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Title of the doctoral dissertation: ...**Structural characterization and conformational changes of RBP3**

Abstract

Vision, perhaps the most crucial sense for human life, is a remarkably complex process that requires a tight orchestration of all the tissues, cells, and biomolecules of the eye. Specifically, the retina, located to the back of the eye, is the region where the light stimulus is converted into electrical impulses that the brain can interpret. The biochemical pathway responsible for this vital process that occurs in the photoreceptors is known as the phototransduction cascade. This process occurs when a photon hits the 11-cis retinal chromophore bound to rhodopsin, causing the conversion of 11-cis retinal into all-trans retinal, ultimately leading to the activation of the enzyme PDE6, hyperpolarization, and extension of the photoreceptors. In order to sustain vision, this biochemical pathway requires constant replenishment of the chromophore. This function is provided by the retinoid visual cycle, which occurs between the Retinal Pigment Epithelium and the photoreceptors through the Interphotoreceptor Matrix. Within the Interphotoreceptor matrix resides a protein known as RBP3, which is involved in the transport of the 11-cis retinal and other retinoid molecules between the photoreceptors and the Retinal Pigment Epithelium. In mammals, RBP3 consist of a single peptide chain of ~1300 amino acids. Since the RBP3 gene has undergone a quadruplication event, within the single peptide chain, it is possible to identify four homologous repeats known as Modules. Each of the Modules represents a functional unit capable of binding retinoids. Despite the important protective role in the retina's physiology, due to its ability to bind retinoids, the proper structural characterization of RBP3 has remained elusive. The target of the thesis is to

provide a high-resolution structure of the protein in order to reveal the mechanism through which RBP3 is capable of maintaining a healthy retina. In order to achieve the target, two strategies were used. Given the size and the high flexibility of the protein and the unlikelihood to obtain suitable crystals, the first approach consisted in the study of the full-length protein through CryoEM in order to obtain a high-resolution structure of the entire architecture of the protein. The RBP3 high flexibility allows for conformational changes of the protein, and Small Angle X-ray Scattering was used to investigate the relationship between the ligand binding and the conformational changes in solution. The second approach consisted of the production of recombinant truncated versions of the protein (e.g., Modules), for crystallization in complex with the ligand in order to identify the retinoid binding sites.