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### The orphan nuclear receptor Nr2e3 plays dual functions in rod photoreceptor differentiation

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During retinal development, rod and cone photoreceptors are generated from common pools of neuroepithelial progenitors. Nrl, Crx, and Nr2e3 are the key transcriptional regulators that control rod differentiation. Nr2e3 is a rod photoreceptor specific orphan nuclear receptor. However, the loss of its function results in enhanced S-cones and rod degeneration in both human (ESCS patients) and mice (rd7) retina. Nr2e3 is not detected in the Nrl−/− retina, which exhibits excess functional S-cones at the expense of rods. Here, we show that, using GFP to tag the newborn rod precursors, some early born rod precursors are transformed into S-opsin-positive cells in the rd7 mouse. On the other hand, forced over-expression of functional Nr2e3 in postmitotic cone precursors induces them to adopt rod pathway at the expense of cone differentiation. However, these new rod-like photoreceptors are not functional, partially due to the lack of rod transducin. The dual functions of Nr2e3 on rod and cone gene regulation are not dependent on Nrl and/or Crx but rely on its expression time and level. Thus, our in vivo studies reveal a critical role of Nr2e3 in rod photoreceptor differentiation during retinal development.

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### Misexpression of neuroD in the developing zebrafish retina: Effect on proliferation and photoreceptor genesis

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NeuroD is a member of a large family of proneural genes and has been implicated in retinal cell genesis, neuronal development, and cell cycle regulation. In the retinas of larval and adult teleosts, neuroD is expressed in two post-mitotic cell populations, a subset of amacrine cells and transiently in nascent cone photoreceptors, and proliferating cells of the rod and cone photoreceptor lineages. In contrast to other vertebrate retinas, neuroD is not expressed in multipotent progenitors, indicating that in teleosts neuroD is not determinative for retinal cell fates. This makes the zebrafish retina a unique system to study the role of neuroD in lineages of cells, which give rise exclusively to photoreceptors. To test the function of neuroD in these cells, lines of zebrafish transgenic for heat shock 70/4:neuroD-EGFP (Hsp:nrd-EGFP) were established for conditional gain-of-function experiments. Heat shock resulted in robust EGFP fluorescence (37°C, 60min) throughout the embryo. Embryos were evaluated by in situ hybridization with probes to neuroD and islet1. Cell type-specific antibodies were used to label amacrine cells, cone, and rod photoreceptors, respectively. Mitotically active cells were labeled with 5mM BrdU and 15% DMSO. Heat shock induces expression of message for neuroD and islet1, a downstream target of neuroD, and results in marked decrease in proliferation compared to controls. We conclude that the Hsp:neuroD-EGFP construct generates functional protein in vivo, and conditional misexpression suggests that neuroD may modulate the mitotic activity of cells in rod and cone photoreceptor lineages.

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### Photoreceptor subtype specification and mosaic patterning in zebrafish

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The well characterized, laminar organization of the seven major cell types of the vertebrate retina has been indispensable for investigating neural development. Unfortunately, the genetic mechanisms underlying the true diversity of neuronal subtypes and their non-random or mosaic arrangements within each lamina remain poorly understood. We have taken advantage of the precisely defined photoreceptor mosaic of the teleost to investigate cell–cell signaling mechanisms essential for subtype specification and patterning in the retina. In zebrafish embryos mutant for the neurogenic gene *mindbomb* (*mib*), cells in the outer nuclear layer adopted a default, red cone fate. Genetic chimera analysis showed that mib mutant cells retained the potential to differentiate into the full repertoire of photoreceptor subtypes consistent with a model of lateral inhibition. However, following genetic perturbations of neurogenesis, the photoreceptor mosaic pattern was quickly re-established in regions of newly differentiated neurons demonstrating that appropriate spacing is not dependent upon propagation of a wave of signaling from a pre-existing photoreceptor cell mosaic. Furthermore, pharmacological inhibition of Notch signaling produced more modest disruptions of retinal organization consistent with the absence of Müller cell maturation. Unexpectedly, the putative radial glial cells of the inner nuclear layer did not differentiate but reentering the cell cycle. Our results demonstrate new context-dependent roles for notch signaling in cone photoreceptor subtype specification, and we are continuing our screen for mutations affecting photoreceptor subtype specification.

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### Role of senseless in late color photoreceptor differentiation in Drosophila

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