

PERSPECTIVES

Combining physiology and genetics in the zebrafish retina

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The zebrafish has recently joined the ranks of *Drosophila* and *C. elegans* as a tractable model for genetic screens (Fishman, 1999). Zebrafish grow fast, can be kept in large numbers in a small space, and are efficiently mutagenized and screened. Genomic resources are made available at an increasing pace. These days, a mutation can be mapped and cloned in a matter of months. Because a mutant hunt is intrinsically unbiased in terms of the classes of genes that will be tagged, it holds the unique potential to discover novel genes or, in our era of genome sequencing, to identify novel functions for known genes. Zebrafish display dozens of innate behaviours in response to light, of which the optomotor and the optokinetic responses are the most widely studied (Brockerhoff *et al.* 1995; Easter & Nicola, 1996). Their retinæ are crisply layered following the typical vertebrate pattern, and the retinal layers are tiled in an almost crystalline fashion by mosaics of different cell types. Electroretinograms are recorded routinely and therefore, not surprisingly, zebrafish are now also being used for a genetic approach to the visual system.

In recent behavioural screens (Brockerhoff *et al.* 1995, 1998; Neuhauss *et al.* 1999), about 20

mutations have been isolated affecting diverse anatomical sites in the retina corresponding to different stages of visual processing (Fig. 1). Some mutations lead to visible developmental changes, others appear to disrupt physiological functions. The field is young, so no gene has been cloned as yet, but the plethora of phenotypes and the relative ease by which they are isolated suggest that the screens conducted so far have only scratched the surface. More sophisticated behavioural assays are likely to reveal more specific mutations. Complementary to further screening and gene cloning, it will remain important to establish baseline data on the retina in wild-type zebrafish and to develop tools to investigate the mutants. A new paper by Connaughton & Nelson in this issue of the *Journal of Physiology* has done just that by characterizing bipolar cells (a class of retinal interneurons transmitting signals from photoreceptors to retinal ganglion cells). Their catalogue of bipolar cell types provides the background against which mutant phenotypes affecting the inner nuclear layer (where bipolar cells are found) can be analysed.

Connaughton & Nelson (2000) recorded from a large sample of bipolar cells in the slice preparation of zebrafish retina. They used dye-filled whole-cell patch pipette electrodes to examine conductance changes evoked by glutamate and several glutamate agonists, and to obtain a morphological picture of the shape and arborizations of each cell. In total, the authors found four principal types of synaptic response. Three of these have been previously reported in other retinæ. A fourth was characterized by a response to kainate but not glutamate. This would be a novel but perplexing finding given that the presynaptic neurons release glutamate. Morphologically the authors report that while some bipolar cell axon terminals stratified exclusively in either the inner or outer regions of the inner plexiform layer, analogous to bipolar cells in rat retina (Euler *et al.* 1996; Hartveit, 1997), 20% stratified in both

sublaminae. Similar multistratification has been observed previously in turtle bipolar cells (Ammermüller & Kolb, 1995). The authors were able to classify the bipolar cells into 13 different types based on the pharmacological and morphological profiles. Comparable numbers of types were similarly identified in turtle and rat.

Genetics aside for the moment, Connaughton & Nelson (2000) gives new information about bipolar cell neurophysiology and raises a number of questions that deserve future attention. When one studies the actual light responses in these zebrafish cells, how will the classification scheme hold up? Will the multistratified cells respond to light as ON- or OFF-cells? What is the function and mechanism of the kainate-sensitive, glutamate-insensitive synaptic inputs? The paper is also likely to be interesting to those who study zebrafish retinal mutants, since it demonstrates for the first time that patch clamp recordings of synaptic responses can be done. Three mutant zebrafish strains, *noa*, *nir* and *drp* (see Fig. 1), have a reduced b-wave in the electroretinogram, indicative of a defect in synaptic transmission from photoreceptors to bipolar cells. It is now feasible to contemplate studying these mutants by recording pharmacological responses directly from the bipolar cells. As more laboratories perform large-scale genetic screens many more potential candidates for defective bipolar cell functions will be identified. It will be interesting to see if mutations can help dissect the distinct functions of this diverse group of neurons and identify the gene products subserving these functions.

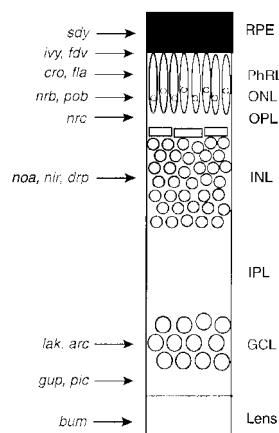


Figure 1. Mutations affecting development and function of the retina in larval zebrafish

A cross-section of a 7-day-old retina is shown, with cell densities and layer thicknesses characteristic for this age. Layers are indicated on the right. GCL, ganglion cell layer; INL and ONL, inner and outer nuclear layer, respectively; IPL and OPL, inner and outer plexiform layer, respectively; PhRL, photoreceptor layer; RPE, retinal pigmented epithelium. Behavioural screens (Brockerhoff *et al.* 1995, 1998; Neuhauss *et al.* 1999), supported by morphological, anatomical and ERG studies, have revealed at least sixteen mutants (three-letter abbreviations on the left) affected at different sites (arrows) in the retina. For *bum*, *cro*, *drp*, *fdv*, *fla*, *gup*, *ivy*, *lak*, *nir*, *pic* and *sdyc*, see Neuhauss *et al.* (1999). For *arc*, *noa*, *nrb*, *nrc* and *pob*, see Brockerhoff *et al.* (1998) and references therein.

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