

Zebrafish on the move: towards a behavior–genetic analysis of vertebrate vision

Herwig Baier

Behavioral screens have uncovered dozens of zebrafish mutants with striking visual defects. In parallel with mutant studies, recent psychophysical experiments indicate that zebrafish are capable of high-level motion processing, previously thought to be restricted to animals with a visual cortex. It should be possible now to devise assays to screen for mutations in visual perception.

Addresses

University of California at San Francisco, Department of Physiology, 513 Parnassus Avenue, S-762, San Francisco, CA 94143-0444, USA;
e-mail: hbaier@itsa.ucsf.edu

Current Opinion in Neurobiology 2000, 10:451–455

0959-4388/00/\$ – see front matter

© 2000 Elsevier Science Ltd. All rights reserved.

Abbreviations

DA-IPC dopaminergic interplexiform cell
OKR optokinetic responses
OMR optomotor responses
RGC retinal ganglion cell

Introduction

The main goal of this review is to draw attention to the success of recent zebrafish screens for point mutations in genes essential for visual development or function [1–10,11*,12*]. Here, I will restrict myself to the description of three mutants with rather striking neurobiological and behavioral phenotypes [11*,12*]. The fact that behavioral mutants

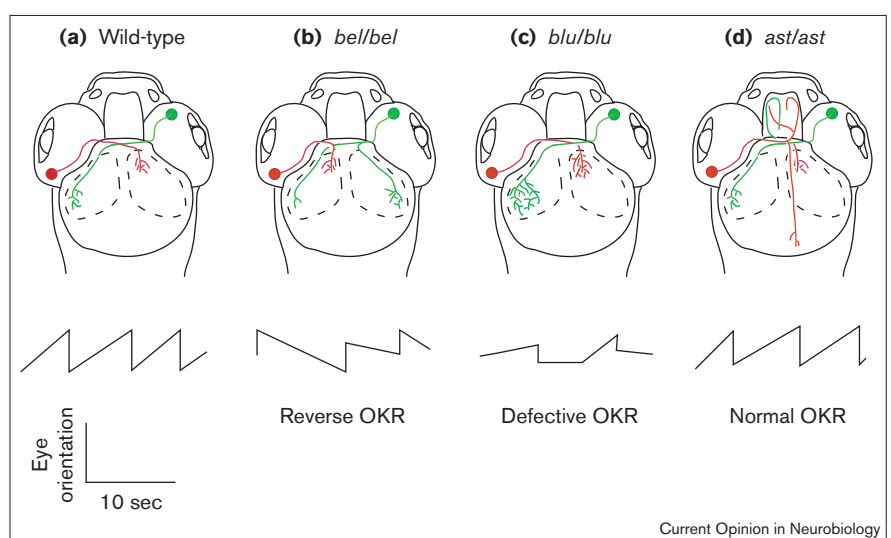
have been found represents major progress and would not have been possible without the persistent efforts of several laboratories to devise rapid, robust, and informative screening assays. This review will highlight three assays, all of which exploit innate behavioral responses by zebrafish to a moving stimulus. These assays test for optokinetic responses (OKR) [5,13,11*], for optomotor responses (OMR) [11*], or for the visually mediated escape response [10,12*]. The OMR assay, in addition to being ideal for high-throughput screens, has also enabled a psychophysical analysis of zebrafish motion vision, which is discussed towards the end of this review.

Behavioral assays reveal visual impairments of mutant zebrafish

In the standard OKR assay [5,13], single fish larvae are immobilized within a dish, which sits on a stable platform inside a rotating drum. The drum is fitted with black and white stripes. As it turns around the dish, the fish move their eyes to pursue the drifting bars. Phases of smooth pursuit are periodically interrupted by fast saccadic eye movements in the opposite direction. When the angle of eye orientation is plotted as a function of time (as in Figure 1, bottom panels), the graph takes a saw-tooth shape for wild-type fish (Figure 1a, bottom). Mutants with retinal dysfunction naturally do not show an OKR, but more subtle mutations have also been identified. The *blumenkohl* (*blu*) mutation, for example, leads to an enlargement of retinotectal arbors [4] and to OKR deficits (Figure 1c; [11*]), whereas the *astray*

Figure 1

Optokinetic behavior of three retinotectal mutants. The top panels show the heads of wild-type and mutant fish larvae and illustrate the organization of their retinotectal projections. The subset of axons that originate in the nasal part of the retina (labeled green, traced only from the left eye) normally projects to the posterior part of the tectum. Axons from the temporal retina (labeled red, traced only from the right eye) normally project to the anterior tectum. The bottom panels show plots of the optokinetic response (OKR), the typical saw-tooth function of eye movements that is produced when viewing a rotating set of stripes. It should be noted that the OKR is probably not mediated by the tectum, but by a smaller visual nucleus not shown here. **(a)** In wild-type fish, all axons project to the contralateral side of the brain and form small arbors within the tectum. Retinotectal mutants show various disruptions of this pattern. **(b)** *Bel* mutant axons project to the ipsilateral tectum; the OKR is reversed. **(c)** *Blu* mutant axons form



larger arbors; the OKR is impaired. **(d)** *Ast* mutant axons project to several ectopic

places in the forebrain and hindbrain; the OKR is nevertheless normal.

(*ast*) mutant, despite severe path-finding errors in the retinotectal projection [3], shows a surprisingly normal OKR (Figure 1d; [11•]). A particularly intriguing case is the *belladonna* (*bel*) mutant, discussed below, which shows an OKR that is opposite to the direction of movement of the grating (Figure 1b; [11•]).

In the OMR assay, fish larvae swim freely in long, narrow chambers. The chambers are placed on top of a computer monitor, which sits on its back (i.e. the screen faces up) [11•]. To evoke the OMR, the fish are shown computer-animated patterns that sweep below them across the screen. Wild-type fish follow the perceived motion of their visual environment. In a flowing river, this behavior helps them stay at one location, preventing them from being carried downstream. The readout of the OMR assay during a screen is quite simple: blind or motion-blind mutants will swim in random directions, while wild-type fish will swim in the direction of the stimulus and accumulate at one end of the chamber. The OMR assay is fast and can handle up to ten clutches, with 10–40 fish each, in parallel [11•]. One of the many mutants whose visual defects have been detected with the OMR assay is the *lakritz* (*lak*) mutant, discussed below, which lacks a large subset of retinal ganglion cells (RGCs). OKR-defective fish usually also fail in the OMR assay [11•].

In the escape assay [10], adult fish are placed into a circular container inside a rotating drum. Except for one black patch, the drum is white. As the drum rotates around the container, the spot will come into view of the fish and will trigger a flight reaction. The test can be made more difficult for the fish by dimming the ambient light, thus decreasing the contrast of the black spot against the background. This way, the visual-sensitivity threshold can be measured, and mutants with decreased sensitivity can be revealed. The escape assay has been used to identify the two dominant *nightblindness* loci, *nba* [10] and *nbb* [12•], of which *nbb* is discussed below.

Cross-wiring of optokinetic responses in the achiasmatic *belladonna* mutant

Belladonna (*bel*) mutant larvae display a striking behavior in the OKR assay [11•]. Their eyes move in episodes of smooth pursuits interrupted by saccades, similar to wild-type fish (Figure 1a); however, the direction of eye movement is opposite to the drift direction of the optical stimulus (Figure 1b). Thus, a clockwise rotation evokes a counterclockwise tracking movement of the eye, and *vice versa*. In *bel* mutants, RGC axons frequently project ipsilaterally [3]. In the tectum, they form a normal retinotopic map, with temporal axons connecting to the anterior part and nasal axons to the posterior part (Figure 1b). A combined behavioral and anatomical analysis [11•,14] has shown that the reverse OKR is tightly correlated with the degree of miswiring of the retinotectal projection. Thus, if most or all of the retinal fibers project ipsilaterally, then the OKR is found to be reversed, whereas it is normal in cases where the projection is predominantly contralateral.

How can ipsilateral misrouting of retinal fibers lead to sign-inversion of the OKR? To answer this question, one must consider the path along which visual information is channeled in order to mediate the OKR. The OKR clearly involves a feedback loop that matches sensory input (movement on the retinal surface) to motor output (muscle control of eye movements). The direction of a horizontally moving grating is either nasal-to-temporal or temporal-to-nasal. For a grating that rotates around the fish (as used here), the direction of movement is opposite for the two eyes, temporal-to-nasal for one and nasal-to-temporal for the other. The brain area that mediates the OKR for a given eye (the fish homolog of the accessory optic system) is located on the contralateral side of the animal. This nucleus feeds the visual input into the oculomotor complex, which commands the eye muscles to turn the eye in register with the stimulus. In *bel* mutants — at least in those with fully penetrant ipsilateral misrouting — the stimulus is disconnected from the recipient eye and drives the movement of the other eye instead. Because this eye sees the motion going in exactly the opposite direction, the OKR is reversed. Thus, in *bel* mutants, the two eyes steer each other's movement. In the OMR assay, in which the stimulus is presented from below, the direction of motion the fish see is the same for both eyes. The miswiring therefore should not affect the behavior. Sure enough, the OMR of *bel* mutants is normal [11•].

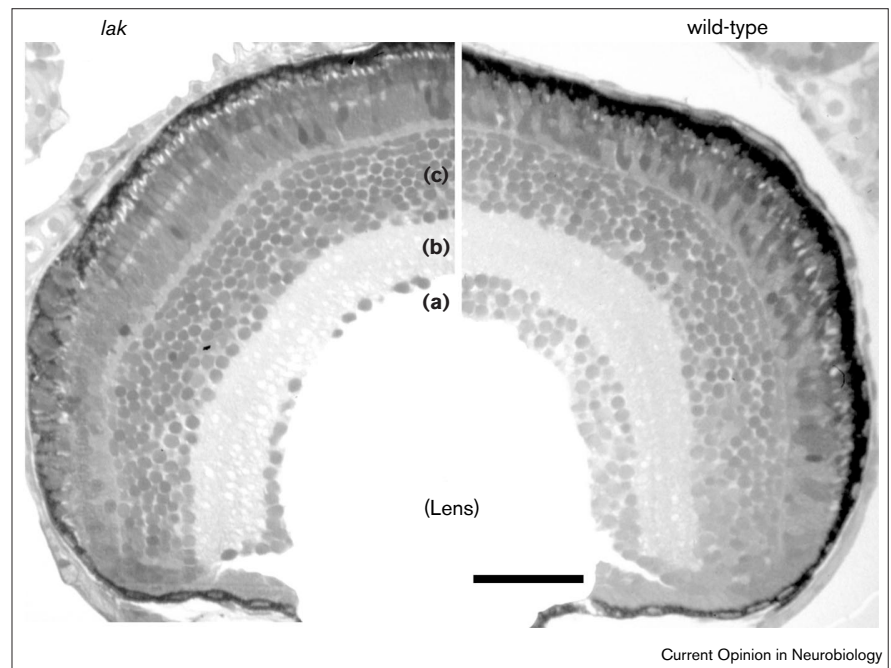
The *lakritz* mutation sheds light on the dark side of blindness

The visual defect of the *lakritz* (*lak*) mutant was first picked up by the OMR assay [11•]. This recessive mutation was originally identified on the basis of a presumed pigment defect [15,16]. Mutants appear more blackish than wild-type fish as a result of a permanent dispersal of melanin granules in their melanophores (black pigment cells); hence their name (German for 'licorice'). Apart from the pigment phenotype, the mutants appear normal morphologically and display no obvious behavioral abnormality [15]. Subsequent behavioral tests have revealed that their blackness is a secondary consequence of their blindness [11•]. Normally, zebrafish adjust their pigment distribution to the ambient light, in a visually controlled neuroendocrine response. Many blind fish fail to do so [11•]. For example, the *blu* mutant (Figure 1c) is also darker, although not as extreme as *lak*. A combined OMR and OKR re-screen of 12 mutants with dispersed pigment has shown that two thirds of them are blind or visually impaired; one third, however, are normal [11•]. A screen for 'black' fish could be a powerful primary screen for blind fish, but its information would be limited without a more direct functional assay.

Sections of the *lak* retina have revealed that 80% of its RGCs are missing (Figure 2; [11•]). This is one of the most specific developmental phenotypes discovered so far. The only good candidate gene for *lak*, based on its similar genotype in the mouse [17,18], seems to be the *Brn-3b* (or *Brn-3.2*) gene, which belongs to the family of POU domain transcription factors. The zebrafish *Brn-3b* gene, however,

Figure 2

The retina of the blind *lak* mutant lacks most retinal ganglion cells. In this composite picture, a methylene-blue stained section of a mutant retina is shown on the left, and a wild-type retina is shown on the right. The *lak* mutation quite specifically prevents development of the majority of retinal ganglion cells (a). The inner plexiform layer (b) is somewhat thinner in the mutant, probably as a result of the absence of ganglion cell dendrites. The inner nuclear layer (c), however, appears to be increased in cell number. Scale bar = 100 μm .



Current Opinion in Neurobiology

maps to a different chromosome than that on which *lak* is located ([19 \bullet]; K Finger, H Baier, unpublished data), indicating that *lak* encodes an as yet unidentified gene. Interestingly, the inner nuclear layer of the *lak* retina, which is populated by bipolar cells and amacrine cells, seems to be enlarged by about the same number of cells that the ganglion cell layer is diminished (Figure 2). The *lak* gene product could be an essential determinant of RGC development, perhaps acting as a switch between ganglion cell and amacrine cell fate.

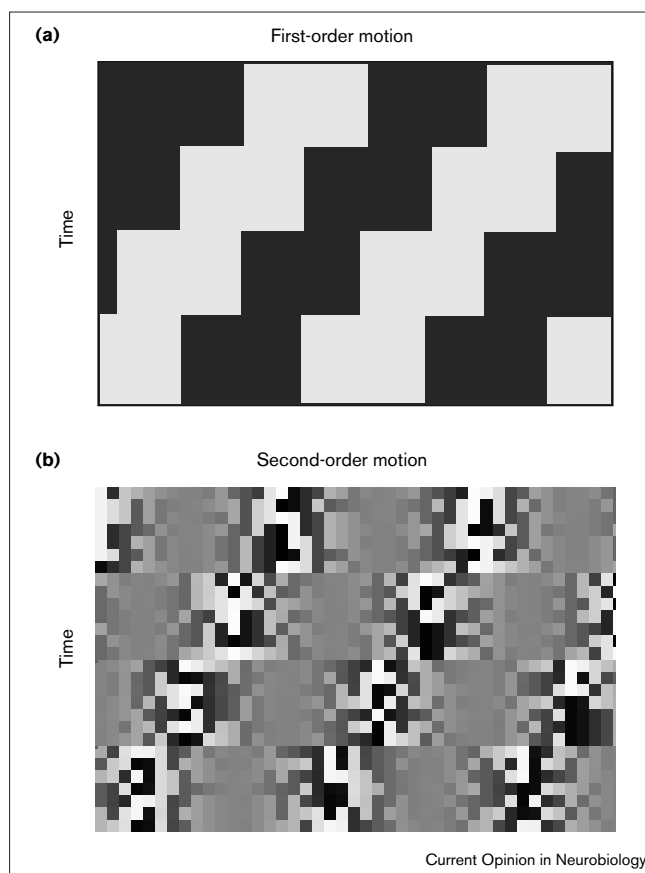
A functional link between olfaction and vision is exposed by the *nbb* mutation

A behavioral screen for dominant adult mutations that disrupt the escape from a threatening object was used to identify a mutant with a unique, somewhat perplexing phenotype. Mutants of the *nightblindness b* locus (*nbb*) exhibit visual thresholds that are elevated by 2–3 log units after prolonged darkness [12 \bullet]. Normally, under dark-adapted conditions, the rod pathway is active, but in *nbb* mutants the RGCs remain quite unresponsive (although the rod response itself is normal), rendering the fish almost blind at night. The loss of visual sensitivity in *nbb* is preceded by a reduction in the number of dopaminergic interplexiform cells (DA-IPCs). The DA-IPC is a teleost-specific type of amacrine cells that releases dopamine, modulating the light-adaptive state of the teleost retina (reviewed in [20]). In a paper that accompanies their *nbb* study, Li and Dowling [21] show that infusion into wild-type eyes of 6-OHDA (6-hydroxy-dopamine), a drug that specifically kills the DA-IPCs, phenocopies the night blindness observed in *nbb* mutants. Infusion of a dopamine

agonist can partially restore the visual sensitivity of DA-IPC-depleted fish, indicating that dopamine is necessary and sufficient for the modulation of particular phases of dark adaptation in the fish retina.

What causes the dysfunction and disappearance of dopaminergic cells in *nbb* mutants? The authors' latest study [12 \bullet] shows that it could be secondary to reduced innervation by centrifugal projection neurons from the distant olfactory bulb. These neurons normally innervate the retina, as well as some other targets in the brain, via the terminal nerve (TN), but do so to a much lesser extent in adult *nbb* mutants. Surgical removal of the olfactory bulb in wild-type fish ablates the TN projection, causing a loss in visual sensitivity similar to that of the *nbb* mutant [12 \bullet]. The most parsimonious explanation of this result is that the TN neurons regulate the function (and perhaps the survival) of DA-IPCs. When the connection is disrupted (by mutation or by olfactory-bulb ablation), dark-adaptation of the inner retina is disturbed.

In teleost fish, amphibians, and other vertebrates, dopamine, applied to the dark-adapted retina, mimics the effects of light on the so-called retinomotor movements in the outer retina. These involve the contraction of cones (bringing their outer segments into the focal plane) and the elongation of rods (out of the focal plane), as well as the dispersal of light-protective melanin granules into the finger-like protrusions of the retinal pigment epithelium, which surrounds the photoreceptor outer segments (for reviews, see [20,22]). Dopamine also suppresses rod input to horizontal cells, whereas it enhances cone input [20].

Figure 3

Zebrafish larvae respond to both first-order and second-order motion stimuli. **(a)** A four-frame movie gives a strong (first-order or Fourier) motion stimulus. The dark–bright square-wave grating is displaced to the left between frames in quarter-cycle steps. **(b)** Second-order (or non-Fourier) motion can be created by moving a sinusoidal contrast envelope across an array of randomly twinkling dots. This stimulus can be purged of Fourier motion artifacts by equilibrating the luminance of the high-contrast and low-contrast areas. The optomotor response of zebrafish larvae is evoked by both motion displays.

Furthermore, goldfish eyes injected with 6-OHDA and therefore presumably depleted of DA-IPCs are more sensitive to light, as shown with a behavioral assay, the dorsal light response [23]. If dopamine, released by the DA-IPCs, triggered a physiological switch from the dark-adapted to the light-adapted state, as suggested by these studies, then one should expect abnormal light-adaptation in *nbb* mutants. However, light adaptation is unaffected in *nbb* mutants, as is the function of rods and cones. This discrepancy deserves further study.

In addition to providing a new twist to the long-standing debate on the role of dopamine in retinal physiology, the *nbb* mutation has revealed a mysterious link between olfaction and vision, which also requires further exploration. The fascinating implication of this work is that a localized disruption in a remote region of the brain can have far-reaching consequences for the function of retinal circuitry.

A mutant screen is uniquely suited to reveal such unexpected connections.

Zebrafish may emerge as a model for psychophysical studies

The OMR assay uses computer-animated stimuli and is easily quantified. It thus invites a psychophysical approach to the study of zebrafish motion perception, which requires control over parameters of the visual stimulus such as spatial and temporal frequencies or contrast. So one may ask how the zebrafish motion pathway compares to the well-studied primate system. The human visual system seems to employ several psychophysically and neurophysiologically separable neural strategies in order to extract motion from a visual scene (see e.g. [24] for a review). A basic ‘first-order’ system will process information in the drift of luminance-variations in an image (the so-called Fourier motion energy) [25]. A grating that is composed of black and white stripes that drift from one end of the computer screen to the other is a typical first-order stimulus (Figure 3a). The ‘second-order’ system picks up movements of texture, contrast, and other higher-order cues (see the example in Figure 3b), whereas it does not process luminance-defined motion. A neural pathway that processes second-order motion has been discovered in the mammalian visual cortex (reviewed in [26]). Zebrafish do not possess a visual cortex, so it was unclear if they perceive second-order motion at all.

A recent study (M Orger, H Baier, unpublished data) has started to address this question. The authors first show that zebrafish larvae vigorously swim in the direction of first-order motion, even when faced with an artificial situation in which edges and shapes move in a direction that is opposite to the luminance-defined motion. This finding establishes that fish and mammalian motion pathways, at a basic level, work in a similar fashion. Next the authors tried several motion displays that had been purged of first-order motion cues. Indeed, second-order cues are also effective in driving the OMR. The strongest response was seen to a drifting column of luminance-reversing pixels. However, the fish also swim in the direction of a drifting contrast envelope that sweeps across an array of twinkling random dots, a standard second-order stimulus (Figure 3b), as well as with other examples of texture-defined motion. Zebrafish have either developed a strategy for high-level motion processing that differs from the strategy used by primates, or the detection of second-order cues is not ‘high-level’ after all, but rather can be accomplished by the basic circuitry of the vertebrate visual system.

Conclusions and outlook

Recent behavioral screens have identified mutants with a broad range of visual phenotypes. The small scale of these screens suggests that the field has only scratched the surface and that larger, better-designed screens should be conducted. It is also necessary to step up the efforts to map and clone the newly discovered mutations. The genomic

resources that allow for efficient cloning of point mutations are now available, most importantly a dense genetic map.

The success of behavior–genetic screens has sparked an interest in the visual capabilities of zebrafish. When we can be more certain what the fish visually perceive, the reasoning goes, we may be able to improve the assays for future screens. Along the way, by analyzing the behavior of wild-type fish in response to diverse stimuli, we hope to learn more about the fundamental features of vertebrate vision, which future mutant screens may then help to dissect.

Acknowledgements

Thanks to many colleagues for sharing preprints and unpublished data. Apologies to everyone whose work could not be considered due to space restrictions. Figure 2 was prepared by using sections provided by T Das and WA Harris. The author is supported by the National Institutes of Health (ROI-EY 12405-02), by a Packard Fellowship for Science and Engineering, by an Alfred P Sloan Research Fellowship, and by a grant from the Sandler Neurogenetics Center.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Malicki J, Neuhauss SC, Schier AF, Solnica-Krezel L, Stemple DL, Stainier DY, Abdelilah S, Zwartkuis F, Rangini Z, Driever W: **Mutations affecting development of the zebrafish retina.** *Development* 1996, **123**:263-273.
 2. Baier H, Klostermann S, Trowe T, Karlstrom RO, Nüsslein-Volhard C, Bonhoeffer F: **Genetic dissection of the retinotectal projection.** *Development* 1996, **123**:415-425.
 3. Karlstrom RO, Trowe T, Klostermann S, Baier H, Brand M, Crawford AD, Grunewald B, Haffter P, Hoffmann H, Meyer SU *et al.*: **Zebrafish mutations affecting retinotectal axon pathfinding.** *Development* 1996, **123**:427-438.
 4. Trowe T, Klostermann S, Baier H, Granato M, Crawford AD, Grunewald B, Hoffmann H, Karlstrom RO, Meyer SU, Müller B *et al.*: **Mutations disrupting the ordering and topographic mapping of axons in the retinotectal projection of the zebrafish, *Danio rerio*.** *Development* 1996, **123**:439-450.
 5. Brockerhoff SE, Hurley JB, Janssen-Bienhold U, Neuhauss SCF, Driever W, Dowling JE: **A behavioral screen for isolating zebrafish mutants with visual system defects.** *Proc Natl Acad Sci USA* 1995, **92**:10545-10549.
 7. Fadool JM, Brockerhoff SE, Hyatt GA, Dowling JE: **Mutations affecting eye morphology in the developing zebrafish (*Danio rerio*).** *Dev Genetics* 1997, **20**:288-295.
 8. Brockerhoff SE, Hurley JB, Niemi GA, Dowling JE: **A new form of inherited red-blindness identified in zebrafish.** *J Neurosci* 1997, **17**:4236-4242.
 9. Brockerhoff SE, Dowling JE, Hurley JB: **Zebrafish retinal mutants.** *Vision Res* 1998, **38**:1335-1339.
 10. Li L, Dowling JE: **A dominant form of inherited retinal degeneration caused by a non-photoreceptor cell-specific mutation.** *Proc Natl Acad Sci USA* 1997, **94**:11645-11650.
 11. Neuhauss SCF, Biehlermaier O, Seeliger MW, Das T, Kohler K, Harris WA, Baier H: **Genetic disorders of vision revealed by a behavioral screen of four-hundred essential loci in zebrafish.** *J Neuroscience* 1999, **19**:8603-8615.
- The authors revisit over 400 zebrafish mutants previously identified on the basis of external morphological features [15]. Both OKR and OMR are tested, and 25 mutants with relatively specific visual-system defects are identified. About half of these mutants show retinal degenerations. The remaining mutants have unique developmental disruptions at various stages of visual processing between the photoreceptors and the tectum, resulting in blindness or more subtle impairments of vision.
12. Li L, Dowling JE: **Disruption of the olfactoretinal centrifugal pathway may relate to the visual system defect in night blindness mutant zebrafish.** *J Neurosci* 2000, **20**:1883-1892.
- Using the visually triggered escape response, an adult zebrafish behavioral screen has picked up a mutation of retinal dark-adaptation. The phenotype reveals that the function and survival of a type of retinal interneurons, the dopaminergic interplexiform cells (DA-IPCs), depends on innervating fibers from the olfactory bulb. This study is a perfect example of the power of a forward-genetic approach to discover unanticipated functional links between distant regions of the brain.
13. Easter SS Jr, Nicola GN: **The development of vision in the zebrafish (*Danio rerio*).** *Dev Biol* 1996, **180**:646-663.
 14. Rick JM, Horschke I, Neuhauss SCF: **Optokinetic behavior is reversed in achiasmatic zebrafish mutant larvae.** *Curr Biol* 2000, in press.
 15. Haffter P, Granato M, Brand M, Mullins MC, Hammerschmidt M, Kane DA, Odenthal J, van Eeden FJ, Jiang YJ, Heisenberg CP *et al.*: **The identification of genes with unique and essential functions in the development of the zebrafish, *Danio rerio*.** *Development* 1996, **123**:1-36.
 16. Kelsh RN, Brand M, Jiang YJ, Heisenberg CP, Lin S, Haffter P, Odenthal J, Mullins MC, van Eeden FJ, Furutani-Seiki M *et al.*: **Zebrafish pigmentation mutations and the processes of neural crest development.** *Development* 1996, **123**:369-389.
 17. Erkman L, McEvilly RJ, Luo L, Ryan AK, Hooshmand F, O'Connell SM, Keithley EM, Rapaport DH, Ryan AF, Rosenfeld MG: **Role of transcription factors Brn-3.1 and Brn-3.2 in auditory and visual system development.** *Nature* 1996, **381**:603-606.
 18. Gan L, Xiang M, Zhou L, Wagner DS, Klein WH, Nathans J: **POU domain factor Brn-3b is required for the development of a large set of retinal ganglion cells.** *Proc Natl Acad Sci USA* 1996, **30**:3920-3925.
 19. Geisler R, Rauch GJ, Baier H, van Bebber F, Broß L, Dekens MP, Finger K, Fricke C, Gates MA, Geiger H *et al.*: **A radiation hybrid map of the zebrafish genome.** *Nat Genet* 1999, **23**:86-89.
- This multi-author project, masterminded by R Geisler and P Haffter, describes an efficient method for locating a cloned gene, expressed sequence tag, or DNA marker, in the zebrafish genome using a radiation-hybrid panel. Over one thousand genes and markers have already been mapped. This paper will boost all areas of zebrafish research because the linkage of a known gene to a given mutation can now be quickly confirmed or excluded on the basis of its chromosomal location.
20. Witkovsky P, Deary A: **Functional roles of dopamine in the vertebrate retina.** *Prog Retinal Res* 1991, **11**:247-292.
 21. Li L, Dowling JE: **Effects of dopamine depletion on visual sensitivity of zebrafish.** *J Neurosci* 2000, **20**:1893-1903.
 22. Wagner HJ, Kirsch M, Douglas RH: **Light dependent and endogenous circadian control of adaptation in teleost retinae.** In *Rhythms In Fish*. Edited by Ali MA. New York: Plenum Press; 1992:255-291.
 23. Lin ZS, Yazulla S: **Depletion of retinal dopamine increases brightness perception in goldfish.** *Vis Neurosci* 1994, **11**:683-693.
 24. Sperling G, Lu ZL: **A systems analysis of visual motion perception.** In *High-Level Motion Processing: Computational, Neurobiological and Psychophysical Perspectives*. Edited by Watanabe T. Cambridge, Massachusetts: Massachusetts Institute of Technology Press; 1998:153-183.
 25. Reichardt W: **Autokorrelationsauswertung als Funktionsprinzip des Zentralnervensystems.** *Z Naturforschung* 1957, **12b**:447-457. [Title translation: Autocorrelation analysis as a functional principle of the central nervous system.]
 26. Baker CL: **Central neural mechanisms for detecting second-order motion.** *Curr Opin Neurobiol* 1999, **9**:461-466.