Targeted misexpression of a *Drosophila* opsin gene leads to altered visual function

Reinhard Feiler, William A. Harris*, Kuno Kirschfeld, Christian Wehrhahn & Charles S. Zuker*

Max-Planck Institut für Biologische Kybernetik, Tübingen, FRG
* Department of Biology and Center for Molecular Genetics, University of California, San Diego, La Jolla, California 92093, USA

Drosophila *mutants transformed with a chimaeric gene that expresses the ocellar visual pigment in the major class of photoreceptor cells of the retina were used to investigate the properties of this minor pigment. The photoreceptor cells in which this opsin was misexpressed showed new spectral characteristics and physiology.*

IN both vertebrates and invertebrates, photoreceptors express specific genes encoding proteins involved in phototransduction and spectral sensitivity[1]. One such set of genes codes for the cell-specific visual pigments. The genes encoding five vertebrate[2-4] and four *Drosophila* opsins[5-10] have recently been isolated and characterized. One of the *Drosophila* opsins, (Rh1), is expressed in the outer six photoreceptor cells (R1-6) of each ommatidium[5,6], two others (Rh3 and Rh4) are expressed in non-overlapping sets of central R7 photoreceptor cells[8-10]. The remaining opsin (Rh2) is not expressed in either the R1-6 or R7 photoreceptors, but may be expressed in some R8 cells[7]. Through the use of *in vitro* mutagenesis and P-element-mediated DNA transformation, visual pigments normally expressed only in a small fraction of the fly's photoreceptors can now be uniquely expressed at high levels in the R1-6 cells which dominate the behavioural, spectral and photochemical properties of the eye[11-13]. Thus, by using such genetically-engineered *Drosophila* strains with cell-specific misexpression of particular opsin genes[14], we are now in a position to study the photochemical and physiological properties of these minor visual pigments, and the behavioural consequences of their expression in different photoreceptor cells.

Most of the *Drosophila* opsins except for Rh1, which was first identified by mutations in the *ninaE* locus[15], have been identified solely on molecular genetic criteria[5-10]. The Rh2 visual pigment is a good example of such molecule: it was identified and isolated by cross homology to an Rh1 complementary DNA probe[7]. In the present study, we have analysed flies in which the promoter of the Rh1 opsin was linked to the structural gene of Rh2 (ref. 14). This fusion construct has been reintroduced into the germ line of mutant host flies ($ninaE^{117}$) carrying an internal deletion of the endogeneous *Rh1* structural gene[6]. The only visual pigment expressed in the outer six photoreceptors of the transformed flies is the one driven by the newly introduced chimaeric gene. By expressing and studying the Rh2 photopigment in R1-6 cells we are thus able to determine whether its spectral characteristics match what was known about the R8 cells, or whether it represents a new opsin.

This general strategy of misexpressing components involved in phototransduction allows one to dissect which properties of a photoreceptor are intrinsic to it and which are a function of the genetically transplanted component. For example, R1-6 and R7 cells all show prolonged depolarizing afterpotentials (PDAs) when there is a significant net conversion of rhodopsin to metarhodopsin[16-18]. This phenomenon, however, has not been seen either in R8 (ref. 17) or ocellar photoreceptors[19]. Is this a property of the visual pigment or the downstream transduction machinery? The UV-sensitizing[20] and the long-wavelength absorbing pigments[21], which are present in only certain subclasses of photoreceptors, lead to changes in the spectral sensitivity of the cell in which they are expressed[22-24]. Can these molecules work with any visual pigment or are they specialized to interact with only one?

In this article we use high resolution microspectrophotometry and physiological recordings to determine the spectral sensitivity and photochemistry of the Rh2 visual pigment misexpressed in the R1-6 photoreceptor cells. Also, we have studied the visually guided optomotor behaviour of the transformed flies. Finally, we have used spectral measurements and RNA expression data in various mutant strains to demonstrate that Rh2 corresponds to the ocellar photopigment. In related studies, Pollock and Benzer[25] and Mismer *et al.*[26] have recently shown that the *Rh2* gene is indeed expressed in the ocelli.

Cell-directed misexpression

Drosophila transformed with chimaeric genes consisting of transcriptional fusions between the promoter element of the *Rh1* gene and unrelated reporter sequences express the reporters only in the six outer photoreceptor cells of the adult retina[27]. Zuker *et al.*[14] have recently generated flies transformed with a P-element vector containing a fusion between the Rh1 promoter and the structural gene encoding the Rh2 visual pigment. These flies, $ninaE;P[Rh1+2]$ have a deletion of their endogenous *Rh1* gene ($ninaE^{117}$)[6] and now functionally express the *Rh2* gene instead of *Rh1* in the R1-6 cells. Figure 1 shows an *in situ* hybridization to a frozen tissue section through the head of a $ninaE;P[Rh1+2]$ fly, demonstrating that Rh2 is a major transcript in their R1-6 photoreceptor cells.

Photochemical properties

Drosophila visual pigments, like those of most invertebrates, are photoconvertible from a photoactive rhodopsin form (R) (also referred to as xanthopsin[28]) into a thermally stable metarhodopsin from (M) (or metaxanthopsin). For the major visual pigment, Rh1, the R-form absorbs maximally at 480 nm and interconverts with an M-form which absorbs maximally at 580 nm (ref. 22). To determine the spectral absorption maxima (λ_{max}s) of these two states for the Rh2 photopigment, we carried out microspectrophotometric measurements through the eye of $ninaE;P[Rh1+2]$ flies. All experiments were carried out on white-eyed flies (genetic background w^{1118}) to eliminate any effects of screening pigments. We relied on the generation of difference spectra which compares absorption properties after differential bleaching near the two (R and M) λ_{max}s of the visual pigment molecule. We found (by trial and error) that maximal difference spectra (Fig. 2) were produced when 524 and 402 nm were the two bleaching wavelengths. With the same protocol,

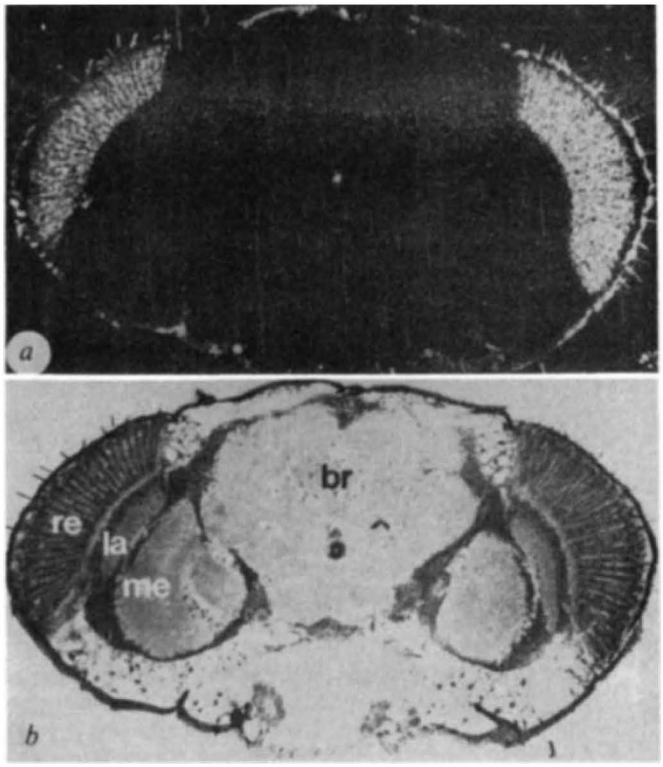

Fig. 1 *In situ* localization of Rh2 transcript in *ninaE*; *P*[*Rh1+2*] flies. A 600-nucleotide *Eco*R1-*Bam*H1 restriction fragment of Rh2 was radiolabelled and hybridized to frozen tissue sections of adult *ninaE*; *P*[*Rh1+2*] heads as described previously[7]. Panel *a* shows a darkfield view of a cross-section with a strong signal over the retinas of the compound eyes, demonstrating high levels of Rh2 expression in the six outer photoreceptors (R1-6) of each facet. Panel *b* is a brightfield view of the same section indicating the location of the retinas, optic lobes and brain. Note that the signal in *a* is present only over the retina. Abbreviations: la, lamina; me, medulla; br, brain; re, retina.

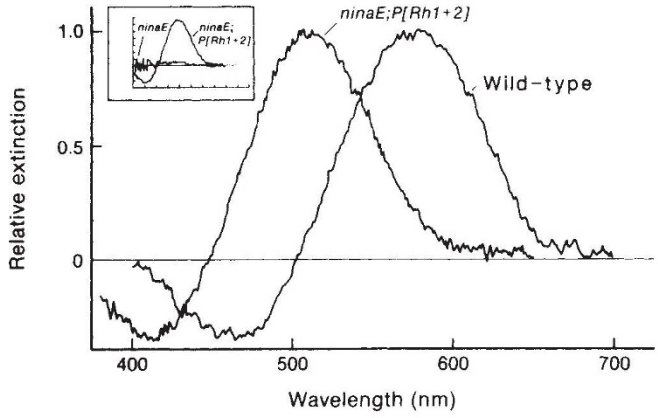

Fig. 2 Difference spectra derived from microspectrophotometry of the retina of control and *ninaE*; *P*[*Rh1+2*] flies. All experiments were carried out on white-eyed flies (genetic background w^{1118}) to eliminate any effects of screening pigments. In this and subsequent figures, for simplicity control flies (w^{1118}; $ninaE^{+}$) are referred to as wild-type. Heads from living animals were cut off, placed in Ringers solution and mounted between coverslips, with eyes directed to the objective of the microspectrophotometer (Leitz MPV II, equipped with Zeiss-ultrafluar optics, combined with Oriel monochromator 7240). Light passing through one of the eyes was selected by the measuring diaphragm. The setup was driven by an IBM AT03 computer via a Data Translation multifunction board DT 2801-A. Difference spectra, experimental details described by Kirschfeld *et al.*[21], were determined as follows: First, the eye was illuminated with strong light of wavelength λ_1, shifting the equilibrium of the two pigment states (R and M). Afterwards, transmission was measured continuously through the spectrum. The measuring light did not modify the equilibrium between the two pigment states significantly. Thereafter, another strong light of wavelength λ_2 was used to shift the pigment into a different equilibrium. Then, the absorption was measured again through the spectrum. The difference spectrum $\Delta E(\lambda)$ was calculated according to $\Delta E(\lambda) = [\log(I_{\lambda_1}(\lambda)/I_{\lambda_2}(\lambda)]$, whereby $I(\lambda)$ are the intensities transmitted after adaptation to light of λ_1 or λ_2, respectively. In the case of wild-type flies, λ_1 and λ_2 were 584 nm and 456 nm. For *ninaE*; *P*[*Rh1+2*] transformants, λ_1 and λ_2 were 524 nm and 402 nm. Relative extinctions, each normalized to 1.0 were plotted against wavelength. The isosbestic point (0-crossing) as well as the entire curve for the Rh2 photopigment in *ninaE*; *P*[*Rh1+2*] flies is shifted about 60 nm to the shorter wavelengths when compared to the Rh1 photopigment of wild-type flies. The inset shows that host *ninaE* flies do not display any appreciable difference spectrum. The *ninaE*; *P*[*Rh1+2*] curve in the inset is replotted simply to show the relative scale of the two curves.

untransformed $ninaE^{117}$ flies showed no difference spectrum (Fig. 2, inset). This is consistent with the fact that they contain no visual pigment molecules in their R1-6 cells[6]. Normal flies or flies transformed with a wild-type copy of the *Rh1* gene (data not shown), display a difference spectrum shifted considerably to the longer wavelengths compared to that of Rh2. The relative extinction spectra of the two Rh2 pigment states (R and M) (Fig. 3) were determined from the difference spectra as described in the figure legend. These curves show that the photoactive R-form (confirmed by sensitivity measurements below) of the pigment has a peak sensitivity at ~420 nm whereas the M form has a maximal absorption near 520 nm. Thus it is clear that the Rh2 photopigment has distinct photochemical properties from Rh1, and that it is therefore a novel but functional photopigment.

Photoreceptors with misexpressed opsin

Having determined the spectral properties of the Rh2 photopigment *in situ*, we carried out electrophysiological recordings of the light-evoked response in *ninaE*; *P*[*Rh1+2*] transformed flies to determine spectral sensitivity. Figure 4 shows spectral sensitivity of control flies and of transformed flies using the 'light clamping' technique of Franceschini[29]. Consistent with the microspectrophotometric analysis, flies expressing Rh2 opsin in their R1-6 cells show a broad peak of sensitivity near 420 nm. In contrast, control flies show a sensitivity peak near 480 nm. This result confirms that the 420-nm absorbing form of Rh2 corresponds to the visually active (R) state of the pigment.

In addition to the sensitivity peak near 480 nm, R1-6 cells expressing Rh1 have a characteristic triplet of sensitivity peaks in the UV region of the spectrum. This is the signature of a previously identified UV-sensitizing pigment, 3-hydroxy-retinol[20,28], whose function is to extend the range of response of R1-6 cells by absorbing photons in the UV and transferring the energy to the visual pigment molecule. Interestingly, R1-6 cells expressing Rh2 also display this triplet of UV sensitivity peaks (Fig. 4). Thus, the sensitizing pigment normally produced by the R1-6 cells is capable of efficiently sensitizing Rh2 opsin. The slight shift to the shorter wavelengths of the UV triplet in ocellar versus R1-6 photoreceptors may indicate that the sensitizing pigment differs in the two cell types.

Normally, R1-6 cells, like many invertebrate photoreceptors, undergo a prolonged depolarizing afterpotential (PDA) that persists after the cessation of the stimulus whenever a substantial amount of rhodopsin (R) is converted to the dark stable metarhodopsin (M)[16,18]. Its physiological basis may be the result of the continued activity of an internal transmitter in the phototransduction cascade[30]. The PDA can be suppressed by photoconverting M back to R. Figure 5 shows that R1-6 cells from

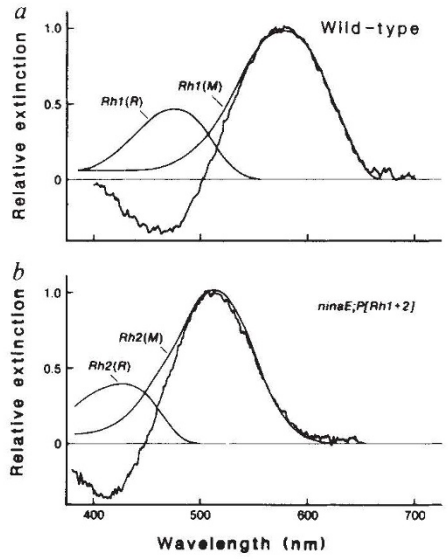

Fig. 3 The spectra of the R and M forms of the Rh1 (*a*) and Rh2 (*b*) photopigments. To determine the relative extinction spectra of the two pigment states (R and M), we fitted the difference spectra by shifting pigment absorptions on a $\lambda^{1/4}$ scale (see Barlow[43]). The fit was done using the Gauss-Newton curve fitting option of ASYSTANT (Macmillan Software, New York). As is typical with invertebrate pigments the M form has a higher extinction coefficient and absorbs at longer wavelengths than the corresponding R form. From these data we conclude that the peak sensitivity of the Rh2 photopigment is near 420 nm and that it interconverts to a M form which absorbs maximally at approximately 520 nm. Rh (R) and Rh (M) stand for photopigment R and M form, respectively.

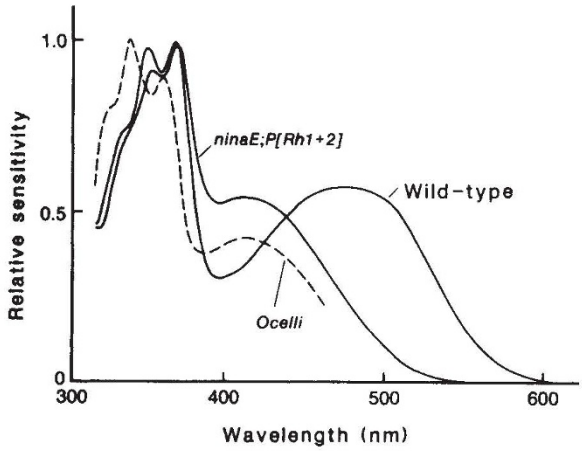

Fig. 4 Spectral sensitivity of control and transformed *ninaE*;*P*[*Rh1+2*] flies. The method applied is based on the 'light-clamp' technique of Franceschini[29]. A quartz neutral density wedge (optical density 0–4) is rotated in the path of the stimulating light in such a way that the electroretinogram (ERG) is constant during the scan through the spectrum. To get high resolution spectra (1-nm bandpass) a double monochromator (Zeiss MM12) was used. This was crucial for resolving the vibrational fine structure of the sensitizing pigment. To increase the signal-to-noise ratio of the photoresponse, we chopped the stimulus light (5–20 Hz) as a.c.-signals of photoreceptors are more stable than d.c.-signals. The amplitude of the ERG was determined by digitally integrating over the area of the a.c.-signal. The triplet of sensitivity peaks in the UV is evident in both wild-type and transformed flies, implying that the sensitizing pigment (3-OH-retinal)[20,28] works to augment the UV sensitivity of the Rh1 or the Rh2 photopigment when either is expressed in R1–6 photoreceptors. The main broad peak of Rh2 sensitivity, near 420 nm, correlates well with our microspectrophotometric analysis of the R form of the Rh2 photopigment. The wild-type sensitivity profile shows a main peak near 480 nm, also in accord with the microspectrophotometric analysis of the R form of Rh1. The dotted curve shows the sensitivity profile of the ocelli of *Calliphora*[31]. Note that there is a good match in spectral profile between this ocellar and the Rh2 photopigment of *Drosophila*.

wild-type flies (panel *a*) undergo a PDA when illuminated with strong blue (480-nm) light. As expected, these cells are refractory to subsequent PDA inducing stimuli. The remaining response is due to activity in R7 and R8 cells[16]. Strong orange light (580 nm), which converts M back to R, suppresses the PDA and brings the cells back to their resting or dark-adapted state. Figure 5 (panel *c*) shows that *ninaE*;*P*[*Rh1+2*] flies do not undergo a PDA when illuminated with the same light stimuli. This, however, is not surprising because 480-nm light is closer to the M peak than the R peak of the Rh2 photopigment (see Fig. 3). To ascertain whether R1–6 cells expressing Rh2 opsin are capable of supporting a PDA, we tested both 420-nm and 520-nm light as they represent the maxima of the R and M forms of the Rh2 visual pigment (Fig. 3). Indeed, Fig. 5 (panel *d*) demonstrates that strong 420-nm light triggers a PDA which can be suppressed specifically by strong 520-nm light. In control flies (panel *b*), both 420-nm and 480-nm bleaching lights lead to a PDA, but unlike in *ninaE*;*P*[*Rh1+2*] flies 520-nm light is incapable of converting a sufficient amount of M back to R to successfully suppress the PDA. Thus, it is clear that Rh2 is capable of generating a PDA in the novel environment of the R1–6 cells but that the wavelengths needed to elicit and suppress the PDA are specifically tuned to the R and M forms of the Rh2 opsin. The finding that Rh2 triggers a PDA when in R1–6 cells, which requires a substantial amount of R to M conversion[30], demonstrates that most Rh2 pigment molecules are active and contribute to transduction in these flies.

Rh2 is an ocellar photopigment

Both the microspectrophotometric and the electroretinogram data suggested that Rh2 displays a spectral sensitivity which is very similar to the ocellar photopigment described for *Musca*[31], *Calliphora*[31] and *Drosophila*[19]. The reported spectral sensitivities of R8 cells studied in these same species are shifted to the red compared to R1–6 (refs 11, 22, 23). In contrast, the Rh2 photopigment shows a sensitivity peak which is shifted significantly to the blue region of the spectrum and has, unlike the R8 cell spectral sensitivity, a pronounced contribution by a UV-sensitizing pigment just as the ocelli[31] (Figs 3 and 4). Therefore, we entertained the possibility that Rh2 is an ocellar photopigment and not a major R8 photopigment.

To examine this possibility we studied the expression of Rh2 in *Drosophila* lines that lack particular photoreceptor cell types. We extracted poly(A)$^+$ RNA from the heads of normal flies and the heads of mutants that included *eyes absent*[32] (lacks eyes only), *no-ocelli*[33] (lacks ocelli only), and *sine oculis*[34] (lacks eyes

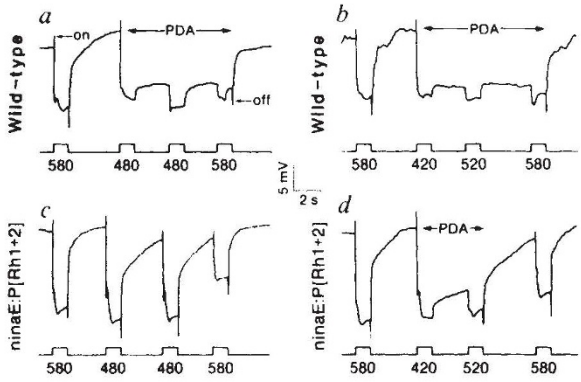

Fig. 5 Prolonged depolarizing afterpotentials (PDAs) in wild-type and *ninaE*;*P*[*Rh1+2*] flies. Electroretinograms recorded with a wick electrode on the surface of the eye were measured during and after brief stimulations of strong light (5×10^{16} photons cm^{-2} s^{-1}) of the indicated wavelengths. Panel *a* shows that intense 580-nm light elicits a maximal receptor potential but no PDA in wild-type flies. Stimulation with 480 nm (the absorption maximum of Rh1(R)), leads to a PDA in control but not in *ninaE*;*P*[*Rh1+2*] flies (panel *b*). Further 480-nm stimulation of control flies leads to only a small response primarily from R7 and R8 (ref. 16). Finally, bright 580-nm light (absorption maximum of Rh1(M)), terminates the PDA and resensitizes the R1-6 photoreceptors. Panel *d* shows that it is possible to elicit a PDA in *ninaE*;*P*[*Rh1+2*] flies but that different wavelengths of stimulating light are necessary. Intense 420-nm light (absorption maximum of Rh2(R)), selectively converts R to M in both Rh1 and Rh2 and leads to a PDA in both specimens. This PDA in *ninaE*;*P*[*Rh1+2*] flies is selectively terminated by strong 520-nm light (absorption maximum of Rh2(M)). In contrast, 580-nm light is required to terminate the PDA of control flies. Lower traces indicate the location and duration of light stimulation. Numbers refers to wavelength (±10 nm).

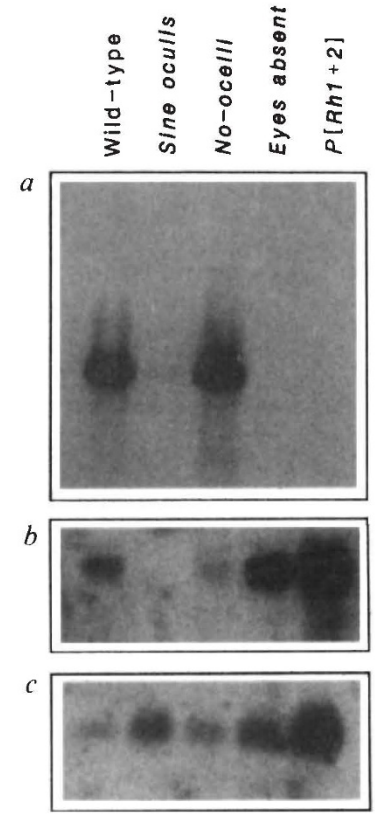

Fig. 6 Rh2 is an ocellar photopigment. Poly(A)$^+$ RNAs were extracted from adult heads of wild-type (3 μg per lane), *sine oculis* (6 μg per lane), *no-ocelli* (3 μg per lane) and *eyes absent* (6 μg per lane) mutant flies as described by Zuker *et al.*[5]. Total poly(A)$^+$ RNA was also isolated from *ninaE* flies transformed with the [*Rh1+2*] chimaeric gene. The RNAs were gel-fractionated, blotted and hybridized to probes specific for the *Rh*1 (panel *a*) or the *Rh2* (*panel b*) genes. The RNA blot shown in panel *c* was hybridized to a radiolabelled probe encoding the ribosomal protein rp49 (ref. 44) to control for the integrity of the RNAs. Rh1 transcript is expressed at normal levels in the heads of flies containing a full complement of R1-6 photoreceptors (wild-type and *no-ocelli*) but is dramatically decreased (*sine oculis*) or absent (*eyes absent*) in flies lacking R1-6 cells. The small amount of transcript seen in *sine oculis* reflects the incomplete penetrance of the mutation. Panel *b* demonstrates that Rh2 transcript is present at normal levels in heads containing ocelli whether or not they contain retinas (wild-type and *eyes absent*). Rh2 transcript, however, is absent or severely reduced in flies lacking ocelli irrespective of the presence of retinas (*sine oculis* and *no-ocelli*).

and ocelli). Figure 6 shows RNA blots hybridized with probes specific for the *Rh1* (panel *a*) and *Rh2* (panel *b*) genes. As can be seen, Rh2 transcript is present in fly heads containing ocelli, whether or not they have compound eyes (wild-type and *eyes absent*). This is in contrast to the expression profile for the Rh1 opsin, where transcript is only seen in fly heads containing intact retinas whether or not they have ocelli (wild-type and *no-ocelli*). These results demonstrate that most Rh2 expression is restricted to the ocelli. Direct observation of the sites of expression of the *Rh2* gene by *in situ* hybridization to tissue sections are in agreement with this result by showing that Rh2 is abundantly expressed in the ocelli[25]. Similar results have recently been obtained by analysing the site of expression of a Rh2-β-galactosidase fusion protein[26]. Taken together, these results conclusively demonstrate that Rh2 corresponds to the ocellar visual pigment of *Drosophila*.

Optomotor behaviour of *ninaE*;*P*[*Rh1+2*] flies

R1-6 cells in *Drosophila* share a common photopigment, send axons that synapse in the first optic ganglion, the lamina, and drive optomotor behaviour[12]. R7 and R8 cells have different photopigments, synapse in the second optic ganglion, the medulla, and do not drive optomotor behaviour[13]. To determine whether the Rh2 opsin is capable of mediating optomotor behaviour through R1-6 cells, we examined optomotor responses in white-eyed control and *ninaE*;*P*[*Rh1+2*] flies. Test flies were put into a *Drosophila* watch glass surrounded by a concentric cylinder carrying a periodic grating of spatial wavelength 90°. The cylinder could be rotated in both directions eliciting movement stimuli of opposite sign. Normal flies follow the direction of motion and generate walking traces which reflect the direction of the moving stimulus (Fig. 8*a*). Mutant *ninaE* flies do not respond to the motion of the striped pattern (Fig. 8*b*). This is expected as all 'optomotor input' is mediated through the R1-6 cells[13] and these flies lack all visual pigment in these cells[35]. But, when expressing the Rh2 photopigment in their R1-6 cells, these same host flies clearly regain optomotor response. Therefore, the R1-6 photoreceptors of *ninaE*;*P*[*Rh1+2*] flies, though they express a visual pigment of a different class of cell, must still make the central connections necessary to mediate an optomotor response. It is interesting that in larger male flies R7 and R8 cells at the anterior margin of the retina appear to have the same visual pigment as is present in R1-6 cells[24,36]. These cells do not, like normal R7 and R8, send axons to the medulla, but like R1-6, they project axons to the lamina[37]. This would suggest a direct correlation between photopigment type and central wiring. Our results, however, indicate that the genetic control of the central wiring of a photoreceptor is clearly not dependent on the type of opsin the cell expresses. These two cellular characters must thus be regulated independently.

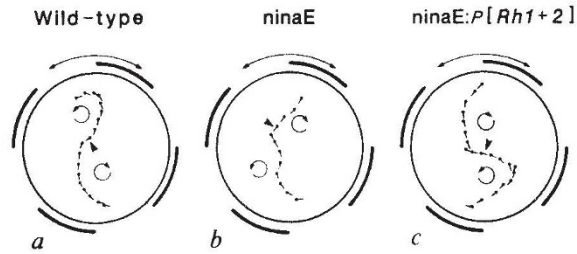

Fig. 7 Optomotor behaviour of white-eyed (wild-type) flies (*a*), *ninaE* mutants (*b*), and *ninaE*;*P*[*Rh1+2*] transformants (*c*). A TV camera connected to a video recorder was positioned above the vial monitoring the movements of fly and stimulus. Fifty walking traces of five flies of each genotype were reconstructed frame by frame (1 per 100 ms). Each point represents the position of the fly's head at a given instant and the line the direction of its body. For clarity, only every fifth frame is shown. When the fly was near the middle of the watch glass (arrowhead) the rotation of the striped drum was reversed. Small curved arrows indicate the sense of direction of the drum. Thus in *a* it is clear that this wild-type fly follows the sense of the rotating pattern. In *b* a *ninaE* fly shows no optomotor response. In *c* a transformed *ninaE*;*P*[*Rh1+2*] fly regains normal optomotor behaviour.

Conclusions

In this article we analyse the spectral properties of the Rh2 photopigment which was first identified solely on the basis of DNA sequence homology with the gene encoding the major *Drosophila* opsin (Rh1)[7]. Cowman *et al.*[7] have previously shown that Rh2 is not expressed in R1-6 or R7 cells but appears to be expressed in the retina at low levels in a subpopulation of R8 photoreceptors. Through a variety of experimental procedures, including microspectrophotometry, electrophysiology and expression studies, we have demonstrated that Rh2 is an ocellar photopigment. The remaining signal of Rh2 in flies without ocelli (Fig. 6*b*) may reflect the presence of a homologous, cross-hybridizing opsin, or the incomplete penetrance of the *no-ocelli* phenotype (that is, that there are remnants of ocelli in homozygous mutant flies).

Zuker *et al.*[14] have shown that R1-6 cells expressing Rh2 are capable of responding to light. This indicates that there must be common features in the transduction process in the ocellar and R1-6 photoreceptors. This finding is supported by the existence of a number of mutants in *Drosophila* that affect visual function in all photoreceptor cell classes[38]. There are, however, a number of mutants that affect only specific subsets of photoreceptor cells[11,39], indicating that there must also be features unique to the different cell types. We have shown that the Rh2 opsin when expressed in R1-6 cells can support the production of a PDA, a result that may be particularly interesting because a previous study showed that *Drosophila* ocellar photoreceptors do not undergo a PDA when illuminated with strong light of a variety of wavelengths[19]. Thus the ocellar photoreceptors using the same visual pigment molecule may be incapable of supporting a PDA. Alternatively the narrow spectral window of illumination which we found to be necessary to maximally photoconvert Rh2 (R) to Rh2 (M) may account for the inability of the previous study to generate a PDA in the ocelli.

In addition to rescuing phototransduction of host *ninaE* flies, the transformed chimaeric gene leads to the recovery of more central visual functions such as optomotor behaviour. We expect that the action spectrum of the optomotor response of *ninaE*;*P*[*Rh1+2*] flies will reflect the new spectral sensitivity of their R1-6 cells. This opens the possibility of tuning an animal's visual behaviour through site-directed *in vitro* mutagenesis of the visual pigment molecule.

In conclusion, we have shown that it is possible to characterize in detail a minor photopigment by misexpressing it in a well defined photoreceptor cell type. The possibility of functionally dissecting individual components of the phototransduction machinery by isolating and reintroducing the genes of interest into the intact organism under the control cell-type-specific promoters[14,27,40] should provide significant insights into the mechanisms underlying visual function[41,42].

We thank Driszlav Mismer for help with the *ninaE*;*P*[*Rh1+2*] flies and to Seymour Benzer, Jeff Hall, Drislav Mismer, John Pollock, Gerry Rubin and Nick Spitzer for critiques of the manuscript. John Pollack and Seymour Benzer kindly provided the *no-ocelli* stock. We thank Robert W. Hardy for his help in obtaining the PDA data. This work was supported by grants from the NIH (to W.A.H.) and National Eye Institute (to C.Z.) and an Alexander von Humboldt Stiftung research fellowship (to W.A.H.).

Received 16 March; accepted 10 May 1988.

1. Fain, A. & Szuts, E. Z. *Photoreceptors: Their Role in Vision* (Cambridge University Press, 1982).
2. Nathans, J. & Hogness, D. S. *Cell* **34**, 807-814 (1983).
3. Nathans, J. & Hogness, D. S. *Proc. natn. Acad. Sci. U.S.A.* **81**, 4851-4855 (1984).
4. Nathans, J., Thomas, D. & Hogness, D. S. *Science* **232**, 193-202 (1986).
5. Zuker, C. S., Cowman, A. F. & Rubin, G. M. *Cell* **40**, 851-858 (1985).
6. O'Tousa, J. E. *et al. Cell* **40**, 839-850 (1985).
7. Cowman, A. C., Zuker, C. S. & Rubin, G. M. *Cell* **44**, 705-710 (1986).
8. Zuker, C., Montell, C., Jones, K., Laverty, T. & Rubin, G. *J. Neurosci.* **7**, 1550-1558 (1987).
9. Montell, C., Jones, K., Zuker, C. & Rubin, G. *J. Neurosci.* **7**, 1558-1566 (1987).
10. Fryxell, K. & Meyerowitz, E. *EMBO J.* **6**, 443-451 (1987).
11. Harris, W. A., Stark, W. S. & Walker, J. A. *J. Physiol., Lond.* **256**, 415-439 (1976).
12. Heisenberg, M. & Buchner, E. *J. comp. Physiol.* **117**, 127-162 (1977).
13. Heisenberg, M. & Wolf, R. in *Vision in Drosophila: Genetics of Microbehavior, Studies of Brain Function* (ed. Braitenberg, V) (Springer, Berlin, 1984).
14. Zuker, C., Mismer, D., Hardy, R. & Rubin, G. *Cell* **55**, 475-482 (1988).
15. Scavarda, N. J., O'Tonsa, J. & Pak, W. L. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4441-4445 (1983).
16. Minke, B., Wu, C.-F. & Pak, W. L. *J. comp. Physiol.* **98**, 345-355 (1975).
17. Stark, W. S. *J. comp. Physiol.* **115**, 47-59 (1977).
18. Hillman, P., Hockstein, S. & Minke, B. *Physiol. Rev.* **63**, 668-672 (1983).
19. Hu, K. G., Reichert, H. & Stark, W. S. *J. comp. Physiol.* **126**, 15-24 (1978).
20. Kirschfeld, K., Franceschini, N. & Minke, B. *Nature* **269**, 386-390 (1977).
21. Kirschfeld, K., Hardie, R., Lenz, G. & Vogt, K. *J. comp. Physiol.* **A162**, 421-433 (1988).
22. Hardie, R. C. *Prog. Sensory Physiol.* **5**, 1-80 (1985).
23. Franceschini, N. *Neurocsi. Res. Suppl.* **2**, 517-549 (1985).
24. Hardie, R. C. *Trends Neurosci.* **9**, 419-423 (1986).
25. Pollock, J. A. & Benzer, S. *Nature* **333**, 779-782 (1988).
26. Mismer, D., Michael, M., Laverty, T. & Rubin, G. *Genetics* (in the press).
27. Mismer, D. & Rubin, G. *Genetics* **116**, 565-578 (1987).
28. Vogt, K. & Kirschfeld, K. *Naturwissen* **71**, 211-213 (1984).
29. Franceschini, N. *Invest. Opthalmol. Suppl.* **May 5**, (1979).
30. Minke, B. in *The Molecular Mechanism of Photoreception, Dahlem Konferenzen* (ed. Steive, H.) 241-205 (Springer, Berlin, 1986).
31. Kirschfeld, K., Feiler, R. & Vogt, K. *J. comp. Physiol.* (in the press).
32. Sved, J. *Drosoph. Info. Serv.* **73**, 169 (1986).
33. Ashburner, M., Aaron, C. S. & Subota, T. *Genetics* **102**, 421-435 (1982).
34. Milani, M. *Drosoph. Info. Serv.* **14**, 52 (1941).
35. Johnson, E. C. & Pak, W. L. *J. gen. Physiol.* **88**, 651-673 (1986).
36. Hardie, R. C., Franceschini, N. & McIntyre, P. D. *J. comp. Physiol.* **133**, 23-39 (1979).
37. Hardie, R. C. *Cell Tissue Res.* **233**, 1-21 (1983).
38. Pak, W. L. in *Neurogenetics, Genetic Approaches to the Nervous System* (ed. Breakfield, X. D.) 66-99 (Elsevier, New York, 1979).
39. Larrivee, D. C., Conrad, S., Stephenson, R. S. & Pak, W. L. *J. gen. Physiol.* **78**, 521-545 (1981).
40. Rubin, G. M. *Trends Neurosci.* **8**, 231-233 (1985).
41. Stryer, L. *A. Rev. Neurosci.* **9**, 87-119 (1986).
42. Stieve, H. (ed.) *The Molecular Mechanism of Photoreception, Dahlem Konferenzen* (Springer, Berlin, 1986).
43. Barlow, H. *Vision Res.* **22**, 635-643 (1982).
44. O'Connell, P. & Rosbash, M. *Nucleic Acid Res.* **12**, 5495-5513 (1984).