Sperm membrane protein dynamics during the acrosome reaction

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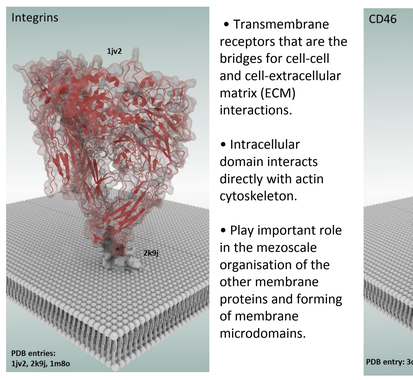
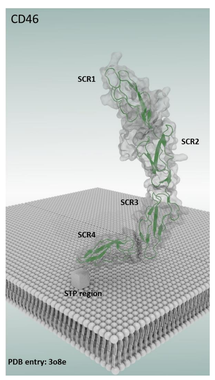
Sperm membrane protein relocation is a key process in human and mammalian reproduction. During sperm acrosome reaction (AR), some key molecules responsible for binding and fusion with the egg plasma membrane have to be relocated to the sperm surface and even more specifically to the equatorial segment, where fusion process takes a place. In previous studies, the unique presence of complement regulatory protein CD46 in sperm acrosomal membrane was shown. CD46 protein has been shown to play an important role in the acrosomal stability and its proper expression and protein abundance in the acrosomal membrane is important for the balanced ability of individual sperm cell and whole sperm population to undergo precisely timed acrosome reaction. However, the mechanism of CD46 action in sperm and its connection with other sperm proteins has not been explored further. Here, using fluorescence microscopy we demonstrate the specific localization and relocation pattern of the CD46 molecule during capacitation and acrosome reaction. Furthermore, using Duolink assay we uncovered the specific interaction of CD46 protein molecules with other specific membrane proteins, which belong to the integrin protein superfamily. Finally, the specific topological patterns of CD46 and integrin protein clusters within the acrosomal cap, equatorial and post-acrosomal regions of the sperm head have been examined by STED super-resolution microscopy. Our results suggest, that CD46 and integrins specifically interact during the process of acrosome reaction and their interaction is vital for their joint relocation. This relocation then leads to the establishing of specific membrane protein content in the equatorial region of the sperm head, which later interact with its counterpart on the egg plasma membrane and thus facilitate sperm-egg fusion. Since recent findings demonstrate the active role of the actin cytoskeleton on the process of sperm membrane protein relocation, our ongoing experiments are also focused on the role of ERM (ezrin/radixin/moesin) protein family in the recruitment and rearrangement of the actin cytoskeleton during active relocation of selected protein receptor candidates in human, mice and boar sperm. We hope, that our new experimental findings together with the extensive use of super-resolution data for 3D models and simulations will bring a new important insight into the physiological processes occurring during the final stages of mammalian fertilization.



• Transmembrane receptors that are the bridges for cell-cell and cell-extracellular matrix (ECM) interactions.

• Intracellular domain interacts directly with actin cytoskeleton.

• Play important role in the mezoscale organisation of the other membrane proteins and forming of membrane microdomains.

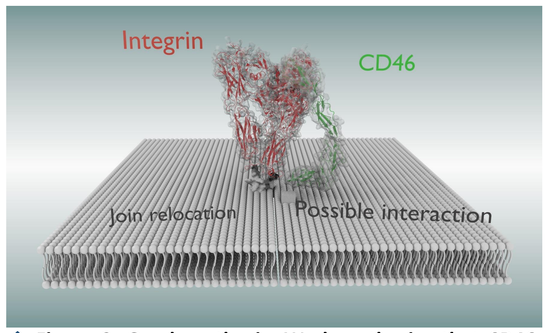


• Membrane protein playing important role during complement regulation in immune system.

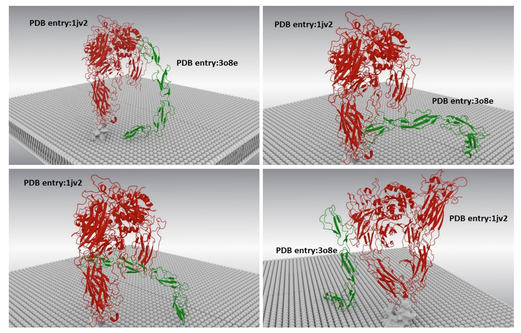
• Present in large ammnounts in acrosomal mebrane and responsible for its stability.

• Suspected role in the sperm-oocyte interaction.

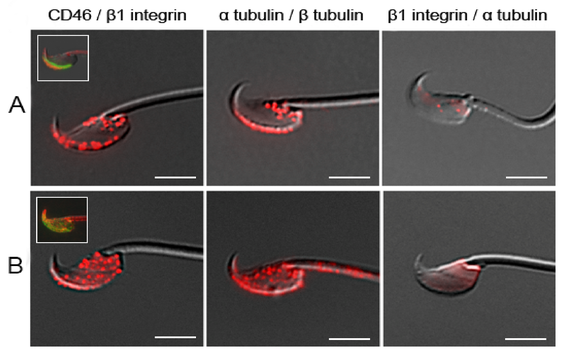
↑ **Figure 1: Proteins of interest.**



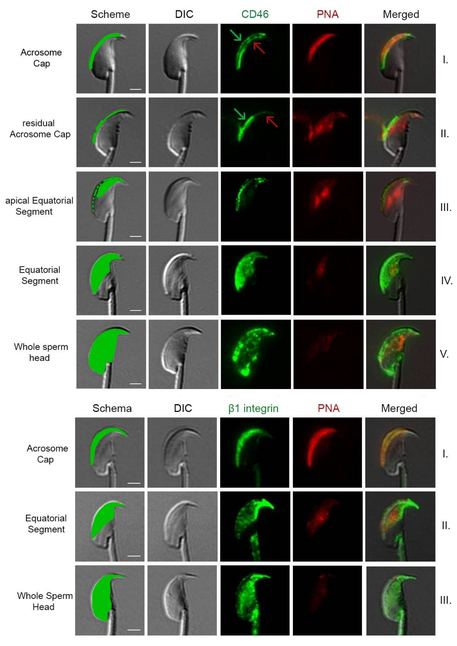
↑ **Figure 2: Our hypothesis: We hypothesize that CD46 and Integrin molecules interact during AR ad this interaction is needed for their join relocation from acrosomal cap to other parts of sperm head.**



↑ **Figure 3: Protein interaction model. ClusPro super – computer simulations of potential interaction sites of CD46 and integrin molecules with manual membrane docking.**

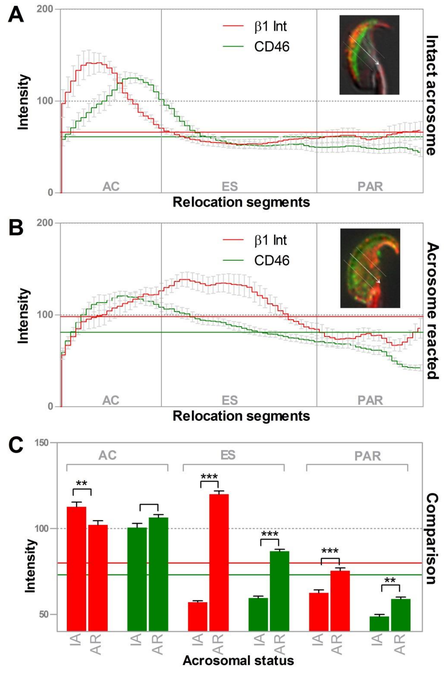


↑ **Figure 4: DuoLink protein interaction analysis showing the topological proximity of proteins of interest in mouse sperm head and tail.**

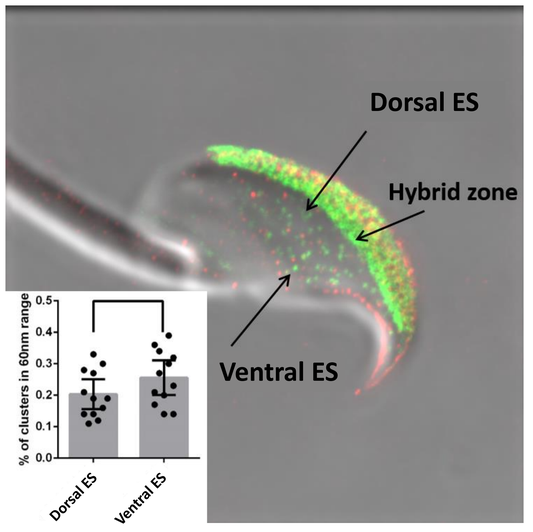


← **Figure 5: Progress of CD46 and β1 integrin relocation prior and during AR.**
(a) CD46 (green), PNA lectin (red). (b) β1 integrin (green), PNA lectin (red). The first column represents a schematic illustration of (a) CD46, (b) β1 integrin localization in the acrosome intact sperm (line I) and in sperm during the AR progress. (a) CD46 detection in intact acrosomal membranes (line I) and residual (line II) the outer acrosomal membrane (see the green arrow), the inner acrosomal membrane begins to emerge (see the red arrow). The CD46 relocation progress during the AR is seen across the apical equatorial segment towards the whole equatorial segment and the whole sperm head.

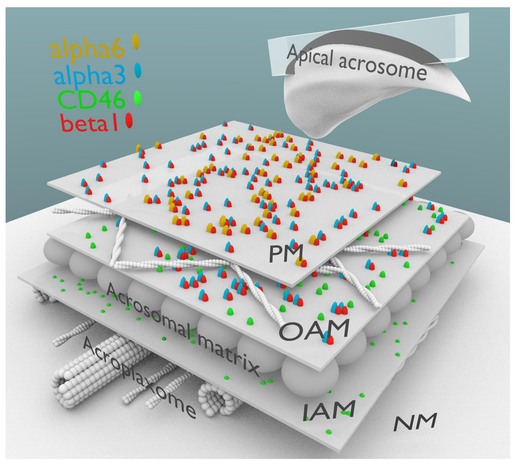
← **(b) β1integrin is relocated across the apical equatorial segment towards the whole equatorial segment and the whole sperm head. In contrast to CD46, the residual acrosome cap and the apical equatorial segments were not detected. Scale bar represents 2 µm.**



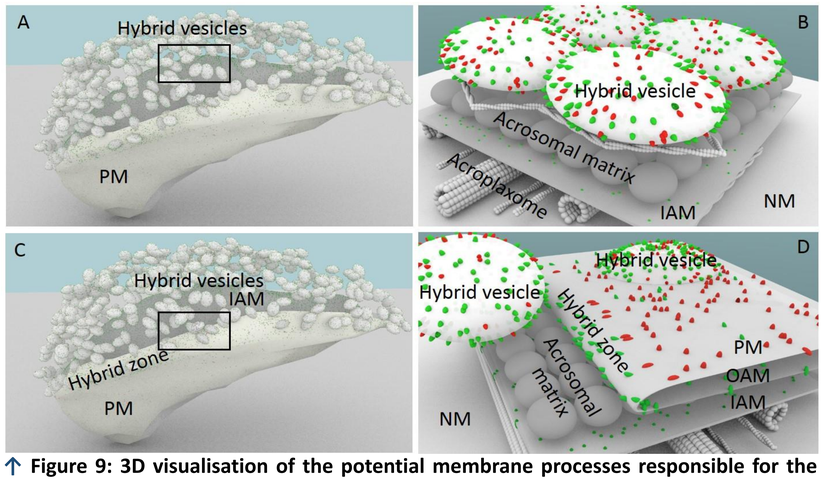
← **Figure 6: Statistical analysis of the relocation process: (a) Coloured lines with error bars represent relative fluorescent intensities of β1 integrin (red) and CD46 (green) among the individual segments of the sperm head in 10 sperm with an intact acrosome. Horizontal coloured lines represent the arithmetic means of the fluorescent intensities for β1 integrin (red) and CD46 (green). (b) Coloured lines with error bars represent the relative fluorescent intensities of β1 integrin (red) and CD46 (green) among the individual segments of the sperm head in 10 acrosome reacted sperm. (c) Statistical comparison of the relative fluorescent intensities of β1 integrin (red) and CD46 (green) among individual segments of the sperm head between the acrosome-intact and acrosome-reacted sperm, horizontal coloured lines the represent arithmetic means of the fluorescent intensities for β1 integrin (red) and CD46 (green). Error bars denote SEM. AC – Acrosome Cap, ES – Equatorial Segment, PAR – Post-Acrosomal Region. p value equal or lower than 0.05 was considered to be significant, $p \leq 0.05$*, $p \leq 0.01$**, $p \leq 0.001$***.**



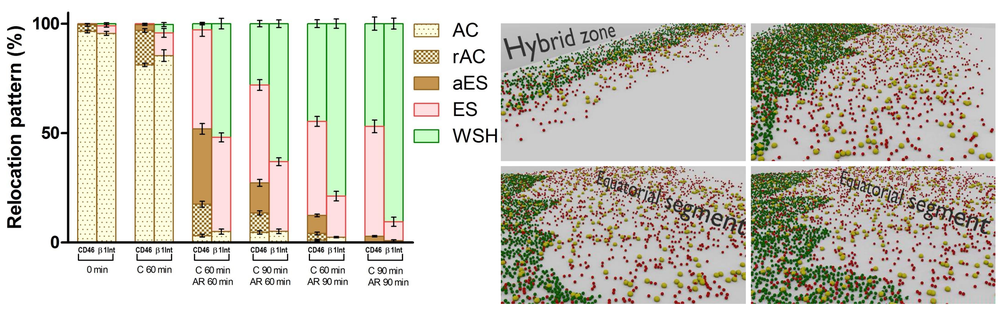
↑ **Figure 7: STED super-resolution microscopy of protein relocation. Picture of sperm head during the initial phase of AR. The topological distance analysis of the CD46 (green) and β1integrin clusters among dorsal and ventral equatorial segment (ES) is represented in inserted bar chart (preliminary data).**



↑ **Figure 8: 3D cartoon summarizing the localization of CD46 and α3, α6 and β1 integrins among different membrane structures of the intact sperm head in apical acrosomal area). PM – plasma membrane, OAM – outer acrosomal membrane, IAM – inner acrosomal membrane, NM – nuclear membrane.**



↑ **Figure 9: 3D visualisation of the potential membrane processes responsible for the relocation of the proteins from the acrosomal sperm area to the other compartments of the sperm head (AB;CD). In hybrid zone, plasma and outer acrosomal membrane fusion results in the creation of the shelf-like membrane structure, where proteins from both membranes are suspected to be able to freely relocate to the other one. Furthermore, the hybrid vesicles are suspected to be able to refuse with the intact plasma membrane of other compartments of the sperm head.**



↑ **Figure 10 ↑ a) Statistical analysis of the relocation process depicted in figure 5 and ↑ (b) snapshots from the simulation of the differential relocation speed of CD46 and β1 integrin based on the quantitative data sets from the population of sperm presented in figures 5 and 10a visually approximate the most likely scenario of the relocation process in the average sperm cell (CD46; integrin; complex).**

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