

life's boundaries

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There is only one way of propagating the species, and that is by mating. However, for many animals, mating usually implies hordes of sperm all fighting to get their nucleus into one egg. The same goes for humans. It is perhaps an odd thing in the first place for Nature to have devised what seems to be an uneconomical procedure, and if an oocyte is fertilised by more than one spermatozoon, the ensuing zygote is not viable. So it was necessary to develop some *modus operandi* by which one sperm is allowed in, while the others are kept out. In fact, over time, animals have armed themselves with more than one strategy to avoid polyspermy. One of the most definitive is to act upon the zone which surrounds an oocyte – the zona pellucida – by making it impenetrable the moment one sperm has wriggled its way through it. Scientists have known for many years that this particular region changes its structure following fertilisation but they didn't know what caused the change. Until they discovered a protease, which has been dubbed ovastacin.



Sperm and ovum (2006), by Holly Roberts

Courtesy of the artist

There are many ways of dealing with polyspermy. Different animals use different methods; some even use more than one. Take the sea urchin for instance. Sea urchins use two types of polyspermy block: electrical and mechanical. Initially, the oocyte is charged negatively and the sperm positively – thus creating favourable ground for gamete fusion. However, upon fertilisation, the sea urchin oocyte becomes positively charged causing other eager sperm to bounce off it. This change in the electrical state of the oocyte is almost instantaneous. An additional mechanical change

occurs later, whereby the zona pellucida surrounding the oocyte's membrane changes structure and becomes impenetrable to sperm – a system used by mammals too. Mice use three post fertilisation blocks: one which prevents a second sperm from fusing with the oocyte's membrane, a second which stops sperm from penetrating the zona pellucida, and yet a third which stops sperm from actually binding to the zona pellucida in the first place. What is more, besides these forms of polyspermy block, many organisms reduce the number of sperm further upstream in the process, as the gametes make their convoluted way to the oocyte.

In mice, the nature of the zona pellucida changes swiftly following fertilisation, making it impossible for any additional sperm to penetrate it – though many get trapped in it. It is a fact that has been known for many years but scientists didn't know what was happening on the molecular level. Until they discovered ovastacin. Ovastacin is a proteolytic enzyme which belongs to the astacin family of metalloproteases, and is found predominantly in ovaries. Proteolytic enzymes are of huge importance since they are at the basis of all sorts of molecular regulation – and are therefore involved in instances such as cell cycle progression, tissue morphogenesis, cell proliferation, ovulation and apoptosis to name a few. In short, proteolytic enzymes are paramount in keeping an organism alive and kicking. Processed in the Golgi apparatus

during oogenesis, ovastacin is stored in cortical granules. Upon sperm-oocyte fusion, hundreds of cortical granules float to the oocyte inner membrane, where they fuse and spill out their contents – amongst them ovastacin – into the zona pellucida.

The zona pellucida is a protective sheath which surrounds the surface of the oocyte. It is composed mainly of three glycoproteins – ZP1, ZP2 and ZP3 – that form a sort of scaffold through which the sperm have to travel in order to reach the oocyte's plasma membrane. If a sperm manages to cross the entire zone, it then fuses to the oocyte per se and releases its nucleus into the egg's cytoplasm. Meanwhile, to scare off the fusion of a second sperm, the contents of the cortical granules are released into the zona pellucida and set about modifying its overall structure. In doing so, they are actually erecting a barrier to polyspermy. Ovastacin is directly involved in this transformation. How? Before fertilisation, sperm are attracted to the oocyte's surface and bind to the zona pellucida by way of ZP2. Immediately following fertilisation, ovastacin is released and cleaves ZP2, thereby demolishing the means for additional sperm to dock to the zona pellucida.

Surprisingly, besides being involved in the process of post fertilisation and polyspermy block, ovastacin also has a role in promoting

gamete fusion. Indeed, oocyte membrane-bound ovastacin – also known as SAS1B for Sperm Acrosomal SLLP1 Binding – is thought to bind tightly to SLLP1 which is found on the sperm's acrosome thus bringing the two gametes even closer. So far, SAS1B (or ovastacin) seems to be the only oocyte metalloproteinase which is known to be directly involved in sperm-oocyte fusion.

Anything which affects the binding of sperm to an oocyte's zona pellucida, or indeed its plasma membrane, is of huge interest to those carrying out research in the field of infertility studies. The more scientists are in the know of what is happening on the molecular level of fertilisation, the more they can tackle aspects of procreation and imagine novel means of contraception. Ovastacin may prove to be a good candidate. For instance, inhibiting ovastacin activity altogether could turn out to be an effective contraceptive. The metalloprotease could also prove to be relevant within the scope of fertility tests. As an example, the precocious release of ovastacin could cause premature cleavage of ZP2. The mouse model is indeed an excellent model to design contraceptive strategies. However, there is still a long way to go. And mice are not men... This said, when one realises the many opportunities there are for a sperm and an egg to miss each other, it is a wonder an encounter ever happens at all.

Also read *Protein Spotlight issue 62, "Shackled Sperm"*
 Protein Spotlight issue 93, "Molecular Chastity"
 Protein Spotlight issue 115, "Love at First Smell"

Cross-references to UniProt

Astacin-like metalloendopeptidase, *Mus musculus* (Mouse) : Q6HA09
Astacin-like metalloendopeptidase, *Homo sapiens* (Human) : Q6HA08

References

1. Burkart A.D., Xiong B., Baibakov B., Jimenez-Movilla M., Dean J.
Ovastacin, a cortical granule protease, cleaves ZP2 in the zona pellucida to prevent polyspermy
Journal of Cell Biology 197:37-44(2012)
PMID: 22472438
2. Sachdev M., Mandal A., Mulders S., Digilio L.C., Panneerdoss S., Suryavathi V., Pires E., Klotz K.L., Hermens L., Herrero M.B., Flickinger C.J., van Duin M., Herr J.C.
Oocyte specific oolemmal SAS1B involved in sperm binding through intra-acrosomal SLLP1 during fertilization
Developmental Biology 363:40-51(2012)
PMID: 22206759