Ancient default activators of terminal photoreceptor differentiation in the pancrustacean compound eye: The homeodomain transcription factors Otd and Pph13

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Keywords: Photoreceptor, Rhabdomere, Orthodenticle, Pph13, *Drosophila*, *Tribolium*, *Daphnia*, Evolution, Development, cis-regulatory DNA, enhancer, transcription factor, eye, retina, Hazy, Munster, Rhodopsin Conserved Sequence I (RCSI)

ABSTRACT

The origin of the *Drosophila* compound eye predates the ancestor of Pancrustacea, the arthropod clade that includes insects and Crustaceans. Recent studies in emerging model systems for pancrustacean development - the red flour beetle *Tribolium castaneum* and water flea *Daphnia pulex* - have begun to shed light on the evolutionary conservation of transcriptional mechanisms found for the *Drosophila* compound eye. Here, we discuss the conserved roles of the transcription factors Otd and Pph13, which complement each other in two terminal events of photoreceptor differentiation: rhabdomere morphogenesis and transcriptional default activation of opsin gene expression. The synthesis of these data allows us to frame an evolutionary developmental model of the earliest events that generated the wavelength-specific photoreceptor subtypes of pancrustacean compound eyes.

The pancrustacean compound eye: evolutionary conservation and cellular organization

Compound eyes represent one of the most frequently evolved eye types in animals [1]. Their particularly elaborate version in insects and crustaceans (Pancrustacea) ranks among the most capable and complex known visual organs [2]. Remarkably, the compound eyes of some arthropod fossils from the Cambrium parallel extant species in structural detail [3,4]. Thus, the sophistication of the compound eye in present day pancrustacean species dates back hundreds of millions of years. Comparative developmental genetic studies have now elucidated the first conserved gene regulatory mechanisms that characterize this ancient but highly advanced ground state.

The key characteristic of compound eyes is their assembly from serially homologous visual subunits, called ommatidia. In most pancrustacean species, the core of a single ommatidium consists of 8 photoreceptor cells, 4 cone cells, and 2 primary pigment cells (Fig. 1) [5]. Surrounding this core, a more variable set of interommatidial pigment cells provides the optical insulation of individual ommatidia, while the cone cells and primary pigment cells form the individual lenses of each ommatidium. This system focuses light from a specific point of the environment on the underlying photoreceptors, which translate the photic information on the environment into neural signals to the brain.

The light capture takes place in a specialized apical membrane compartment of the photoreceptors, the rhabdomere, which houses the light-sensitive, seven-transmembrane G-protein coupled opsin receptor proteins and the downstream phospholipase C-based phototransduction machinery. In most cases, a single photoreceptor specifically expresses one of several opsin genes, which differ in wavelength-sensitivity (WS), falling into three subfamilies: green or long wavelength-sensitive (LWS), blue or short wavelength-sensitive (SWS-B), and ultraviolet short wavelength-sensitive (SWS-UV) [6].

Together with structural features, the expression of specific opsin paralogs defines distinct subtypes among the eight photoreceptors of a single ommatidium. Some of these subtypes are deeply conserved. This includes the dichotomy between outer vs inner photoreceptors. The six outer photoreceptors R1-R6 are often characterized by rhabdomeres that span the entire depth of the retina and are arranged around the two inner photoreceptors R7 and

R8. The rhabdomeres of the latter are centrally-positioned, with that of the R7 photoreceptor usually located distally to that of the R8 photoreceptor. As recently reviewed in new molecular detail [7], these basic photoreceptor subtypes have further diversified in lineage- or species-specific ways. Thus, while uniform from the outside due to conserved ancient traits in cellular organization [8,9], pancrustacean compound eyes are also highly diverse in their complex neural organization, providing excellent opportunities for studying the developmental basis of the adaptive evolution of receptor diversity. Fundamental insights into this issue have been gained through the progress in understanding the molecular regulation of rhabdomere formation and differential opsin expression in *Drosophila* [10,11] and functional comparative studies in a broader range of pancrustacean model systems.

Opsin expression, photoreceptor subtypes, and ommatidial subtypes in fruit flies, flour beetles, and water fleas

In addition to *Drosophila*, studies of compound eye development have been initiated in emerging pancrustacean model species that include the red flour beetle *Tribolium castaneum* [12], the grasshopper *Schistocerca americana* [13], the two-spotted cricket *Gryllus bimaculatus* [14,15], and, most recently, the water fleas *Daphnia pulex* and *Daphnia magna* [16]. Not surprisingly, these models differ in many visually-relevant features, given their diversified visual ecologies. Water fleas, for instance, are cyclopic, possessing only a single median compound eye in contrast to the typical pair of lateral compound eyes in most pancrustaceans (Fig. 2). Moreover, the cyclopic water flea compound eye consists of less than 20 ommatidia. This contrasts with close to 100 ommatidia in each of the two lateral compound eyes of *Tribolium* and about 750 ommatidia per compound eye in *Drosophila* (Fig. 2) [17–19]. Further remarkable is the that this variation in eye size correlates very little with the diversity of opsin genes expressed in the eyes of these species.

Only two opsin genes, for instance, are expressed in the *Tribolium* compound eye, with a singleton LWS opsin present in all photoreceptors, and a singleton SWS-UV opsin selectively expressed in the inner R7 photoreceptors (Fig. 2). The resulting co-expression of two opsins in the R7 photoreceptors is a derived exception to the ‘one-receptor’ rule typically required for

wavelength discrimination in visual systems [20] and likely reflects the crepuscular ecology of *Tribolium* [21].

Astoundingly, the small-eyed *Daphnia* represents a much more complex model for differential opsin expression. One SWS-UV, three SWS-B, and 25 LWS opsins have identified from its genome [16,22]. While our understanding of the visual organization of *Daphnia* is still very limited, it is reasonable to speculate that this diversity of *Daphnia* opsins reflects the dynamic spectral characteristics of the freshwater environments to which this species adapted [23]. Physiological evidence suggests that, like in *Drosophila*, the *Daphnia* opsins are differentially expressed among photoreceptors within individual ommatidia and possibly in different combinations across the eye [16,19,24]. Consistent with this, recent molecular studies revealed that specific LWS opsins and the singleton SWS-UV opsin are indeed differentially expressed [22]. Moreover, there is molecular evidence that temporal and environmental variables impact the differential expression of *Daphnia* opsin genes [16]. Notwithstanding these first insights, presence and organization of photoreceptor subtypes and ommatidial subtypes in the *Daphnia* compound eye await to be elucidated.

In comparison to *Daphnia*, *Drosophila* expresses a moderate number of five opsin paralogs in the compound eye: two LWS (*Rh1/ninaE* and *Rh6*), one SWS-B (*Rh5*), and two SWS-UV opsins (*Rh3* and *Rh4*) (Fig. 2). However, the differential expression of these genes defines no less than six photoreceptor subtypes and possibly five ommatidial subtypes [7]. For the purposes of this review, it is sufficient to focus on the yellow and pale subtypes of ommatidia, which fill most of the retina (Fig. 1 and 2). All *Drosophila* ommatidia, including yellow and pale ommatidia, express the LWS opsin *Rh1* in the outer photoreceptors R1-R6. It thus is the differential expression of opsins *Rh3-Rh6* in the inner photoreceptors R7 and R8 that distinguishes the ommatidial subtypes [9]. Yellow ommatidia express the SWS-UV opsin *Rh4* in the R7 photoreceptor and the green-sensitive *Rh6* in the R8 photoreceptors, while pale ommatidia express the SWS-UV opsin *Rh3* in the R7 photoreceptor and SWS-B opsin *Rh5* in the R8 photoreceptor (Fig. 2). Together, this translates into five opsin-defined photoreceptor subtypes in the yellow and pale ommatidia of the *Drosophila* eye: outer photoreceptors (*Rh1*-positive), pale R7s (*Rh3*-positive), yellow R7s (*Rh4*-positive), pale R8s (*Rh5*-positive) and yellow R8s (*Rh6*-positive).

The role of this complex mosaic of photoreceptor subtype-specific opsin expression in the visual ecology of *Drosophila* remains poorly understood [25,26]. At the physiological level, it has been established that different eye regions and distinct ommatidial subtypes serve specialized visual tasks. This includes the detection of polarized light in specific areas of the eye [27,28], the achromatic tracking of motion by the monochromatic outer photoreceptors R1-R6 [29,30], and the discrimination of colors by the spectrally diverse inner photoreceptors R7 and R8 [29,30]. Interestingly, recent work has demonstrated crosstalk between these motion and color vision systems [30–32]. Moreover, there is evidence that the contribution of outer and inner photoreceptors to motion vs color vision varies across species [33]. Given this complexity and diversity of pancrustacean compound eyes, it is fascinating to note the deep conservation of the basic outer vs inner photoreceptor subtypes [8,9]. As described below, the same can now also be stated regarding the transcriptional mechanisms that generate the retinal mosaics of pancrustacean compound eyes.

Conserved default activation of SWS opsin expression by Otd

The complexity of differential opsin expression in the *Drosophila* compound eye has served as an efficient system for studying the transcriptional mechanisms involved in differential gene expression during cell fate differentiation. These efforts uncovered a surprisingly simple core logic of the gene regulatory network that controls the photoreceptor subtype-specific expression of opsin genes *Rh3*, *Rh5*, and *Rh6* (Fig. 3 and 4) [10]. Each of these genes, while highly restricted to specific inner photoreceptors in the mature eye, has the default potential to be activated in all eight photoreceptors within an ommatidium. Thus, instead of photoreceptor-specific gene activation, it is transcriptional repression in incompatible photoreceptor subtypes that restricts opsin expression to the correct photoreceptor subtype.

The default activation and its transcriptional facilitators have been found to be deeply conserved in *Tribolium* and *Daphnia*. The default activation of the *Drosophila* SWS opsins *Rh3* and *Rh5* is mediated through direct activation by Otd (Orthodenticle) [34], the single member of the Otx K50 homeodomain transcription factor family in *Drosophila* (Fig. 3) [34]. The ancientness of this factor is amply reflected in the level of its complex pleiotropic

involvement in neurogenesis, including photoreceptor subtype specification. In the eye, in addition to promoting SWS opsin expression (Fig. 3 and 4), Otd promotes the pale R8 photoreceptor fate through regulation of the Hippo signaling pathway [35], indirectly represses *Rh3*, *Rh5*, and *Rh6* in outer and yR7 photoreceptors by promoting the expression of the transcriptional repressor Defective proventriculus (Dve) [34,36], and indirectly represses *Rh6* in the pale R8 photoreceptors by activating the large Maf transcription factor Traffic Jam (Tj) [35]. The sum of these Otd-involving mechanisms ultimately filters Otd-mediated default activation of opsins *Rh3* and *Rh5* to specific expression in the pale subsets of R7 and R8 photoreceptors (Fig. 3).

In addition to this extraordinary pleiotropy, the challenge of probing for evolutionary conservation of Otd-dependent functions has been exacerbated by the convoluted orthology relationships between the Otx factors of *Drosophila*, *Tribolium*, and *Daphnia* [22]. Both *Daphnia* and *Tribolium* possess two *otd* genes due to apparently independent duplications, while *Drosophila* has lost one *otd* homolog. Notwithstanding this dynamic gene family history, select *otd* homologs from *Tribolium* and *Daphnia* can rescue the activation of the SWS-UV opsin *Rh3* in *Drosophila* pR7 photoreceptors [22]. Moreover, one of the *Tribolium* *otd* homologs (*otd2*) rescues the activation of the SWS-B opsin *Rh5* [22]. Further documenting a conserved function of Otd factors in SWS-UV activation, knockdown of the *Tribolium* *otd* genes revealed that the *otd2* paralog is essential for the expression of SWS-UV opsin in *Tribolium* [22]. Intriguingly, three K50 Otd candidate binding sites can be detected in the upstream region of the *Tribolium* SWS-UV opsin gene (A.Z. personal observation). This suggests that the SWS-UV opsin gene is directly activated by *Tribolium* Otd2, equivalent to the activation of SWS-UV opsin *Rh3* by Otd in *Drosophila* (Fig. 3). Of further note, the downregulation of *Tribolium* Otd2 does not affect the activation of the *Tribolium* LWS opsin [22]. This is consistent with the Otd-specific activation of SWS opsins in *Drosophila* (Fig. 3) and speaks for an evolutionarily conserved split in the transcriptional default activation of SWS vs LWS opsin genes by Otd and a second factor, which has been identified as well.

Conserved default activation of LWS opsin by Pph13

The photoreceptor-wide transcriptional default activation of the green-sensitive LWS opsin *Rh6* in the *Drosophila* eye is provided by the Aristaless-related (Arx) homeodomain transcription factor PvuII-PstI homology 13 (Pph13) [37]. Similar to the case of Otd and the Rh3 and Rh5 opsins, this Pph13-mediated default activation is filtered to the selective expression of Rh6 specifically in yellow R8 photoreceptors through concomitant transcriptional repression in the incompatible photoreceptor fates (Fig. 4). This involves the transcription factor Dve in outer photoreceptors R1-R6 [34,36], the transcription factor Prospero (Pros) in R7 photoreceptors [38], and the transcription factor Tj in the pale R8 photoreceptors [35].

Recent studies in *Daphnia* and *Tribolium* reveal that the role of Pph13 as a default activator of LWS opsin expression is indeed also ancestral in pancrustacean eye development [22]. Circumstantial evidence for this function already existed, in part, based on the evolutionary conservation of the Pph13 binding site within a previously defined photoreceptor-specific cis-regulatory element, called the Rhodopsin core sequence I (RCSI) or P3 site. This 11 bp long palindromic homeodomain binding site was identified based on its conserved presence in the promoters of all six *Drosophila* opsin genes (Fig. 3 and 4) [39,40] and drives reporter gene expression in all photoreceptors in the *Drosophila* compound eye [34,39,41]. The latter could also be demonstrated for transgenic moths and beetles [42–45], presenting the first evidence that a conserved photoreceptor-selective factor is responsible for the RCSI/P3 mediated transcriptional activation of opsin expression.

Consistent with this role, Pph13 is highly restricted to photoreceptors in *Drosophila*, recognizes a portion of the RCSI/P3 site present in the RCSI/P3 reporter constructs, and is essential for mediating RCSI/P3 reporter gene expression in *Drosophila* [37]. Further analysis of Pph13-dependent binding properties, however, revealed higher binding affinity to a specific subset of RCSI/P3 sites - that found in the LWS opsin *Rh6*, rather than those in the SWS opsins *Rh3* and *Rh5* in *Drosophila*. Consistent with this specificity for the *Rh6* opsin, mutations in Pph13 lead to loss of Rh6 expression in the *Drosophila* compound eye, while Rh3 and Rh5 remain unaffected [37].

The recent studies in *Tribolium* and *Daphnia* have uncovered putative RCSI elements with Pph13 binding sites in a subgroup of the *Daphnia* LWS opsin gene regulatory sequences (the B lineage) and in both of the two opsin genes of *Tribolium* [22]. Similar to the situation in

Drosophila, however, *Tribolium* Pph13 protein binds to the RCSI site of the LWS opsin with higher affinity than the RCSI site of the SWS-UV opsin [22]. Demonstrating a functionally conserved capacity of Pph13 in LWS opsin activation, both *Tribolium* and *Daphnia* Pph13 specifically rescue expression of the LWS *Rh6* opsin in *Drosophila* mutants lacking endogenous *Pph13* activity [22]. Furthermore, reducing *Pph13* in *Tribolium* diminishes LWS but not SWS-UV transcript levels, thus mirroring the misregulation of opsin expression in *Pph13* mutant *Drosophila* eyes. Taken together, these findings identify Pph13 as an ancestral activator of LWS opsin expression in the pancrustacean retina through an LWS opsin-restricted RCSI site (Fig. 4).

Conserved cooperativity of Otd and Pph13 in the transcriptional activation of rhabdomere formation

In addition to their roles in opsin regulation, Otd and Pph13 are also both required for the proper formation of the opsin-containing rhabdomere compartment of photoreceptor cells [37,46]. Rhabdomeres form through initial expansion of the apical photoreceptor membrane into densely packed microvillar stacks that eventually extend along the proximodistal axis of the mature photoreceptor [47]. *Drosophila* eyes mutant for *otd* or *Pph13* exhibit partial defects in rhabdomere morphogenesis [48,49], while eyes mutant for both factors are completely void of rhabdomere structures [37]. Thus, in the *Drosophila* eye, *otd* and *Pph13* function additively to regulate rhabdomere formation.

Comparative studies in *Tribolium* and *Daphnia* have now established that this involvement of *otd* and *Pph13* in rhabdomere formation is likewise ancient for the pancrustacean eye [22]. Misexpression of both of the *otd* genes of *Tribolium* and the *Daphnia otd1* homolog exhibit equivalent capacities to rescue *otd* mutant rhabdomeres in *Drosophila*. Similarly, expression of either the *Tribolium* or *Daphnia* Pph13 homologs in *Drosophila Pph13* mutant photoreceptors rescues rhabdomere morphology close to wild type organization.

As a final demonstration, downregulating the *otd* orthologs or *Pph13* abolishes rhabdomere formation in *Tribolium*, documenting the conserved involvement of these factors for rhabdomere formation in both flies and beetles [22].

A developmental model of photoreceptor subtype evolution

The roles of Otd and Pph13 in both differential opsin regulation and rhabdomere formation are likely no coincidence. Besides harvesting light, opsins also represent structural components of the rhabdomere, which is documented by the fact that reduced opsin expression levels leads to a failure in rhabdomere development [50,51]. This link leads to an integrated model of the evolution of rhabdomere structure and differential opsin expression in the ancestral pancrustacean retina (Fig. 5). The role of Otd in the transcriptional activation of photosensitive protein components likely preceded the pancrustacean ancestor. This conjecture is based on the involvement of Otx factors in the development of the ciliary photoreceptors of vertebrates, the capacity of vertebrate Otx factors to rescue Otd functions in the *Drosophila* eye, and the multilayered master regulatory roles of Otd in pancrustacean photoreceptor differentiation [22,34,35,37,46,48,52,53]. It is therefore reasonable to assume that an ancestral Otx homolog was the regulator of the prototype photosensitive membrane compartment that harvested light through opsins in the earliest animal photoreceptors.

The joining of Pph13 at a later stage of rhabdomeric photoreceptor evolution follows from the absence of Pph13 orthologs outside protostomes, which suggests a younger evolutionary age, in line with the exceptionally specific function of this factor to photoreceptors. It is thus tempting to speculate that Pph13 joined Otd in the upstream activation of rhabdomere-related genes during the evolution of a more elaborate rhabdomere organization that required extended cis-regulatory input. During this transition, Pph13 likely acquired the role of the LWS opsin expression activator through recognition of a subset of RCS/P3 sites, while Otd retained its ancestral role in activating opsin genes for the SWS opsin precursor. Consistent with the findings of a recent comprehensive analysis of pancrustacean opsin gene family evolution [54], the latter likely subsequently gave rise to the SWS-B and SWS-UV opsin subfamilies in the pancrustacean retina, both of which inherited the dependence on activation by Otd. This division of labor might have been the beginning of differential opsin expression in photoreceptor subtypes, which experienced further refinements in different lineages, likely through repressor mechanisms like those that shape the retinal mosaic of *Drosophila*.

Besides explaining the complementary roles of Otd and Pph13 in opsin regulation and rhabdomere development, this model provides an evolutionary explanation for the default activation of opsins through Otd and Pph13 in the *Drosophila* retina. In further support of the model, Pph13-specific RCSI sites have been found in the orthologs of the photoreceptor-specific G protein β -subunit 76C gene (G β 76C) in *Drosophila*, *Tribolium* and *Daphnia* [22], revealing an extended range of conserved rhabdomeric target genes of Pph13 [49]. Similarly, a broader range of putative direct Otd target genes with rhabdomere-specific functions such as Arrestin 2 and Calphotin has been identified in *Drosophila* [55].

Perspectives

The model outlined above has several predictions inviting future studies. One obvious prediction is the conservation of the Pph13 and Otd opsin default activation cassettes in other pancrustacean compound eyes. Interestingly, a recent study on RCSI/P3 reporter gene expression in the butterfly species *Bicyclus anynana* concluded that Pph13 is absent from Lepidoptera [56]. Indeed, preliminary analysis confirms the absence of detectable Pph13 orthologs in most lepidopteran genome and transcriptome databases. The Glanville fritillary *Melitaea cinxia* whole genome shotgun sequence contig 12241, however, contains the coding sequence for an unambiguous Pph13 homeodomain (M.F., personal observation). Pph13 is thus likely also conserved in Lepidoptera and may have escaped detection in other moth and butterfly species so far due to the fact that the extent of its conserved sequence is confined to the homeodomain. In support of this speculation, the detection of Pph13 in the *Daphnia* genome also required targeted effort [22].

The evolutionary developmental model of photoreceptor subtype diversification presented here further predicts the conservation of Otd as the default activator specifically of SWS opsins vs conservation of Pph13 as default activator of LWS opsins. Given the efficient detectability of Otd- and Pph13-specific binding sites and the compact organization of the cis-regulatory DNA of opsin genes, this prediction can be tested by bioinformatic studies in additional pancrustacean species. This objective will be aided by a new generation of binding

site prediction programs [57–59]. The same tools should be further valuable for testing the postulated direct regulation of structural rhabdomere protein genes by Otd and Pph13 [22,55].

Evolutionary conservation can also be hypothesized for the repressive mechanisms that restrict the execution of the photoreceptor-wide default activities of Pph13 and Otd to photoreceptor subtypes. One such candidate mechanism is the repression of default LWS opsin expression in R7 photoreceptors by Pros (Fig. 4). The loss of this single input is an attractive explanation for the exceptional co-expression LWS and SWS-UV opsin in the R7 photoreceptors of the *Tribolium* eye (Fig. 2 and 4).

The RNAi knockdown approach, which has been highly efficient for characterizing gene functions [60], has recently been complemented by CRISPR-mediated gene knockout protocols in *Tribolium* and *Daphnia* [61,62]. This expanded range of genetic interference approaches can be expected to expedite the functional analysis of additional regulators in these models of pancrustacean compound eye development. The resulting insights are likely to benefit the investigation of other remarkable visual systems in the Pancrustacea such as recently characterized in dragonflies and stomatopod Crustacea, which display exceptional ommatidial diversity [63–66].

Compound eyes are also ancestral body plan components of the other two major arthropod subphyla - Chelicerates and Myriapods. It is therefore equally exciting to note a new generation of molecular developmental studies in the structurally and evolutionarily complex visual systems of chelicerates [67–69], opening further testing grounds for examining the conserved roles of Otd and Pph13 in photoreceptor differentiation.

ACKNOWLEDGEMENTS

We thank Cassandra Extavour and Leslie Pick for the invitation to contribute this review, Riyue Bao for help with compiling information on transcription factor binding sites in the regulatory DNA of *Drosophila* opsin genes, Donna Kashian for the picture of *Daphnia magna*, and the two anonymous reviewers for excellent comments. This work was supported by funds from NIH grant EY022687 (TAC), and NSF grants IOS-0951886 (MF) and IOS-1353267 (ACZ).

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Fig. 1. Cellular organization of the ommatidium in the pancrustacean compound eye. Color code and abbreviations of cell types: Photoreceptors R1-R8 = light grey; Cone cells (CC) = turquoise; Primary pigment cells (1'PC) = yellow; Interommatidial cells (IOCs) = brown. The subcellular localization of the wavelength-specific opsin proteins in the photoreceptor rhabdomeres is indicated by green, blue, and magenta background shading. The examples above illustrate the difference between the pale and yellow subtype ommatidia in the *Drosophila* eye. In both subtypes, the outer photoreceptors R1-R6 express the LWS opsin Rh1. In pale ommatidia, the inner R7 photoreceptor expresses SWS-UV opsin Rh3 while the inner R8 photoreceptor expresses SWS-B opsin, Rh5. In yellow ommatidia, the R7 photoreceptor also expresses a SWS-UV opsin, Rh4, while the inner R8 photoreceptor expresses LWS opsin Rh6. The photoreceptor subtypes also differ by position, rhabdomere localization, and the depth of axonal projection in addition to the differential expression of opsin genes. The basic arrangements of both photoreceptor and accessory cells (cone cells, primary pigment cells, and interommatidial cells) as well as of inner and outer photoreceptor cell fates are highly conserved in pancrustacean compound eyes [33].

Fig. 2. Compound eye organization, ommatidial subtypes, and opsin gene repertoires in *Drosophila*, *Tribolium*, and *Daphnia*. See text for details on the differential expression of opsin paralogs in the ommatidia schematics shown for *Drosophila* and *Tribolium*.

A

B

Fig. 3. Cis-regulatory evidence of the conserved role of Otd in the photoreceptor-wide transcriptional activation of SWS opsin expression. White boxes indicate sequence elements that bind transcriptional activators. Black boxes indicate sequence elements that bind transcriptional repressors. Bold gene names indicate functions that have been found conserved between *Drosophila* and *Tribolium* based on [22]. Arrangement of binding sites does not scale with their relative positions in the native cis-regulatory DNAs.

Fig. 4. Cis-regulatory evidence of the conserved role of Pph13 in the photoreceptor-wide transcriptional activation of LWS opsin expression. White boxes indicate sequence elements that bind transcriptional activators. Black boxes indicate sequence elements that bind transcriptional repressors. Grey box indicates presumed ancestral sequence element in the ancestral LWS opsin gene precursor hypothesized to be absent due to loss in *Tribolium*. Bold gene names indicate functions that have been demonstrated to be conserved between *Drosophila*, *Tribolium*, and *Daphnia* based on [22]. The capacity of *Tribolium* and *Daphnia* orthologs to rescue specific opsin regulation functions in *Drosophila* is indicated by superscripts. Arrangement of binding sites does not equal their relative positions in the native cis-regulatory DNAs. Activating input from the additional transcription factor Senseless (Sens) in yellow R8 photoreceptors based on [70].

Fig. 5. A developmental model of pancrustacean photoreceptor subtype evolution through coordinated regulation of rhabdomeric proteins by Pph13 and Otd. Arrows represent direct transcriptional activation of target gene classes listed in grey. See text for further details.