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Migration Pattern Dynamics during Choroid Fissure Closure in Zebrafish

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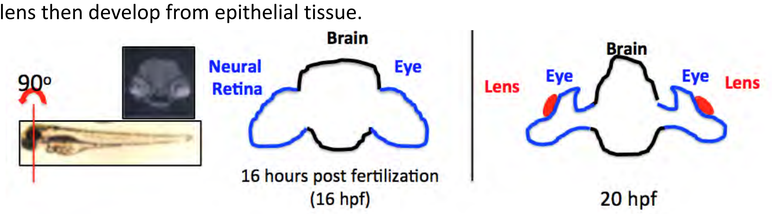
Abstract

Successful closure of the choroid fissure (CF) is essential for proper development of the vertebrate eye. The impermanent structure forms as the optic cup surrounds the invaginating lens allowing hyaloid vasculature to enter the developing eye. If the choroid fissure closure (CFC) fails to close, a coloboma develops associated with approximately 3-11% of childhood blindness worldwide. The CF cells are distinct prior to fusion as they remain undifferentiated until fusion of the opposing sides and have unique N-cadherin expression. During CFC, cells must breakdown basement membrane to allow fusion between both sides producing a seamless ventral retina. Both breakdown and fusion of the apposing sides of the CF initiate from the central region and proceeds bi-directionally. It is unknown however where or if the CF cells migrate away from an aligned fusion point. *in vivo* confocal microscopy of transgenic *Hsp701:*Gal4*;UAS:*Kaede zebrafish embryos during CFC allows distinctive contrast of photoconverted cells to give insight to their spatial temporal movement. We partitioned our analysis into three distinct regions along the proximal/distal axis of the CF to determine if cellular movement at the choroid fissure edges maintained distinct cellular migration patterns prior to differentiation. Preliminary analysis from 44 to 48 hpf in the proximal and distal regions demonstrate movement distinct from those within the central region. In both proximal and distal CF, upper (dorsal) CF cells move towards the central CF changing their proximal/distal axis in the opposite direction of fusion. Lower (ventral) CF cells move further ventrally towards the apposing side in order to fuse the CF. This contrasts with the central CF upper and lower cells that move directly towards the apposed sides. These results further support the hypothesis that cells at the CF edge are regulated differentially from the remaining differentiating retina.

Zebrafish Choroid Fissure Closure

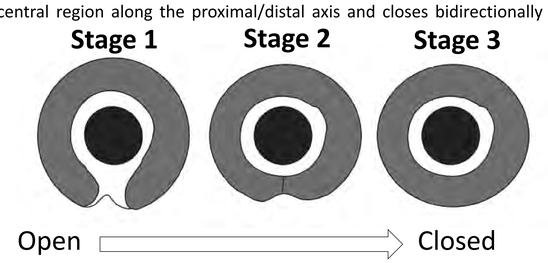
Eye Development of Zebra Fish

The eye develops from neural tissue and bulges out to create the eye shape. The lens then develop from epithelial tissue.



Choroid Fissure Closure

The choroid fissure (CF) is a transient opening at the last major morphogenesis change in eye. The mechanism of the CF closure has three major steps. The first stage involves proliferation and elongation of the cells at the CF. The second step is the breakdown of the laminin basement membrane. The last is the fusion of the two apposed sides into one seamless structure. The closure begins in the central region along the proximal/distal axis and closes bidirectionally in a zipper-like fashion.



Improper Choroid Fissure Closure

If the CF does not properly close it will result in a coloboma. A coloboma is visible as a tear through the iris however there is no retinal tissue present within the opening. Colobomas are associated with 3-11% of childhood blindness and tend to present as a phenotype of multiple syndromes.

Brown, *et al,* 2009

Wildtype Coloboma

UV Activation of CF Cells and Cell Tracking

Figure 1

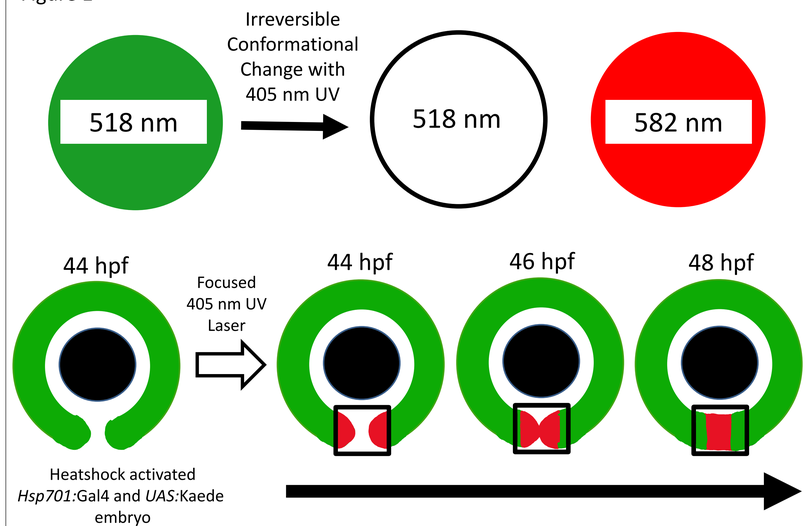


Figure 1. *Hsp701:*Gal4 and *UAS:*Kaede heatshock embryos are UV activated in the CF cells. Before exposure to a 405 nm UV laser, Kaede emits at the 518 nm wavelength (green). The UV laser allows for conformational change of the Kaede protein shifting the emission to 582 nm (red). Precise photoconversion was done on the confocal to activate the cells at the CF. Z-stack images of CF were taken every 15 minutes after the one exposure and sectioned into 2 µm slices. Analysis was done using ImageJ.

Successful Photoconversion of Transgenic Embryo

Figure 2

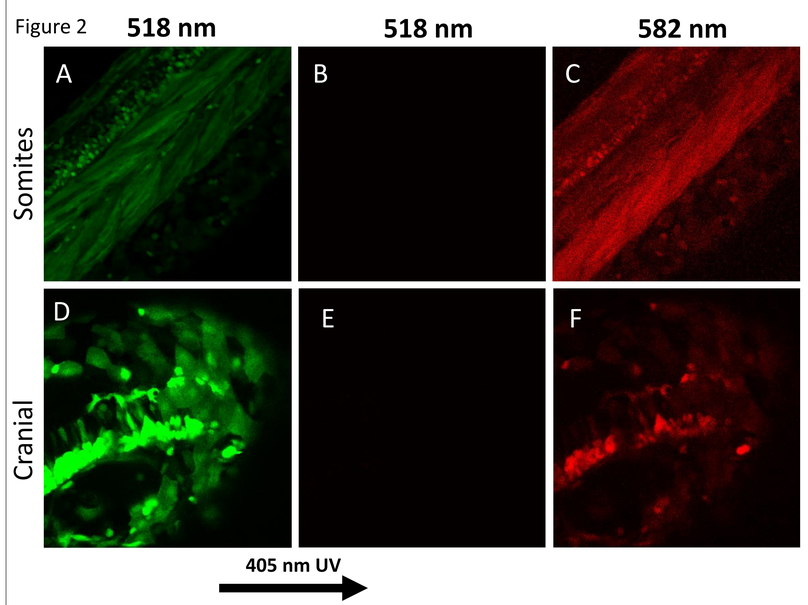
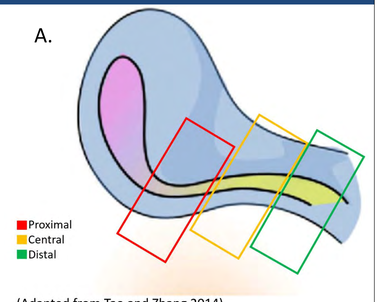


Figure 2. Successful heatshock activation and photoconversion of Kaede protein. A, B, and C demonstrates conversion of somites. D, E, and F visualize the photoconversion of the skull. Kaede is ubiquitously expressed with no phenotypic abnormalities.

Movement of Choroid Fissure Cells

Figure 3

Figure 3a. Spatial model of developing eye with open CF prior to fusion**.** Analysis was sectioned into 3 regions along the proximal/distal axis of the CF. The proximal region is shown in red, the central in yellow, and the distal in green. Figure 4 uses the same categorization .



(Adapted from Tao and Zhang 2014)

Figure 4

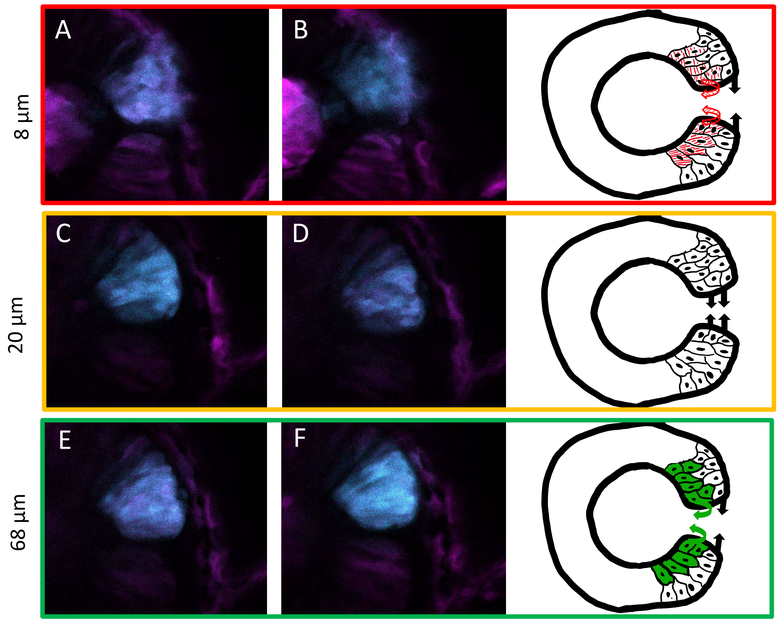


Figure 4. All analyses were done using ImageJ and Excel. For ease of viewing green was pseudocolored magenta, while red was pseudocolored cyan. Both the proximal (A and B - red) and distal (E and F - green) regions have dorsal (upper) CF cells that leave their initial position on the axis and move towards the central region. While the lower (ventral) cells remain within their same point on the axis and approach the apposed side. This was measured with ImageJ, but visually seen with a decrease in fluorescence in the proximal region and an increase in the distal region over time. Central CF cells remain in their same position along the axis and approach the apposed side. All cells are visible throughout the imaging period. This provides evidence as to why the CF closes at the central region first.

Future Directions

- What are the fate of these cells at their final position after fusion?
- What factors are involved that initiate these unique dynamics?

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