Characterization of CRISP2 in human spermatozoa

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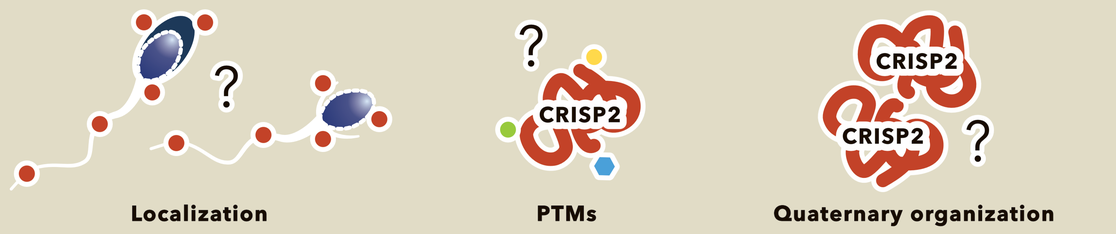
State of the art

Fertilization is a fascinating and complex process involving protein interactions between spermatozoa and oocytes[1]. Among the involved proteins are the **Cystein-RIch Secretory Proteins (CRISPs)**[2], which comprise three members in humans. While CRISP1 and 3 are secreted by the epididymal epithelium and associated on the surface of the spermatozoa, **CRISP2** is expressed inside the spermatozoa during spermatogenesis[3,4]. However, its exact **localization** inside the mature spermatozoon is debated[5-8]. Contrary to CRISP1 and 3, CRISP2 is thought to be non-glycosylated[9], but the presence of other **post-translational modifications (PTMs)** has not yet been investigated.

Aim



In the present study, we contributed to the characterization of native CRISP2 in human spermatozoa by investigating its localization, PTMs and quaternary organization.

Localization of CRISP2 in human spermatozoa

Using immunofluorescence, we localized CRISP2 mainly in the principal piece of the flagellum and at the base of the sperm head. A faint signal is also observed within the acrosome.

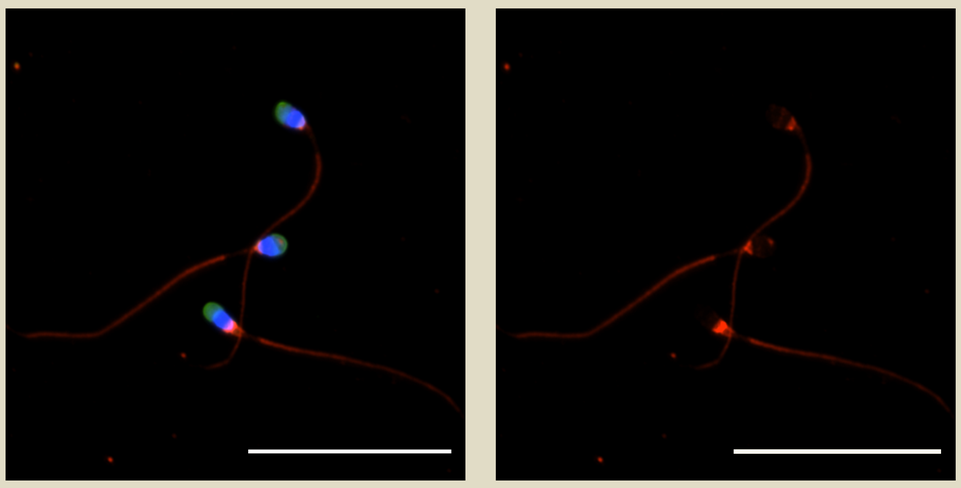
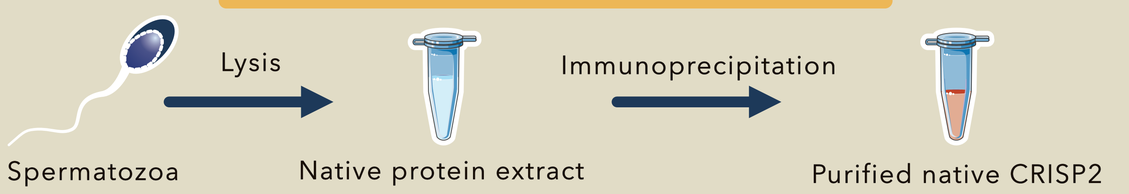


Figure 1: Localization of CRISP2 within uncapacitated human sperm cells. Human spermatozoa were fixed with 4% paraformaldehyde, permeabilized with 0.3% Triton-X-100, treated with hot citric acid-EDTA solution for epitope retrieval and stained with anti-CRISP2 antibodies. **Red**: CRISP2, **blue**: DAPI staining of the nucleus, **green**: PSA-FITC staining of the acrosome. **Scale** = 50 µm.

CRISP2 quaternary structure



A sperm lysate, obtained with a non-denaturing buffer, as well as CRISP2 immunoprecipitated from that lysate were resolved by native-page followed by Western blot analysis to detect CRISP2 (Fig. 2 A, B). CRISP2 was detected as two main bands and a faint one in the upper part of the gel, indicating that it forms oligomers. These oligomers were not dissociated in the presence of NaCl, DTT, EDTA, Triton-X-100 nor urea (Figure 2 A).

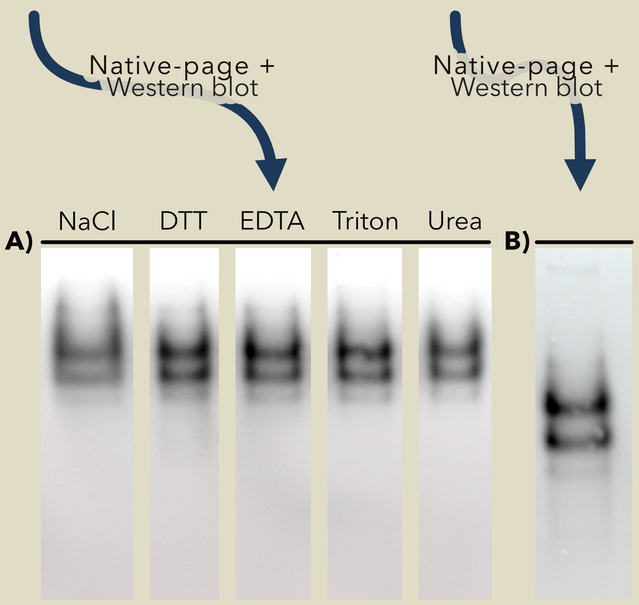
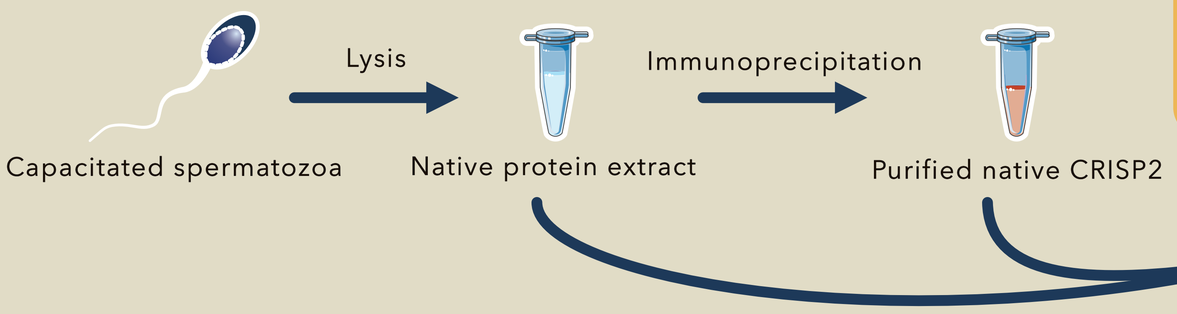


Figure 2: Western blot analysis of uncapacitated sperm lysate and immunoprecipitated CRISP2 under native condition or in the presence of 1 M NaCl to disrupt ionic bounds, 100 mM DTT to disrupt disulfide bounds, 24 mM EDTA to chelate metallic ions, 1 % Triton-X-100 to disrupt hydrophobic interactions, or 4 M urea to disrupt hydrogen bounds. A : 12% native-gel, B: 10% native-gel.

Post-translational modification of human CRISP2



Sperm lysate and CRISP2 immunoprecipitated from that lysate were resolved by SDS-page followed by Western blot analysis to detect CRISP2 and its potential PTMs. No O- or N-glycosylations nor phosphotyrosines were detected in the IP fraction, indicating that CRISP2 does not display those PTMs. The labelling obtained in the crude lysate for other proteins demonstrates that the absence of labelling of CRISP2 is not due to the inability of the antibody or the lectin to recognize its target.

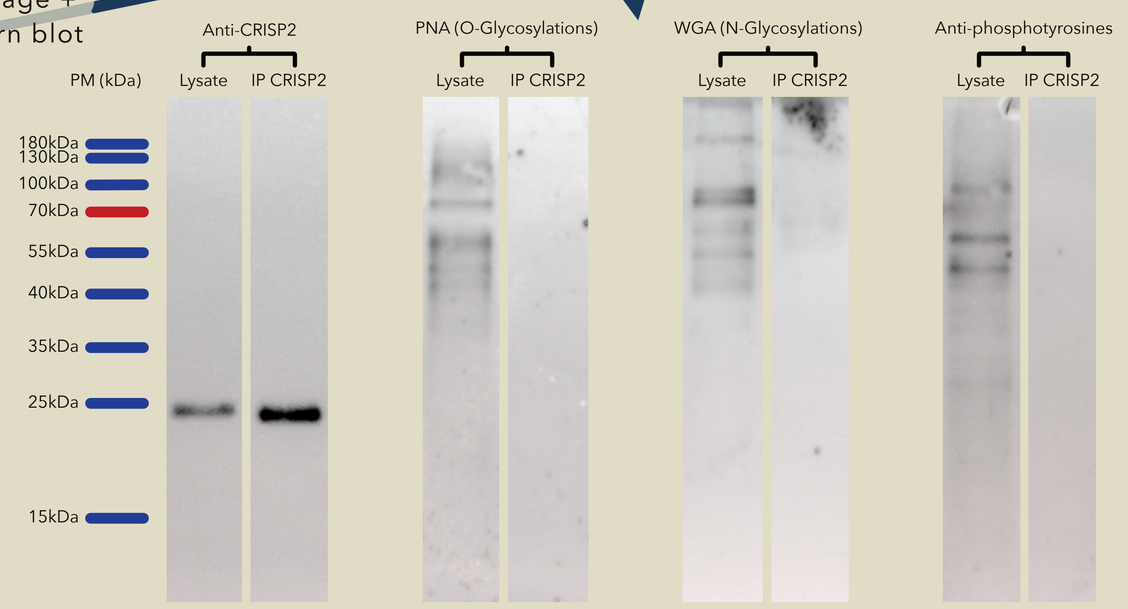


Figure 3: Western blot analysis of CRISP2 PTMs. The different fractions were separated by SDS-page (12%) and then analyzed by Western blot using specific antibodies or lectins. The crude lysate was used as positive control. O-glycosylations were detected with Peanut Agglutinin (PNA), N-Glycosylations were detected with Wheat Germ Agglutinin (WGA) and phosphotyrosines were detected with specific antibody.

What's next?

Here, we **localized CRISP2** within the head and the tail of the spermatozoa. The next step for this part is to localize CRISP2 at different time points during the capacitation (a maturation of the spermatozoa required for oocyte fertilization) and after acrosome reaction. We also showed that CRISP2 exists as **strongly stabilized oligomers** within the spermatozoa. Blue-Native-PAGE will be used to estimate the size of these oligomers. Finally, for the first time, PTMs of human CRISP2 were investigated. Our preliminary results suggest that human CRISP2 bear **no glycosylation or phosphotyrosines**.

[1] Gahlay, G. K., & Rajput, N. (2020). *Biol Reprod*, *103*(6). **[2.]** Gonzalez, S. N., Sulzyk, V., Weigel Munoz, M., & Cuasnicu, P. S. (2021). *Front Cell Dev Biol*, *9*, 800351. **[3.]** Maeda, T., J. Nishida, and Y. Nakanishi. Dev Growth Differ, 1999. **41**(6): p. 715-22. **[4.]** Jamsai, D., et al.. Hum Reprod, 2008. **23**(9): p. 2151-9. **[5.]** Nimlamool, W., B.S. Bean, and L.J. Lowe-Krentz. Mol Reprod Dev, 2013. **80**(6): p. 488-502. **[6.]** Busso, D., et al.. Mol Hum Reprod, 2005. **11**(4): p. 299-305. **[7.]** O'Bryan, M. K., Sebire, K., Meinhardt, A., Edgar, K., Keah, H. H., Hearn, M. T., & de Kretser, D. M. (2001). Mol Reprod Dev, 58(1), 116-125. **[8.]** Zhang, M., Bromfield, E. G., Veenendaal, T., Klumperman, J., Helms, J. B., & Gadella, B. M. (2021). Biol Reprod, 105(5), 1160-1170. **[9.]** Hardy, D. M., Huang, T. T., Jr., Driscoll, W. J., Tung, K. K., & Wild, G. C. (1988). *Biol Reprod*, *38*(2), 423-437.