Review Bachelor Thesis Bahareh Attar

The Bachelor-Thesis of Bahareh Attar with the title “Biochemical analysis of Orai1 mutants derived from a cancer database” involves structural and mechanistic insights of a distinct plasma membrane (PM) located protein called Orai1. Orai1, in conjunction with Stromal Interaction Molecule 1 (STIM1), a protein found in the endoplasmic reticulum (ER) membrane, constitutes the calcium-release-activated calcium (CRAC) channel complex. Within this system Orai proteins are the pore forming unit responsible for precisely controlled Calcium (Ca2+) influx over the PM. Mrs. Atta initiated her Bachelor's thesis by providing a comprehensive introduction highlighting the significance of Ca^{2+} in cellular systems, followed by an exposition on specific Ca^{2+}-binding proteins and their inherent properties. Subsequent chapters include, detailed representations of the two key players of the CRAC channel systems, STIM1 and Orai1. A pivotal emphasis was then placed on the existing structural characteristics of the Orai1 protein in its closed and open conformations.

Throughout Mrs. Attar´s thesis, different methods were explained including bioinformatic tools like PyMOL and biochemistry experiments (SDS-page, Western-blot). Mrs. Atta used the different available PDB structures of open and closed *drosophila melanogaster d*Orai proteins. Using different PyMOL settings/commands she compared and analyzed the structural features of the desired X-ray crystal structures. As the structures include different mutations (K163W and H206A), which represent the closed (K163W in *d*Orai) and open (H206A in *d*Orai) conformation of the channel, Mrs. Atta tried to explain and highlight the influence of the mutations on the channel composition. In pursuit of an intricate comprehension of the H206A structure, corresponding to H134A in hOrai, the thesis also encompassed an elucidation of the pore-widening mechanism attributed to the H134A mutation in *human* Orai1, as elucidated in the work by Frischauf et al.. There, biochemical analysis including SDS-PAGE and Western blots were used and the results of this paper are discussed and interpreted in the work of Mrs. Atta, specifically focusing on the K163W mutation in *d*Orai (R91W in *h*Orai).

Overall, Mrs. Attar presented an overview of the different available Orai crystal structures, comparing and interpreting the results of open and closed channel conformations. For this she used PyMOL as a useful tool to represent differences in the channel composition. As an additional readout, she included results from published studies of *h*Orai1 H134A focusing on biochemical analysis.

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