

Featured Publication Note

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Neural lineage and cell division in the zebrafish retina *in vivo*

The research of Professor William Harris and colleagues from the Department of Anatomy at the University of Cambridge, UK, focuses on the molecular embryogenesis of the visual system. Zebrafish (*Danio rerio*) potentially offer a novel approach to elucidating mechanisms of retinal development as well as degeneration by providing genetic tractability in a vertebrate with a retina similar to humans.

The *ath5* gene encodes a basic helix-loop-helix transcription factor, necessary for the development of retinal ganglion cells (RGCs), yet not all *ath5*-positive cells differentiate as RGCs. This study investigates the development of RGCs using an *ath5*:GFP transgenic line of zebrafish in combination with 3D time-lapse microscopy to determine reproducible features in lineages of *ath5*-expressing progenitors.

Sections through 5 day-old post-fertilized retinas, when all cell layers are differentiated, showed GFP-positive cells not only in the ganglion cell layer (GCL) as expected, but also in the inner nuclear layer (INL) containing horizontal and amacrine cells, and in the outer nuclear layer (ONL) containing photoreceptors (Figure 1 A-C). These results show that RGCs are not the only fate choice of *ath5*-expressing retinal progenitors.

To demonstrate the lineage relationship between *ath5*:GFP-positive RGCs and other *ath5*:GFP-positive cells, Professor Harris and colleagues examined several individually dividing *ath5*:GFP progenitors by 3D time-lapse microscopy. Embryonic retinas were imaged with a confocal laser scanning microscope at 10-15 min intervals and 0.5 μ m optical sections, through a volume up to 50 μ m, for a minimum of 10 h, starting from ~32 h after fertilization. To visualize the acquired data as time-resolved volumes, images were processed using Volocity® Visualization. Cell tracking was performed using Volocity Quantitation.

The results showed that all *ath5*:GFP-progenitors gave rise to two daughter cells. After separation, these two daughter cells re-extended toward the basal surface but then migrated in opposite directions and acquired different morphologies; one differentiating to a RGC (migrating basally) and the other migrating back to the apical surface. Figure 2 shows an example of this lineage pattern.

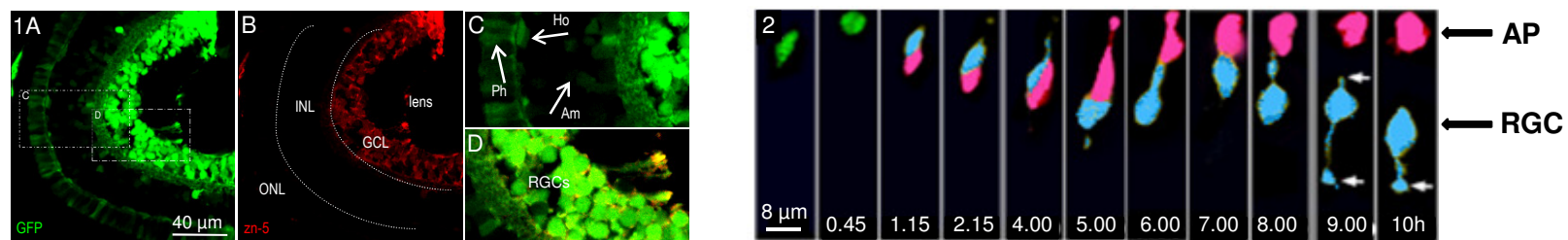


Figure 1: Sections through the central retina of a 5 day-old *ath5*:GFP transgenic embryo. Images were processed using Volocity Visualization. (A) RGC progenitor cells are labeled with GFP. The white boxes indicate the areas shown in C and D. (B) shows all three retinal cell layers, RGCs are labeled with zn-5+ antibody. (C) Some *ath5*:GFP progenitors become photoreceptors (Ph), amacrine (Am), and horizontal (Ho) cells. (D) Overlay of RGCs (red) and GFP-positive (green) progenitors; GCL=ganglion cell layer, INL=inner nuclear layer, ONL=outer nuclear layer.

Figure 2: Time-lapse series showing the lineage of an *ath5*:GFP progenitor. Imaging was started 30-32 h after fertilization, and t=0 corresponds to the time of appearance of *ath5*:GFP. Progenitor is shown in green, daughter cells are shown in pink (AP, apical cells) or blue (RGCs). See also Video 4, available at <http://www.jcb.org/cgi/content/full/jcb.200509098/DC1>, and created in Volocity).