



Neural lineage and cell division in the zebrafish retina in vivo

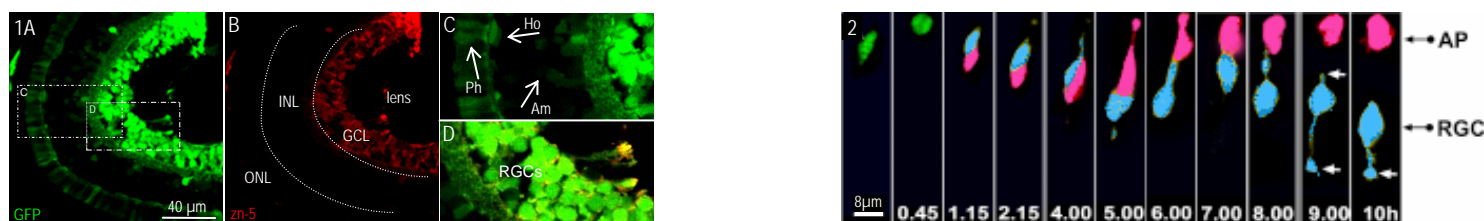


Figure 1 A-D Sections through the central retina of a 5 d old *ath5:GFP* transgenic embryo. Images processed with **Volocity Visualization**.

(A) RGC progenitor cells are labeled with GFP. The white boxes indicate the areas shown in (C, 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)

The research of Prof. 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* promoter, a bHLH transcription factor, is 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-d old postfertilized 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, Harris and colleagues examined several individual dividing *ath5:GFP* progenitors by 3D time-lapse microscopy.

Embryonic retinas were imaged with a confocal laser scanning microscope at 10 to 15 min intervals and 0.5 µm optical sections through a volume up to 50 µm 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 Classification**.

Results showed that all *ath5:GFP*-progenitors gave rise to two daughter cells. After separation, these two daughter cells reextended toward the basal surface but then migrated in opposite directions and acquired different morphologies, one differentiating to a RGC, migrating basally, the other migrating back to the apical surface. Figure 2 shows an example of this lineage pattern (see also Video 4, available at <http://www.jcb.org/cgi/content/full/jcb.200509098/DC1>, created in **Volocity**).

Images reproduced with kind permission of Prof. William A Harris and Dr Lucia Poggi; Application Note based on: Influence on neural lineage and mode of division in zebrafish retina in vivo **The Journal of Cell Biology**, 2005, 171: 991-999