Production and functional testing of probes mimicking human sperm CRISP1, CRISP2, and TMEM95 for oocyte receptor identification

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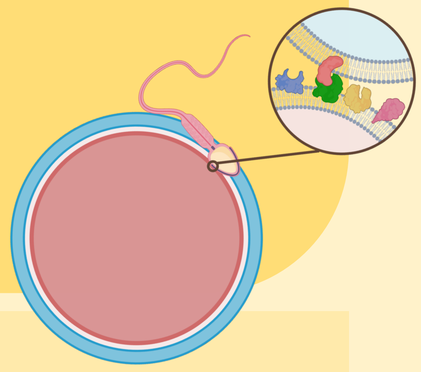

1- State of the art

Fertilization is a complex process involving a series of interactions between a spermatozoon and an oocyte, culminating in membrane binding and fusion. Despite extensive research, the molecular mechanisms governing these interactions remain incompletely understood. To date, ten proteins have been identified as essential for fertilization in gene knockout (KO) models: IZUMO1, SPACA6, TMEM95, TMEM81, FIMP, SOF1, DCST1, and DCST2 on the spermatozoon, and JUNO and CD9 on the oocyte (Fig. 1)[1,2]. Additional proteins associated with the sperm surface, such as CRISP1 and CRISP2, also play key roles in fertilization; however, their KO does not lead to complete infertility. These two proteins are believed to share the same oocyte receptor[3,4].

To date, the IZUMO1–JUNO pair is the only identified and characterized molecular interaction[5]. Recently, in our laboratory, we performed a virtual screening (AlphaFold-Multimer predictions) of the human oocyte surfaceome using CRISP2 as bait. Thirty potential oocyte interactors of CRISP2 were identified with high confidence interface predicted template modelling (ipTM > 0.7).

The objective of this study is to generate high-avidity probes mimicking the human sperm proteins CRISP1, CRISP2, and TMEM95 in order to identify their oocyte receptor.

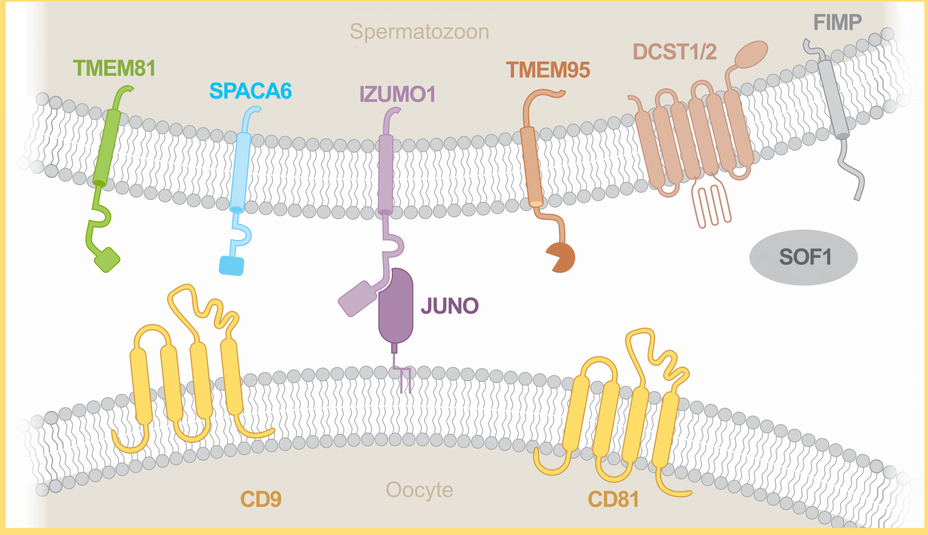

Figure 1. Essential sperm and oocyte proteins involved in gamete binding and fusion. Illustration of the membranes of the two gametes with their identified membrane proteins. The only identified and characterized pair is shown (IZUMO1–JUNO), whereas the receptors for the other proteins remain unknown. Adapted from Deneke & Pauli (2021).

2- Materials & Methods

Based on the strategy used to discover IZUMO1 oocyte receptor[6], the full-length ectodomains of human CRISP1, CRISP2, and TMEM95 were expressed as pentamer scaffolds in Expi293F cells (Fig. 2). Human IZUMO1 was used as a positive control.

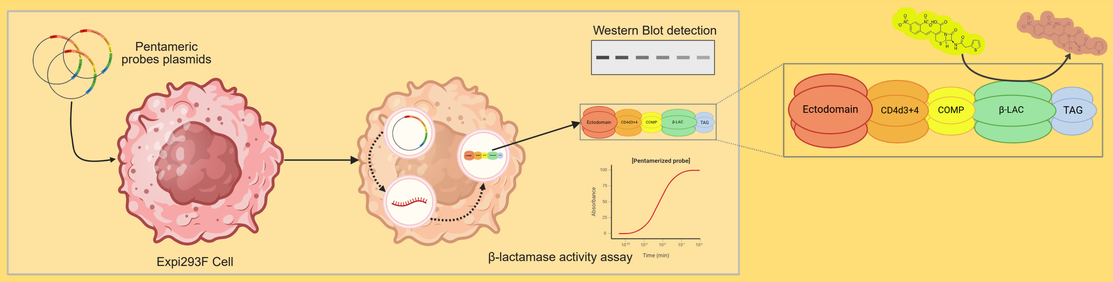

Figure 2. Strategy for producing pentamerized probes mimicking CRISP1, CRISP2, and TMEM95. Illustration of the process for the pentamerized probes, with each monomer composed of five domains: the ectodomain of the protein of interest (red), rat CD4 domains 3-4 (orange) for immunofluorescence detection , Cartilage Oligomeric Matrix Protein (COMP; yellow) for pentamerization, β-lactamase (β-LAC; green) for enzymatic detection, and a double C-terminal tag consisting of 3×FLAG and 6×His (TAG; blue) for Western blot.

Thirteen of the 30 candidate oocyte receptors for CRISP2 identified previously in our lab using AlphaFold predictions were expressed in fusion with a C-terminal FLAG tag in HeLa cells. These cells were then incubated in the presence of (1) biotinylated peptides representing conserved CRISP1/CRISP2 binding regions, and (2) pentamerized probes produced in Expi293F cells (Fig. 3).

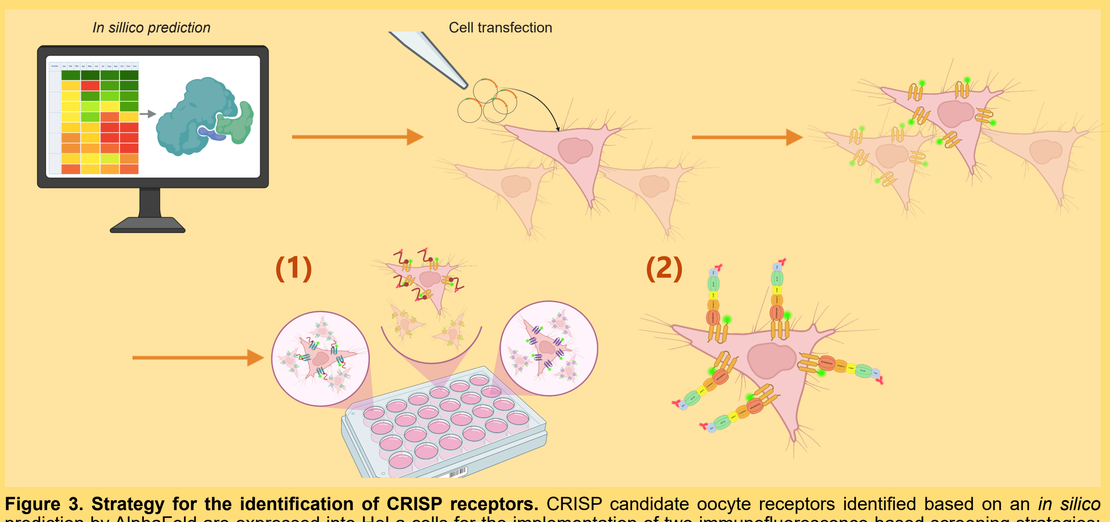

Figure 3. Strategy for the identification of CRISP receptors. CRISP candidate oocyte receptors identified based on an *in silico* prediction by AlphaFold are expressed into HeLa cells for the implementation of two immunofluorescence-based screening strategies: (1) use of biotinylated peptides encoding the binding regions of CRISP1 and CRISP2, and (2) pentameric probes encoding the ectodomains of CRISP1 and CRISP2.

4- Conclusions

The probes for CRISP1, CRISP2, and TMEM95 were successfully produced and are now available for binding assays aimed at identifying their oolemmal receptors.

Preliminary results are promising for IGHG1 as a potential receptor candidate for CRISP proteins, while additional receptor candidates remain to be tested. In the near future, the same virtual screening approach and experimental strategy will be applied to identify and validate receptor candidates for TMEM95.

3- Results

Probe production

Western blot analysis demonstrated the successful production of the different probes in the Expi293F culture medium (Fig. 4).

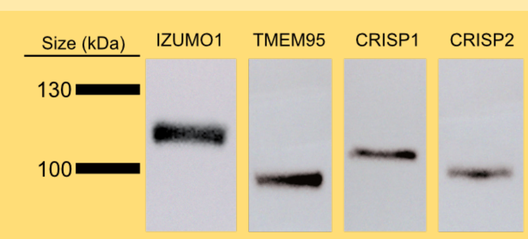

Figure 4. Detection of pentamerized probes in culture medium. Pentamerized probes produced by Expi293F cells were separated by 8% SDS-PAGE and analyzed by Western blot using an anti-Flag antibody to confirm correct production and secretion.

Identification of CRISP oocyte receptor

Thirteen potential candidate oocyte receptors for CRISP2 have been successfully cloned and expressed in HeLa cells, showing clear membrane localization (Fig. 5). Therefore, the first screening approach using biotinylated peptides has been initiated. Preliminary results indicate that IGHG1 displays specific labeling, as the CRISP1 peptide preferentially bound to transfected cells (Fig. 6).

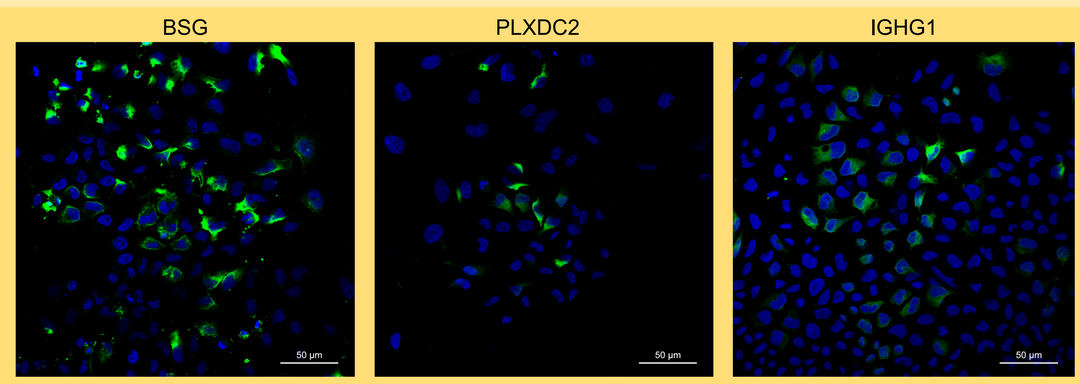

Figure 5. Expression of Flag-tagged candidate oocyte receptors. Confocal microscopy images of HeLa cells expressing BSG, IGHG1, and PLXDC2 fused to a Flag tag. Blue: Hoechst-labeled nuclei, green: oocyte receptors immunolabeled with mouse anti-Flag antibody followed by CoraLite 480 anti-mouse IgG.

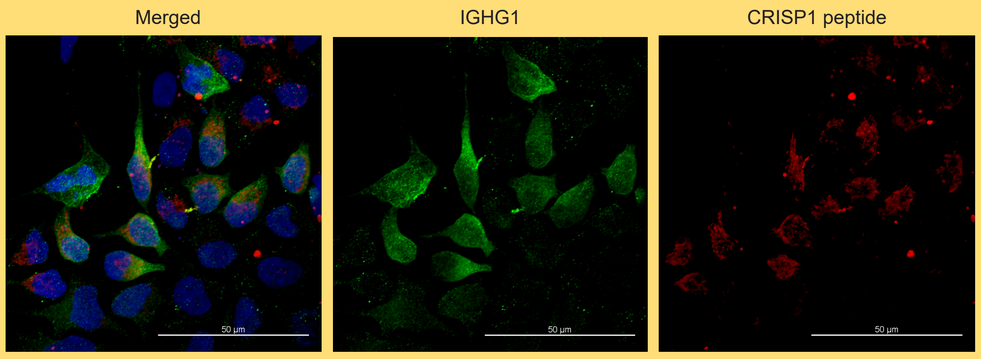

Figure 6. Results from first screening approach for the identification of the CRISPs' receptor. Confocal microscopy images of HeLa cells expressing IGHG1 and incubated with 30 µM biotinylated CRISP1 peptide. Blue: Hoechst-labeled nuclei, green: oocyte receptors immunolabeled with mouse anti-Flag antibody followed by CoraLite 480 anti-mouse IgG. Red: detection of biotinylated CRISP1 peptide with streptavidin-Texas Red.

REFERENCES: [1] Deneke V.E. and Pauli A. (2021) Annu. Rev. Cell Dev. Biol. 37:391–414. [2] Deneke V.E. et al. (2024) Cell 187(25):7066-7078.e22. [3] Curci L. et al. (2020) FASEB J.34(12):15718–33. [4] Gonzalez S.N. et al. (2021) Front. Cell Dev. Biol. 9:800351. [5] Trebichalská & Holubcová (2020) J. Assist. Reprod. Genet. 37(2):243-256. [6] Bianchi E. et al. (2014) Nature 508(7497):483–7.

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