Development of a method to determine protein occupancy of a DNA sequence of interest in the developing retina

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Retinal diseases often involve death of photoreceptor cells, which are the primary light-sensing cells of the eye. Due to the neuronal nature of this tissue, the cells are non-regenerative; thus, the degradation and resulting vision loss are largely irreversible. In order to develop effective treatments and therapies for retinal diseases, it is crucial to understand the formation and development of healthy photoreceptor cells. During development, cell-type specific gene expression is regulated by the binding of transcription factor (TF) proteins to specific sequences of DNA in the genome, termed *cis*-regulatory elements (CREs); determination of TF occupancy of CREs that regulate photoreceptor-specific genes is a key step in elucidating the requirements to generate a healthy photoreceptor cell. This research project seeks to investigate this by developing a method to identify the protein occupants of a DNA sequence of interest in the developing retina. The method entails covalent attachment of a proximity labeling enzyme, APEX2,[1] to a DNA plasmid containing the sequence of interest. Upon electroporation of the APEX2-plasmid complex into the retina and addition of the APEX2 substrates, proteins occupying the sequence of interest should be preferentially labeled with biotin, allowing for their subsequent identification.

To test this method's ability to identify TFs involved in retinal development, it was first necessary to develop an approach for site-specific attachment of APEX2 to the plasmid. The engineered HaloTag protein is known to form a highly specific covalent linkage to haloalkane functional groups;[2] thus, the attachment strategy involves reaction of an APEX2-HaloTag fusion protein with a chloroalkane(CA)-modified plasmid. To generate the CA-modified plasmid, a strand replacement strategy was employed where nickase sites on the plasmid, separated by about 20 base pairs, allow site-specific incorporation of an oligonucleotide containing the desired modification. Preliminary results have shown successful expression and purification of the APEX2-HaloTag fusion protein as well as confirmation of both components' catalytic activities. Additionally, preparation of the CA-modified plasmid has been optimized and shown to be reactive with the APEX2-HaloTag fusion protein to generate the desired APEX2-plasmid complex. The next step is to test if this novel approach can successfully detect TFs known to bind to a previously characterized CRE; if so, it will then be utilized for *de novo* identification of TF occupants of other CREs that are pertinent to retinal development in order to further elucidate the formation of healthy photoreceptor cells.

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