

Bouncer is necessary and sufficient for species-specific fertilization

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Fertilization is the process by which sperm and egg interact, fuse, and eventually form a single cell, the zygote. This process initiates the life of all sexually reproducing organisms. Despite its importance, little is known about its molecular basis.

We discovered a small protein, named Bouncer, which is essential for species-specific fertilization in zebrafish. Bouncer belongs to the lymphocyte antigen-6 (Ly6)/urokinase-type plasminogen activator receptor (uPAR) protein family and is conserved in vertebrates. In zebrafish, Bouncer is highly expressed in the egg, where it localizes to the plasma membrane and to vesicles. Bouncer-deficient eggs, derived from homozygous bouncer knockout females, cannot be fertilized. We demonstrate that this phenotype is due to a block in sperm entry and can be circumvented by intracytoplasmic sperm injection. Closer analysis revealed that Bouncer mediates sperm-egg interaction. Furthermore, Bouncer does not indiscriminately allow sperm entry into the egg but is selective for conspecific sperm. Our work thus reveals the importance of the previously unknown zebrafish protein Bouncer as species-specific fertilization factor.

Curiously, in externally fertilizing species, Bouncer's expression is restricted to oocytes, while in internally fertilizing species, Bouncer's homolog is expressed specifically in testis. Bouncer's closest homolog in mammals is the protein Sperm Acrosome Associated 4 (SPACA4), which has been implied by *in vitro* studies to play a role in fertilization in humans. By generating Spaca4 mutant mice, we show that SPACA4 is important for murine male fertility *in vivo*, suggesting that our findings in fish might also be relevant for human biology.