The Role of Ankyrin-B in the Cornea

Ananth Sastry

The work of a medical scientist is much like the work of a detective. When presented with a problem or "case", one must use contextual clues to form a conjecture, investigate and observe to gather data, and form a final conclusion using deductive reasoning. This past summer, I worked in the lab of Dr. Jeffrey Goldberg, living the life of a sleuth and observing the actions of antibodies and proteins within the cornea of the eye.

The first clue presented by Dr. Nancy Joyce described the actions of a protein called Na, K-ATPase. This protein is responsible for pumping water out of the cornea in the endothelial layer, an action that maintains optical clarity. Diseases affecting the eye and injuries from cataract surgeries often slough off this protein and lead to loss of vision and often blindness. I hoped to identify the bio-molecular pathways that are responsible for keeping the cornea clear.

The second clue provided by Dr. Peter Mohler described another protein called Ankyrin-B, which had shown to be responsible for the localization of Na, K-ATPase in cardiac cells. Ankyrin-B had never been described or observed in the cornea, so I began my investigation there.

I tested for the presence of Ankyrin-B in the corneal endothelial layer of rat eyes using a technique called immunostaining. Using this technique, sections of rat eyes were stained with antibodies specific to the protein we were searching for, and then stained with a non-specific fluorescent antibody that could then be viewed under a microscope. I found that Ankyrin-B was very positive and localized in the corneal endothelium membrane in mature rat eyes, but not in neonatal eyes. This showed that Ankyrin-B was indeed expressed in the endothelial layer and led me to believe that the relationship between these two proteins also existed in the cornea.

To test this further, I stained different rat eyes from immediately after birth to twenty days after birth with antibodies for both Ankyrin-B and Na, K-ATPase. The following figures display some of the results. (Key: Ep- Epithelial layer; Stroma- Stromal layer; En- Endothelial layer; P2- Cornea of rat two days after birth; P20- cornea of rat twenty days after birth)

Ep Stroma En

P2 Ankyrin-B stain

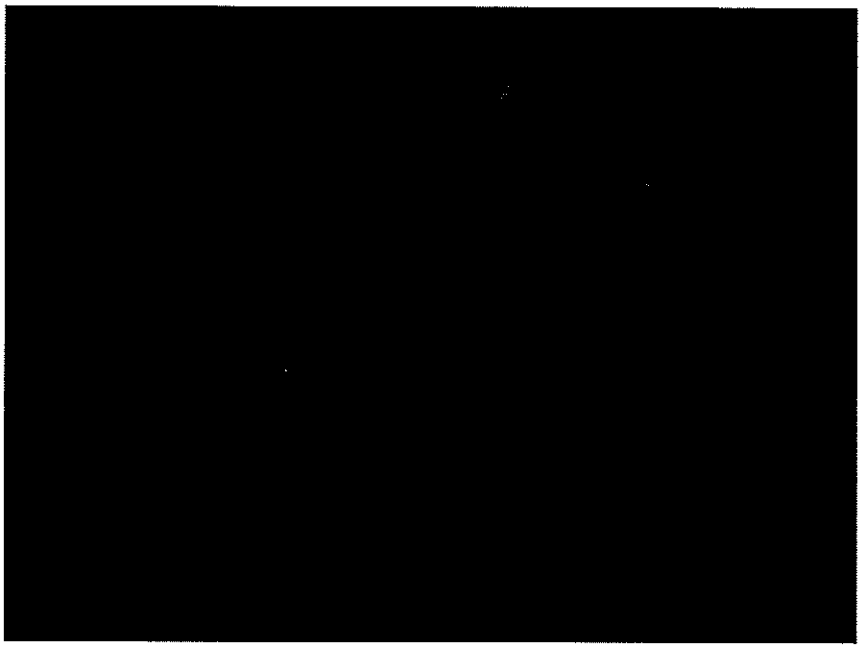
Ep Stroma En

P20 Ankyrin-B Stain

Ep Stroma En

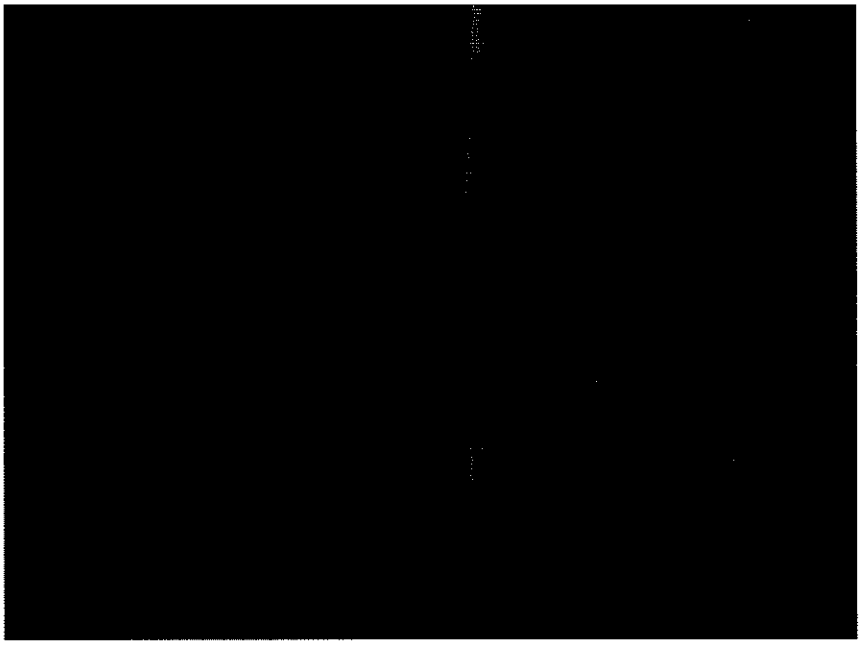
Negative Control for Ankyrin-B

Ep Stroma En



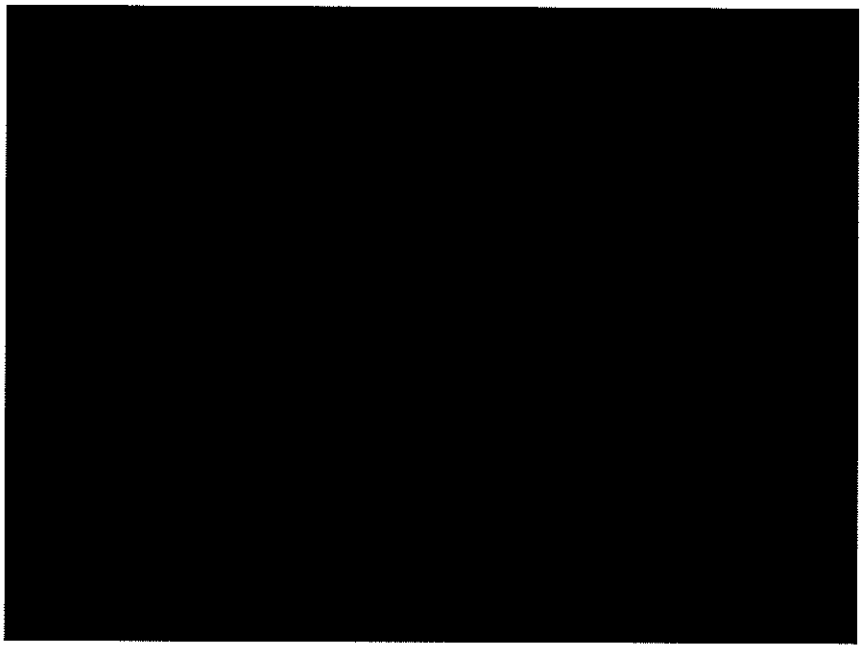
P2 Na, K-ATPase Stain

Ep Stroma En



P20 Na, K-ATPase Stain

Ep Stroma En



Negative Control for Na, K-ATPase

The results show that during development, the expression of Ankyrin-B increases, which also correlates with a parallel increase of expression and localization of Na,K-ATPase in the endothelial layer of the cornea. This suggests that Ankyrin-B is responsible for the localization and expression of Na, K-ATPase in the cornea, and therefore has a significant role in vision. The final steps of my investigation, beginning this fall, will involve the removal of the Ankyrin-B protein and subsequent observation of Na, K-ATPase expression in the cornea. The results of these experiments should, at the very least, lead to a greater knowledge of corneal biology and, we hope, could also lead to new therapies in the treatment of corneal dystrophies. As Sherlock Holmes once told Dr. Watson, "the game is afoot!"