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silent walls

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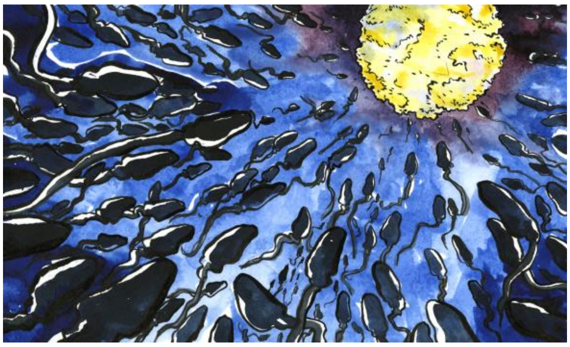
Though it may seem a paradox, life is riddled with barriers. This is because it is sometimes necessary to create dead ends to keep things at a healthy distance. Obstructions of this kind exist at all levels of living matter. Specialized pores are found in membranes surrounding cells but also within cells, to ensure that only specific molecules are able to cross while the transit of others is barred. Aquaporin and sodium channels are two examples through which only water molecules or sodium ions fit, respectively. Another vital barrier is the one that keeps spermatozoa that belongs to one species from fertilizing eggs that belong to another – which would only bring about chaos. Though the mingling of germinal fluids and how life ensues have been discussed since the days of Aristotle, on the molecular level very little is known, still, on how species keep to themselves. A recent find in zebrafish has lifted a veil: scientists discovered a protein on the membranes of zebrafish eggs, which only allows access to zebrafish sperm. They called it Bouncer.



by Fanny Vaucher

Fertilisation is complex. Biologists have been acquainted with advanced technology for well over 20 years now, which has given them the means to become quite intimate with DNA and proteins. However, up until the late 19th century, how living entities emerged from a parent and carrying very similar features, had remained obscure for centuries. In the Middle Ages explanations flirted with metaphysics. Where is what? What penetrates what? What triggers what? When the optical microscope was perfected in 1677, it became possible to observe spermatozoa, and scientists began to focus their attention on germ cells. There were the spermists who believed that one minute living being pre-existed in each spermatozoon waiting for its cue to grow, while the ovists believed they lay waiting in the ovum. By the 1880s, much had been understood about cells, their nucleus and even chromosomes – and the genetic continuity of a species had been clarified. 100 years later, how a spermatozoon meets an ovum, where the genetic heritage lies and how it is handed down to its descendants had almost become common knowledge. What remained vague, however, was how it all happens at the molecular level.

For progeny to be viable, one partner has to mate with a second partner from the same species. In this way, the species itself will survive. How, though, do male and female germ cells recognise each other? How do they make contact, and how do sperm cells enter an egg? Furthermore, an egg must not be fertilised by more than one sperm cell – so what is it that stops other sperm cells from penetrating the egg? There is little doubt that the process of fertilisation must summon hordes of molecules to get things right. In humans, sperm are thought to undergo some sort of preselection in the uterus just to make sure they belong to the same species. Once they reach the egg, they have to forage their way through its coat. The first one that manages to do this triggers off a chemical reaction that hardens the coat, thus making it impossible for other sperm cells to penetrate. The laureate sperm cell then continues to pierce a hole through the egg's membrane to release its nucleus into the cytoplasm. Things are a little different in zebrafish. For one, fertilisation occurs in the external medium. Second, while human sperm can fertilise an egg anywhere on its membrane, zebrafish sperm has to find a specific opening on the egg membrane, known as the micropyle. It is the zebrafish egg that selects a compatible sperm cell thanks to a species-specific receptor on its surface: Bouncer.

Bouncer belongs to the Ly6/uPAR (Ly6/urikinase plasminogen activator receptor) family – so called because Ly6 and uPAR are two of its representatives. This family of proteins features domains whose stamp is a beta-structural core stabilised by disulphide bonds that create three characteristic extended loops, or fingers. Ly6/uPAR proteins are wide-spread and found in mammals, birds, reptiles, amphibians, fish and insects where they are part of the nervous, immune and reproductive systems. The very diverse functions that Ly6/UPAR proteins carry out are probably due to structural differences in the three-fingered domains. In zebrafish, Bouncer is bound to the egg's membrane by way of a GPI anchor.

What exactly does Bouncer do? Zebrafish eggs that have been deprived of Bouncer have no effect on sperm behaviour prior to contact – which means that the protein doesn't act as a cue to attract sperm. Neither does it seem to have a role in maturation once the egg is released into the spawning medium. However, when there is no Bouncer, sperm are neither able to locate the micropyle – where the protein is mainly expressed – nor interact with the egg. This means that Bouncer is no doubt necessary for sperm-egg interaction. Now, what happens to zebrafish eggs whose Bouncer protein is replaced by one from a fish distantly related to them? Say, from the medaka fish which diverged from zebrafish about 200 million years ago. The answer? The medaka Bouncer did not restore fertility to the zebrafish eggs. However, the medaka sperm were able to interact with the zebrafish eggs. The resulting embryos were hybrids with a maternal zebrafish genome and a paternal medaka genome, but they didn't survive early embryogenesis and gastrulation. This implies that Bouncer is both sufficient and necessary for species-specific egg-sperm recognition.

So, Bouncer only lets in sperm that will not disturb the internal harmony – hence the use of "bouncer" to echo the name given to security guards at the entrance of nightclubs and bars, whose job is to deny access to troublemakers. This is the first time scientists have discovered a protein that is actually part of the species-specific barrier – but it does not act on its own. There must be a ligand on the sperm cell that binds specifically to Bouncer, and numerous other proteins will be involved in attracting the sperm to the micropyle and then barring the entrance to those who have arrived too late. Though there is still a lot to understand, species-specific studies like these are essential to grasp how species keep to themselves and how evolution is driven. They are, of course, also crucial in fertility studies and could inspire therapies to help people who seem unable to conceive. If Bouncer is necessary for sperm-egg binding, there is probably a human equivalent which, when deficient, may be one of the causes of human infertility. The hunt is surely underway.

Cross-references to UniProt

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