issue.

In conclusion, the paper by Han et al. (2010) is a major breakthrough in the pursuit of mechanisms involved in mammalian fertilization. Comparable structural studies on other ZP proteins, as well as on other sperm and egg proteins thought to participate in fertilization, may lead to an understanding of mutations that cause infertility, the development of new means of contraception, and other advances in human reproduction.

REFERENCES

Clark, G.F., and Dell, A. (2006). J. Biol. Chem. *281*, 13853–13856.

Florman, H.M., and Ducibella, T. (2006). Mammalian fertilization. In Physiology of Reproduction, J.D. Neill, ed. (New York: Academic Press), pp. 55–112.

Gahlay, G., Gauthier, L., Baibakov, B., Epifano, O., and Dean, J. (2010). Science *329*, 216–219.

Han, L., Monné, M., Okumura, H., Schwend, T., Cherry, A.L., Flot, D., Matsuda, T., and Jovine, L. (2010). Cell *143*, this issue, 404–415.

Jovine, L., Darie, C.C., Litscher, E.S., and Wassarman, P.M. (2005). Annu. Rev. Biochem. *74*, 83–114.

Monné, M., Han, L., Schwend, T., Burendahl, S., and Jovine, L. (2008). Nature *456*, 653–657.

Turner, L.M., and Hoekstra, H.E. (2008). Int. J. Dev. Biol. *52*, 769–780.

Varki, A., Cummings, R.D., Esko, J.D., Freeze, H.H., Hart, G.W., and Etzler, M.E. (2009). Essentials of Glycobiology, Second Edition (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press)., pp 784.

Wassarman, P.M. (2008). J. Biol. Chem. *283*, 24285–24289.

Wassarman, P.M., and Litscher, E.S. (2008). Int. J. Dev. Biol. *52*, 665–676.