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

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1 - Background

Cystein-Rich Secretory Proteins (CRISPs) are well known for their role in sperm function and in fertilization[1, 2]. Humans have 3 of them[3]. Unlike CRISP1 and 3, which are secreted by the epididymal epithelium and associate with the sperm surface[2-4], CRISP2 is expressed inside the spermatozoa during spermatogenesis[1]. Several functions have been described for CRISP2: (a) it interacts with Sertoli cells during spermatogenesis[5], (b) it regulates some ion channels such as CatSper and RyR, involved in sperm motility[6-8] , and (c) it mediates interaction with the oocyte via a still unidentified receptor that it shares with CRISP1[9]. This study aims to identify that receptor via a new approach.

2 - Interaction between CRISP peptides and oocytes

In rats, interaction between CRISP1 and the oocyte is achieved via a short evolutionarily conserved 12-amino acid domain[10]. This peptide is conserved in CRISP2. Here, we confirmed the interaction of synthetic biotinylated CRISP1 and 2 peptides with MII oocytes in humans.

Figure 1: Schematic representation of CRISPs. Those proteins are characterized by two conserved domains (CAP and CRD) and the presence of a signal peptide (SP). Four short regions are highly conserved through evolution (P1-4). P2 is the region necessary for oolemma interaction.

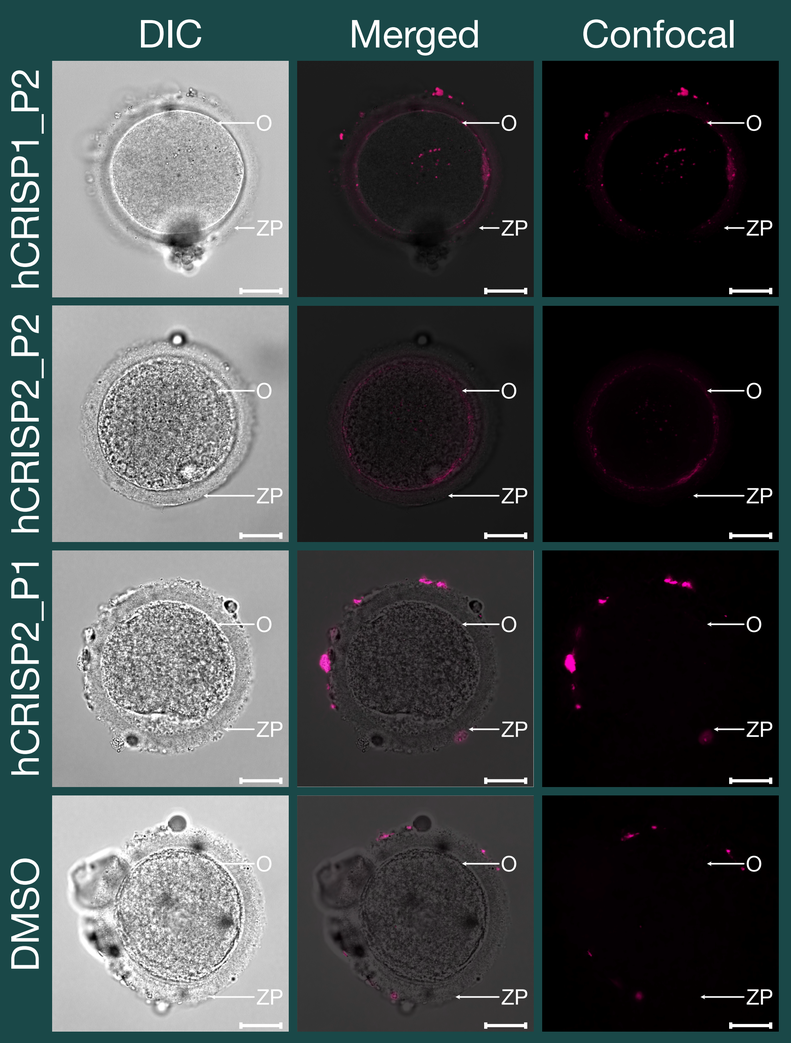


Figure 2: Human MII oocytes were incubated with 30 µM of synthetic biotinylated CRISP1 (hCRISP1_P2) or CRISP2 (hCRISP2_P2) peptides. As a control, oocytes were incubated with another peptide from hCRISP2 (hCRISP1_P1) or DMSO, the vehicle used to dissolve the peptides. The biotinylated peptides were detected with streptavidin-TexasRed. Magenta: CRISP1 or CRISP2 peptide. Images are Z-stacks of pictures from the oocyte equatorial region in confocal and differential interference contrast (DIC) microscopy. Superposition of both is presented in the middle (Merged). O: oolemma ; ZP: zona pellucida. Scale bar = 25 µm.

3 - Development of the repository

Available oocyte proteomes from humans[11, 12] and mice[13-15] were analyzed using the DeepLoc algorithm[16] to identify membrane proteins. Combined with protein topology analysis and literature review, we identified around 300 candidates with at least one ectodomain capable of interacting with CRISP2 from the sperm surface during fertilization. The coding sequences of these proteins will be transferred via Gateway cloning into plasmids designed for GFP-fused protein expression. After transfection, HeLa cells expressing the membrane proteins will be incubated with hCRISP1/2 biotinylated synthetic peptides that have demonstrated interaction with the oolemma (see Figure 2). Interaction will be confirmed by co-detection of GFP and the peptide on the same cell.

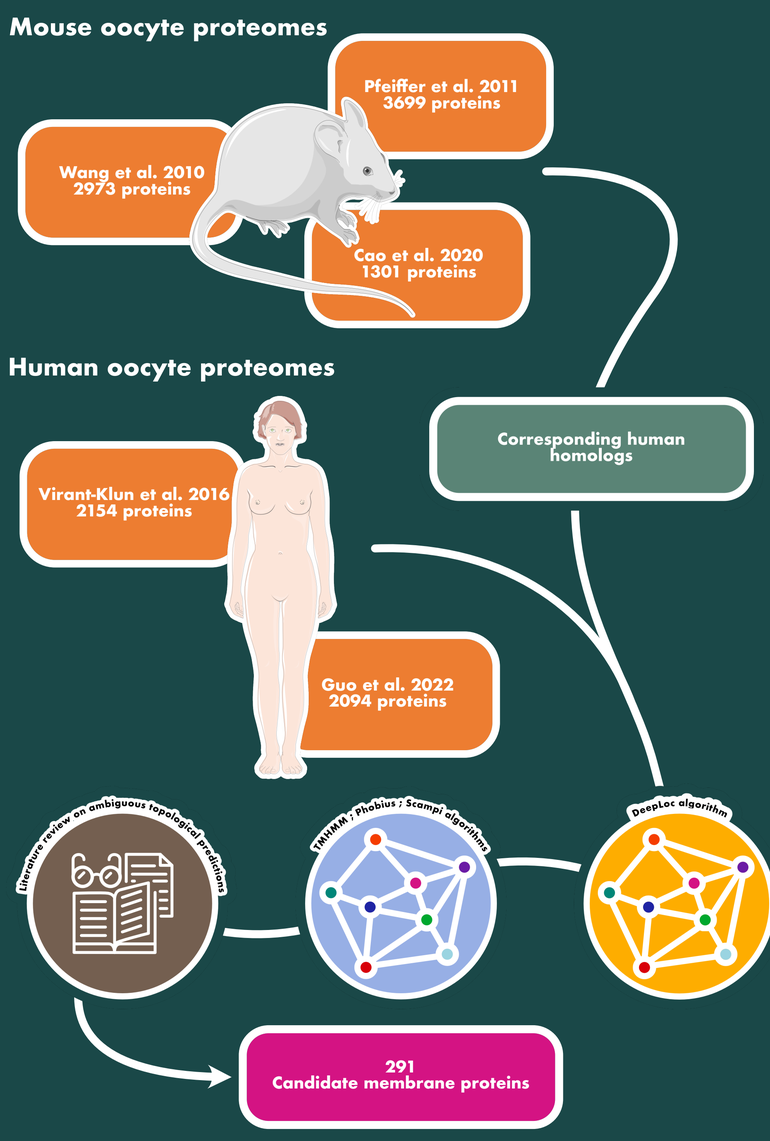
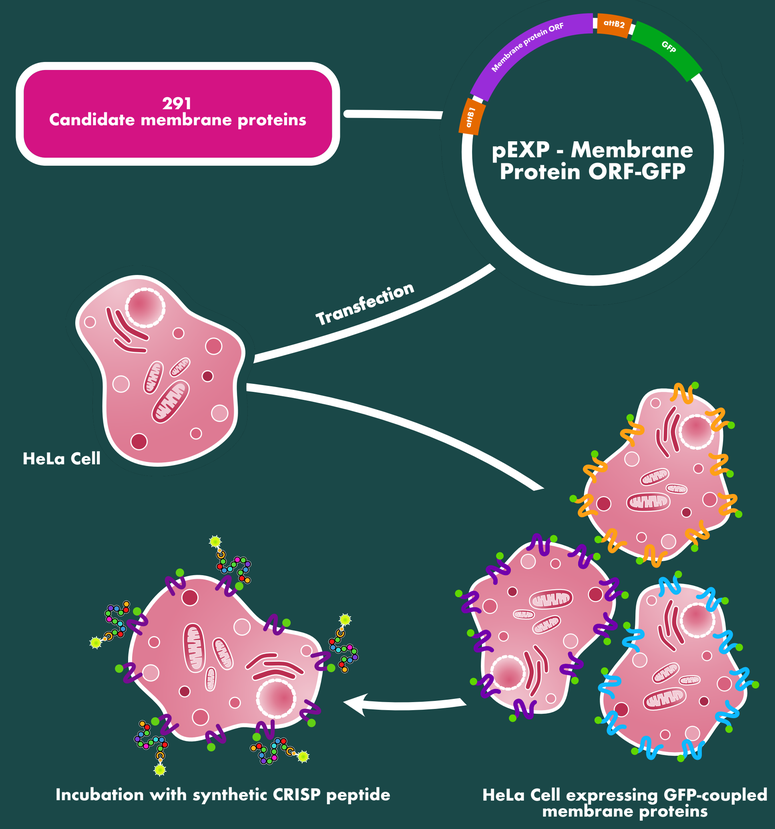


Figure 3: General flow-chart depicting the development of our plasmid repository encoding oocyte membrane proteins fused with GFP. The plasmids are subsequently transfected in HeLa cells, which are used in peptide binding assays to identify potential interactors of human CRISPs.

4 - First screening with CRISP peptides

Using Gateway cloning, we have already generated 55 plasmids coding for GFP-fused membrane proteins. These were transfected into HeLa cells which were then incubated with the CRISP synthetic biotinylated peptides. These peptides did not bind to cells expressing the 55 screened interactors. The sequences coding for the other 250 oocyte membrane proteins will be cloned and tested soon.



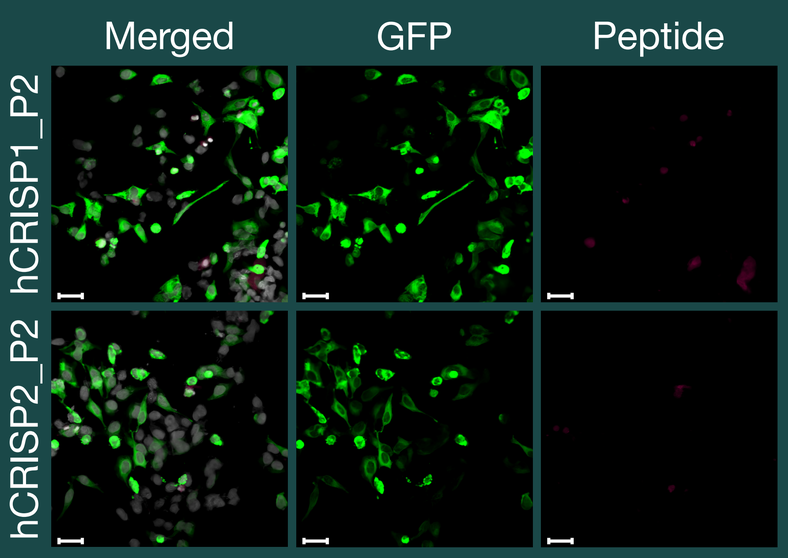


Figure 4: Example of result obainted for the peptide binding assay. Here, HeLa cells were transfected with a plasmid coding for GFP-fused SPINT2 and were then screened with hCRISP1_P2 and hCRISP2_P2 peptides. As a control, untransfected cells were incubated with the same peptides. Gray: Hoechst staining of the nuclei. Green: GFP-fused membrane protein. Magenta: CRISP1 or CRISP2 peptide. Scale bar = 50 µm.

5 - Conclusion & perspectives

In this study, by analyzing human and murine oocyte proteome, we identified around 300 oocyte membrane proteins that could interact with sperm surface proteins. We aim to clone all of them in plasmids to allow their expression in fusion to GFP. Our initial screening on 55 of them did not highlight CRISP oocyte receptor, but we are optimistic that it will be found among the 245 remaining candidates.

6 - References

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