

# Rhodopsin and visual sensitivity: Splendours and miseries of an ancient molecule

Kristian Donner

Department of Biological and Environmental Sciences, P.O.Box 65 (Viikinkaari 1),  
FI-00014 University of Helsinki, Finland; e-mail: [kristian.donner@helsinki.fi](mailto:kristian.donner@helsinki.fi)

In all seeing animals, the first event in phototransduction is activation of rhodopsin, a G-protein-coupled receptor protein, by photoisomerization of its covalently bound prosthetic group, some form of “retinal” (vitamin A aldehyde). The chain of molecular amplification by which this finally results in a change of the membrane potential of the photoreceptor cell differs between taxa and cell types, but none can escape the functional limitations set by rhodopsin, a molecule invented more than 3 billion years ago. Permissible modifications of this protein are restricted by the necessity of retaining basic functions (correct structural relations to the membrane and other proteins, reversible binding of retinal, activation of G protein, quenching of the activated state, etc). Leaving deactivation and regeneration properties aside, the main functional *variables of rhodopsin activation* are (i) the wavelength of maximum absorbance ( $\lambda_{\max}$ ), and (ii) the propensity for being “spontaneously” activated by thermal energy alone. Such “dark” activations cause a random background of false, photon-identical events against which real light has to be detected, and thus set an ultimate limit for the signal-to-noise ratio of dim-light vision. The rate of thermal activations correlates strongly with  $\lambda_{\max}$ , as sensitivity to low-energy (“red”) photons requires a low molecular activation energy, which gives a higher probability of activation by thermal energy alone (Ala-Laurila et al. *Vision Res* 44:2153-58 and *Biophys J* 86:3653-62, 2004). Since evolution has been able to tune rhodopsins to any  $\lambda_{\max}$  value in the so-called visible light range, the non-existence of pigments with  $\lambda_{\max}$  in the “infrared” is evidently due neither to molecular constraints as such, nor (of course) to an absence of interesting light signals in this range, but to the unacceptable rates of thermal activations that would follow.

One main theme of our recent studies has been the scope and molecular mechanisms of “adaptive” tuning of rhodopsin on short evolutionary time scales (down to < 10000 years), using the crustacean *Mysis* group of sibling species as a model. These animals live at least part of the year in very dim light deep in subarctic lakes and seas and possess only one rhodopsin. Postglacial isolation of populations in the Baltic and White Seas and in a large number of Swedish, Finnish and Russian lakes can be accurately dated, and phylogenies and rates of genetic divergence established by (supposedly) neutral molecular markers. The degree of divergence of rhodopsins of allopatric populations of the same and different species, and of sympatric populations of different species, gives a measure of adaptive evolution, constraint and drift in a molecule (gene) subject to very direct functional selection. We find that differences in  $\lambda_{\max}$  between “Sea” and “Lake” populations of two species (*M. relicta* and *M. salemaai*) is on the order 20-30 nm in both, and the shorter  $\lambda_{\max}$  of the “Sea” populations is broadly consistent with maximization of the conceptual signal-to-noise ratio calculated according to the model of Ala-Laurila et al. (*Biophys J*, 2004). In contrast to North American *M. diluviana*, all Scandinavian populations appear to use 100% A2 chromophore to achieve a general red-shift of  $\lambda_{\max}$ , as is reasonable not only in the lakes but also in the coastal seas. While the amino acid sequences of opsins from “Lake” and “Sea” *M. salemaai* show substitutions known (in vertebrate rhodopsins) to produce spectral differences similar to those observed, *M. relicta* seems to have invented a similar adaptive difference by some quite unconventional mechanism.

# Comparative study of retinoids from eyes of two populations of *Mysis relicta* (Crustacea, Mysidacea) with different light damage resistance

T. Feldman, M. Yakovleva, M. Lindström\*, K. Donner\* and M. Ostrovsky.

Institute of Biochemical Physics, Russian Academy of Sciences, Moscow

\*Tvärminne Zoological Station, University of Helsinki

E-mail: [tf@maryno.net](mailto:tf@maryno.net)

Two Finnish populations of the opossum shrimp *Mysis relicta* have been extensively studied. One population occurs in the deep, dark, Lake Pääjärvi, and the other one in the Pojo bay, which is part of the Baltic Sea (Lindström & Nilsson, 1988). Although these two mysid populations belong to the same species and are genetically very similar (Väinölä 1986), their eyes differ markedly in tolerance to medium or strong light and in spectral sensitivity (Lindström & Nilsson 1988, Lindström et al. 1988). The sea population is more resistant to light damage than the lake one. The higher content of ommochromes in the sea populations' eyes can be one reason for their resistance to light damage (Dontsov et al., 1999).

We have investigated a content of polyene chemical compounds in the eyes, which would be phototoxic at certain situations. Using spectral and HPLC techniques we have shown that the quantity and quality of the total carotenoid composition in the eyes of both populations are approximately the same. There is only one small difference between the two populations: the percentage of astaxanthin in the eyes of the sea population is higher than in the lake population. We have, however, found clear differences in the total absorption spectra of retinoid hexane extracts, namely different amounts of a product with absorption maximum at 324 nm. In the lake population, the amount of this product is larger than in the sea population. We have shown that the main product absorbing at 324 nm is a retinol.

Our results support the hypothesis, that animals, which inhabit very dim light environments, need more visual pigment (Donner et al. 1994). Retinol is a precursor of retinal in the visual cycle. So, larger retinol content in the lake shrimp's eyes would be explained by the necessity of more visual pigment for more effective processes of visual pigment regeneration in darkness, where photoregeneration of metarhodopsin to rhodopsin is almost impossible.

The probable mechanisms of light damage to the eye are discussed.

## References

- Donner K.O., Langer H., Lindström M., Schlecht P. (1994). J Comp Physiol A 174:451-459.  
Dontsov A.E., Fedorovich I.B., Lindström M., Ostrovsky M.A. (1999). J.Comp.Physiol.B, V.169. No.3. P.157-164.  
Lindström M, Nilsson HL (1988). J Exp Mar Biol Ecol 120:23-37  
Lindström M, Nilsson HL, Meyer-Rochow VB (1988). Zool Sei 5:743-757.  
Väinölä R. (1986). Ann Zool Fenn 23:207-221.

# Metabolic TRP channel activation in fly photoreceptors

**Gregor Zupančič\*, Andrej Meglič\*, Darko Perovšek‡, Gregor Belušič\***

\*University of Ljubljana, Biotechnical Faculty, Department of Biology, Večna pot 111, 1000

Ljubljana, Slovenia; ‡University clinical centre, Eye clinic, Grablovčeva 46, Ljubljana, Slovenia

Some years ago it was shown that TRP and TRPL channels can be activated by metabolic stress, i.e. the lack of intracellular ATP produced either by severe hypoxia or mitochondrial uncoupling (Agam et al., 2000, *J Neurosci*, **20**: 5748-5755). The mechanism of TRP channel activation through metabolic stress in flies was later shown to be dependent on free DAG present in the plasma membrane due to impaired PIP<sub>2</sub> re-synthesis in the absence of ATP (Hardie et al., 2003, *J Biol Chem*, **278**, 18851-18858). The finding that hypoxia can activate a specific signalling pathway is important, yet the entire physiological significance of this phenomenon is so far unclear. The central question is whether the TRP channel activation by hypoxia is a pathophysiological process or a physiological signalling mechanism. In other systems involving DAG signalling and TRP channels, like endothelial and vascular smooth muscle cells, it was shown for example that such metabolic activation of TRPC6, plays a central part in hypoxic vasoconstriction (Weissmann et al., 2006, *PNAS*, **103**, 19093-19098). It may well be that the same mechanism is present in all the cells expressing TRPC channels activated by DAG and/or its metabolites and that it thus constitutes a novel widespread physiological signalling mechanism. Our main question addressed in the present work was, what is the condition the mitochondria need to be in, in order to bring about the opening of the TRP channels? Another related question we also addressed was whether it is possible for this mechanism to be directly implicated in the light-induced TRP channel activation in insect phototransduction – an implicit question present ever since the discovery of this phenomenon. To address these issues we monitored hypoxic TRP channel activation by measuring the receptor potentials in the blowfly *Calliphora vicina* chalky, as well as extracellular K<sup>+</sup>, Na<sup>+</sup> and Ca<sup>2+</sup> with ion selective electrodes. At the same time we also monitored the redox state of the mitochondrial respiratory pigments (haems a, a<sub>3</sub>, b and c as well as flavoproteins in the respiratory complexes). We did this during the transition from normoxic to anoxic conditions, during a physiological metabolic load (illumination) and in steady-state hypoxic conditions. We applied the time-resolved absorption spectroscopy in combination with a principal components-based spectral deconvolution (Zupančič, 2003, *Pflügers Arch*, **447**, 109-119). In darkness, upon the establishment of anoxia around the animal, the values of V<sub>m</sub>, [K<sup>+</sup>]<sub>o</sub>, [Na<sup>+</sup>]<sub>o</sub> and [Ca<sup>2+</sup>]<sub>o</sub> changed very much as described previously (Agam et al., 2000, *J Neurosci*, **20**, 5748-5755). With constant illumination the changes in [K<sup>+</sup>]<sub>o</sub>, [Na<sup>+</sup>]<sub>o</sub> and [Ca<sup>2+</sup>]<sub>o</sub> started to appear with the latency of 8.06 ± 1.38 s, 10.38 ± 2.48 s and 13.11 ± 3.09 s, respectively (mean ± s.e.m., n=7 if not stated otherwise), after the onset of anoxic conditions. On the other hand the first detectable changes in the respiratory pigments redox states, those of cytochrome c, started at 1.79 ± 0.19 s. Moreover the changes of cytochrome c reached 39.4% ± 5.4 % of the maximal reduction by the time changes in ionic composition were even detectable. Such values are well outside the normal physiological range that occurs during intense illumination, which is 7.4 ± 0.2 % (n=15) and can only be observed when environmental P<sub>O2</sub> falls below 0.02 hPa. We also observed that this is when the cytochromes b and a<sub>3</sub> go from oxidation, induced by the metabolic load due to illumination to reduction due to anoxia. We concluded that this is the key mitochondrial event necessary for metabolic TRP channel activation and that in flies, metabolic stress is unlikely to be a significant contributor to physiological TRP channel activation.

# Identification of the diacylglycerol lipase (DAGL) gene, *inaE*, and the role of DAGL in TRP channel activation in *Drosophila*

Hung-Tat Leung<sup>1</sup>, Julie Tseng-Crank<sup>1</sup>, Eunju Kim<sup>1</sup>, Cecon Mahapatra<sup>1</sup>, Shikoh Shino<sup>1</sup>, Ying Zhou<sup>1,3</sup>, Lingling An<sup>2</sup>, R W Doerge<sup>2</sup>, and William L Pak<sup>1</sup>

<sup>1</sup>Dept of Biol Sciences, Purdue University, W Lafayette, IN USA ([wpak@purdue.edu](mailto:wpak@purdue.edu)); <sup>2</sup>Dept of Statistics, Purdue University; <sup>3</sup>Now at Dept of Biol, U of Massachusetts, Amherst, MA, USA

In *Drosophila*, a phospholipase C-mediated signaling cascade links photoexcitation of rhodopsin to the opening of the TRP/TRPL channels. A lipid product of the cascade, DAG (diacylglycerol), and its metabolite(s), polyunsaturated fatty acids (PUFAs), have both been proposed as potential excitatory messengers (Chyb et al., 1999; Raghu et al., 2000). DAG is metabolized by DAG lipase. However, DAGLs which might play a role in phototransduction had not been previously identified in any organism. In this work, a *Drosophila* DAGL gene, *inaE*, has been identified from mutants that are defective in photoreceptor responses to light. The *inaE*-encoded protein isoforms showed high sequence similarity to known mammalian DAG lipases and exhibited DAG lipase activity *in vitro*. A polyclonal antibody generated against a INAE peptide labeled punctate material distributed throughout the photoreceptor cytoplasm. Some of the labeled puncta were found within the rhabdomeres. The available *inaE* mutants were all relatively mild, making it difficult to decipher the role of DAGL in phototransduction. To get around this problem, we generated (1) *norpA inaE* double mutants, and (2) more severe alleles of *inaE* than those available. To generate the double mutants, a hypomorphic *norpA* allele was used to severely reduce the PLC activity so as to limit the amount of DAG generated but not to eliminate it. Thus, in the *norpA inaE* double mutant, even mild *inaE* mutations would be able to largely block the small amount of DAG generated. The severe *inaE* alleles were generated by remobilizing a P element inserted in the *inaE* gene to induce imprecise excisions. Imprecise excision mutants thus generated were all lethal. Therefore, we generated several mosaic lines homozygous for the imprecise excision events only in the eye (Stowers and Schwarz, 1999). Three mosaic lines of progressive severity, *xl29*, *xl15*, and *xl18*, were chosen for study. In *xl29*, the *inaE* gene was intact, and the deletion that conferred it its lethality was outside the *inaE* gene. *xl15* and *xl18*, respectively, carried ~1.0 and 1.7 kb deletions in the *inaE* gene, but neither was completely null. The results of these studies provided evidence that, while DAG may have a role in facilitating and orchestrating response generation, it alone is not sufficient to generate physiologically normal responses of the photoreceptor.

# Molecular characterization and expression of visual pigments in bees

Johannes Spaethe\* and Adriana D. Briscoe<sup>#</sup>

\* Department of Evolutionary Biology, University of Vienna, Austria,  
johannes.spaethe@unvie.ac.at;

<sup>#</sup>Department of Ecology and Evolutionary Biology, University of California, Irvine, USA,  
abriscoe@uci.edu

Bumblebees exhibit trichromatic colour vision similar to humans. Electrophysiological investigations have identified three receptor types in the complex eye which have their maximum sensitivity at UV, blue and green light, respectively. We cloned and sequenced four opsin genes of the bumblebee *Bombus impatiens*, three of which are expressed in the complex eye. Using an antibody against the UV opsin protein we found three kinds of ommatidia among the UV opsin-expressing photoreceptor cells in the main retina, namely ommatidia containing two UV opsin immunoreactive cells, one or none. UV opsin expression was also detected in the median and the dorsal ocelli. Surprisingly, we also found opsin expression in different parts of the brain. UV opsin immunoreactivity was detected in the proximal rim of the lamina adjacent to the first optic chiasm, which is where studies in honeybees have found expression of the circadian clock protein PERIOD. We also found UV opsin immunoreactivity in the core region of the antennal lobe glomeruli and different clusters of perikarya within the protocerebrum, indicating a putative function of these brain regions in the entrainment of circadian rhythms. A comparison between bumblebees and recently published data of honeybees revealed significant differences in opsin expression patterns.

# Molecular Diversity, Patterns of Expression, and Functional Divergence of Visual Pigments in Stomatopoda (Crustacea)

Megan L. Porter, Michael Bok, Phyllis R. Robinson, and Thomas W. Cronin

Department of Biological Sciences

University of Maryland

Baltimore County, Baltimore MD 21250 USA

[porter@umbc.edu](mailto:porter@umbc.edu), [mikebok@gmail.com](mailto:mikebok@gmail.com), [probinso@umbc.edu](mailto:probinso@umbc.edu), [cronin@umbc.edu](mailto:cronin@umbc.edu)

Stomatopod crustaceans are hypothesized to express up to 16 different visual pigments in a single retina. In this study, we investigate the complement of expressed opsin genes in retinas of seven different species of stomatopods representing a broad taxonomic range (five families, three superfamilies) using degenerate primers targeting crustacean middle wavelength sensitive opsins. Based on microspectrophotometric studies, stomatopods contain up to six photoreceptors with middle-wavelength sensitivity, and correspondingly we hypothesized that the primers utilized would isolate up to six different opsin gene copies per species. A total of 79 unique retinal opsin transcripts were isolated, resulting in seven to fifteen different expressed opsin transcripts in each species. Minimally, the characterized sequence diversity represents four to ten opsin copies in the genome of each species. Phylogenetically, these opsin transcripts form six distinct clusters, grouping with other characterized crustacean opsins and sister to insect middle-wavelength visual pigments. Within these stomatopod opsin groups, intra- and interspecific clusters of highly similar transcripts suggest that there has been rampant, recent opsin gene duplication. Some of the observed molecular diversity is also due to ancient gene duplication events within the stem crustacean lineage. Expression patterns the isolated opsin transcripts were investigated in one species, *Neogonodactylus oerstedii*. Using *in situ* hybridization, individual transcripts were found to be expressed in different middle wavelength photoreceptors, including the peripheral hemispheres of the eyes, as well as both tiered receptors in midband rows two and three, and the receptors in midband rows five and six specialized for polarization sensitivity. The number of transcripts recovered within this species suggests that, at least in some photoreceptors, several highly similar copies of the opsin gene are being expressed. Using evolutionary trace analysis, 11 amino acid sites were identified as functionally divergent among stomatopod opsins.

Remarkably, these 11 sites form tight clusters in two regions of the opsin protein known to be functionally important: six in the chromophore binding pocket and five at the cytoplasmic interface of cytoplasmic loop III and transmembrane helix VI. Eight of these sites (five in the chromophore binding pocket and three in cytoplasmic loop III) show amino acid differences among the polarization sensitive receptors and the receptors specialized for color vision. These two clusters of sites, and the distributions of residues among different classes of receptors, indicate that stomatopod opsins have diverged both with respect to spectral tuning and to metarhodopsin II function.