Development of a Plasmid Repository Encoding Oocyte Membrane Proteins to Unveil Novel Sperm-Oocytes Interactions: A Focus on Human CRISP

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In an era marked by heightened fertility concerns, exploring the intricate interaction between sperm and oocyte proteins is a captivating pursuit. While several sperm proteins have been identified as essential for fertilization, the corresponding protein partner on the oocyte membrane (oolemma) is known only for a subset of these proteins. In this context, Cystein-RIch Secretory Proteins (CRISP) are particularly interesting as they are primarily expressed in the male genital tract of mammals and are known to play key roles in fertilization. In humans, three CRISP have been identified. CRISP1 and 3 are secreted by the epithelium of the epididymis and associate with the surface of spermatozoa during their epididymal transit, whereas CRISP2 is of testicular origin and expressed ab initio in spermatozoa. Both CRISP1 and CRISP2 are involved in gamete fusion, interacting with common and yet unidentified complementary sites in the oolemma. Interestingly, in rats, CRISP1 interaction with the oolemma was shown to be mediated by a small region of 12 amino acids corresponding to an evolutionarily conserved structural motif among CRISP.

In this study, we analyzed published human and murine oocyte proteomes using various software tools for predicting sub-cellular localization and topology. This analysis led to the compilation of a catalog of around 300 oocyte membrane proteins, each featuring at least one ecto-domain. Using Gateway cloning, the coding sequences of these proteins were integrated into vectors allowing the expression of the proteins in fusion with GFP. These plasmids were transfected into HeLa cells, each pool of cells expressing a membrane oocyte protein fused with GFP. To identify CRISP oocyte interactor(s), these cells are screened with synthetic peptides of both human CRISP1 and CRISP2 corresponding to the 12 amino acid region involved in the interaction with the oolemma. We first validated the use of these peptides on human oocytes. Our findings demonstrated that CRISP1 and CRISP2 peptides exhibited binding affinity to the oolemma, whereas a control peptide from a different region of CRISP2 failed to bind.

We believe our plasmid library and methodology will contribute to identifying egg CRISP interactor(s) as well as to uncover novel protein interactions between oocytes and spermatozoa.