

# Light and the evolution of vision

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## Abstract

It might seem a little ridiculous to cover the period over which vision evolved, perhaps 1.5 billion years, in only 3000 words. Yet, if we examine the photoreceptor molecules of the most basic eukaryote protists and even before that, in those of prokaryote bacteria and cyanobacteria, we see how similar they are to those of mammalian rod and cone photoreceptor opsins and the photoreceptive molecules of light sensitive ganglion cells. This shows us much with regard the development of vision once these proteins existed, but there is much more to discover about the evolution of even more primitive vision systems.

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## Introduction

Darwin is often quoted as seeing the development of the eye as a significant difficulty for his theory of evolution by natural selection. He writes in *The Origin of Species*:

*To suppose that the eye, with all its inimitable contrivances.... could have been formed by natural selection, seems, I freely confess, absurd in the highest possible degree.*<sup>1</sup>

But what is omitted by many is his answer to this conundrum, a few sentences further on:

*Reason tells me, that if numerous gradations from a simple and imperfect eye to one complex and perfect can be shown to exist.... and if such variations should be useful to any animal.... then the difficulty of believing that a perfect and complex eye could be formed by natural selection, should not be considered as subversive of the theory.*

Indeed, he notes further.

*How a nerve comes to be sensitive to light, hardly concerns us more than how life itself first*

*originated; but....as some of the lowest organisms....are known to be sensitive to light, it does not seem impossible that certain elements...should become aggregated and developed into nerves endowed with this special sensibility.*

In a later letter to J.D. Hooker written in 1871 Darwin remarks:

*But if (and oh! what a big if!) we could conceive in some warm little pond, with all sorts of ammonia and....salts, light, heat, electricity.... present, that a proteine [sic].... was chemically formed ready to undergo still more complex changes...<sup>2</sup>*

and so perhaps a warm little pond would be a reasonable place to start as we look at the evolution of vision. I wonder if you remember pond dipping as a child? As far as I remember we only looked at macroscopic life-forms—water boatmen, pond skaters and the like. But if we had happened to look under a microscope no doubt we would have found two organisms, *Euglena* (Figure 1a) and *Chlamydomonas* (Figure 1b).

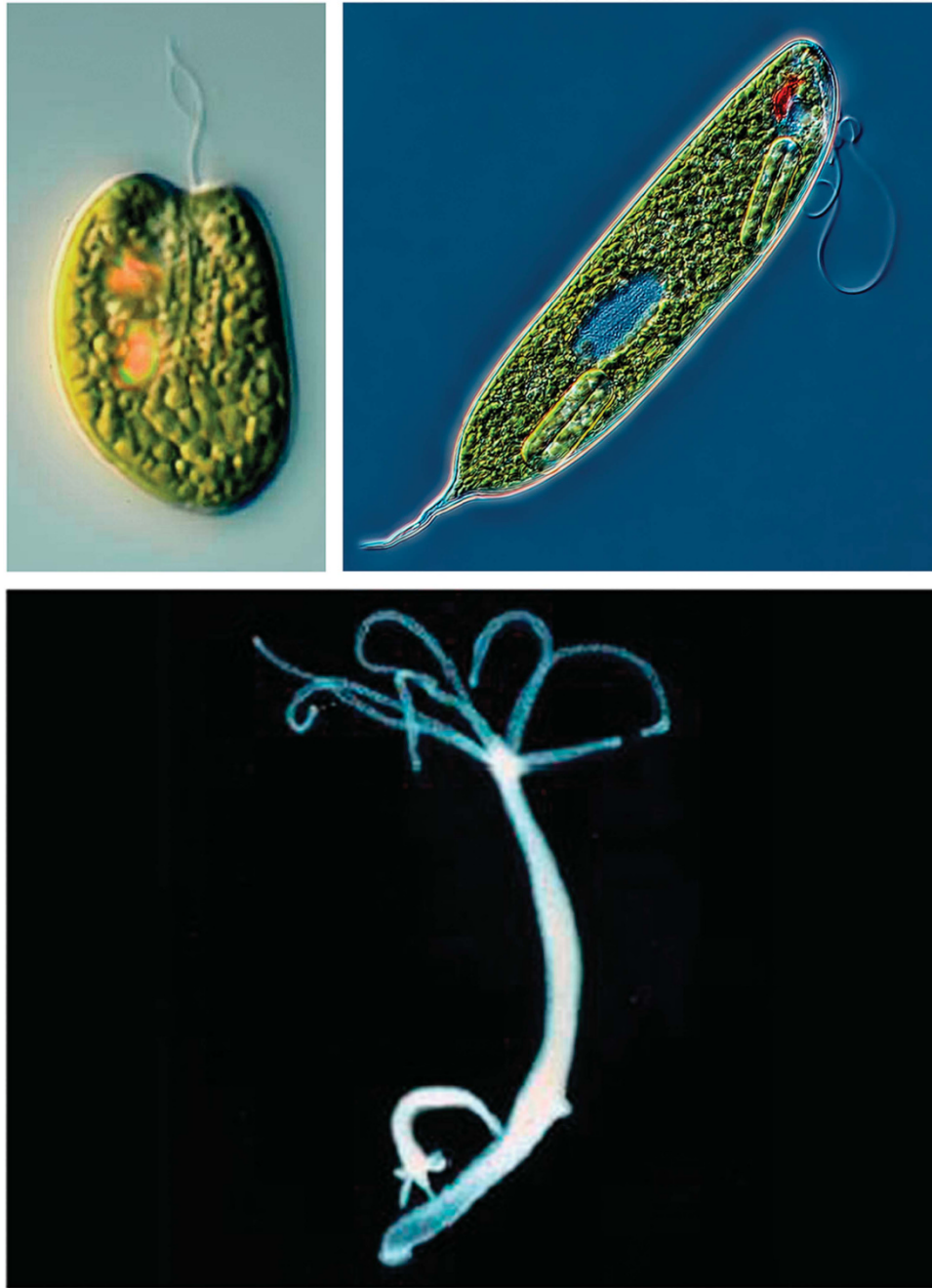
## *Euglena and Chlamydomonas*

Both these unicellular protists have a clear orange-coloured eyespot. *Euglena gracilis* exists as a photosynthesising autotroph but at low light intensity it can survive as a heterotroph ingesting plant material. Neither strictly plant nor animal, it occupies a third kingdom as a protist. *Chlamydomonas reinhardtii* might more strictly be defined as a green alga in the plant kingdom. Phototaxis is essential for both organisms; moving towards light upon which they depend for energy and nutrition, yet also undergoing negative phototaxis to protect themselves against too intense a source of illumination. The eyespot is not the photoreceptor itself but rather a mass of carotenoid pigment shading the photoreceptor from light from one direction. This demonstrates the essential components of any visual system; any photosensitive organism needs a photoreceptor that detects the light. But that

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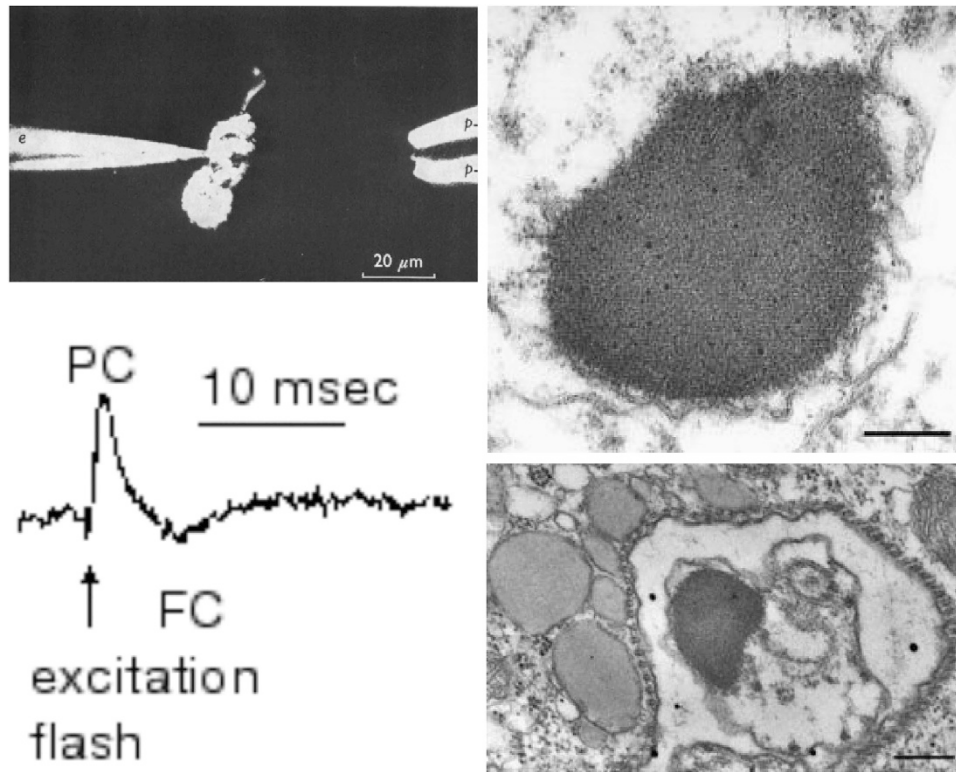


**Figure 1** Top left *Chlamydomonas reinhardtii*. Top right *Euglena gracilis*. Bottom *Hydra vulgaris*.

alone would not allow the organism to determine the direction of the light source. A pigment spot reduces the illumination from one direction, or changes the wavelength of the incident light falling on the photoreceptor, thus allowing the organism to move in the direction of the light or away from it. So third, a mechanism to promote movement is essential. To detect the light is one thing but to move towards or away from it requires a motor system; the flagellae in *Chlamydomonas* and *Euglena*. But also a mechanism is required by which

detection of light can be translated into a change in flagellar movement, generally an ion flux of one kind or another.

In *Euglena* the photoreceptor, a tightly packed mass of crystalline protein, is located next to the eyespot (Figure 2a). It has been estimated that the photoreceptor contains around  $2 \times 10^7$  molecules of a rhodopsin-like protein.<sup>3</sup> Around  $10^8$  photons impinging on this crystal saturate the protein with a maximum absorption at around 500–525 nm.<sup>4,5</sup> Photostimulation leads to positive



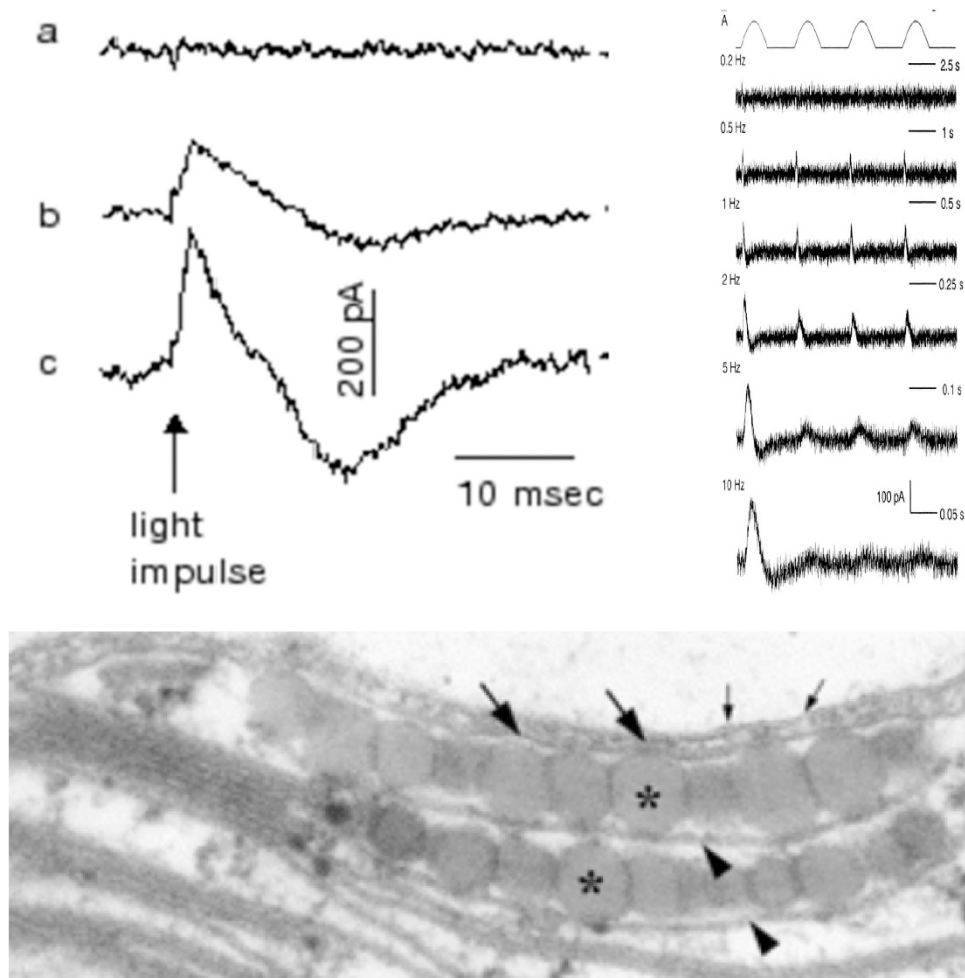
**Figure 2** Photoreceptor of *Euglena gracilis*, left: measuring the photoelectric effect after Nichols and Rikmenspoel (1977) right: photoreceptor rhodopsin crystal positioned next to emergent flagellum after Barsanti *et al* (2012).

phototaxis with the ion flux giving what appears surprisingly like an electroretinogram (Figure 2b).

In *Chlamydomonas* (Figure 1b) the eyespot apparatus consists of thylakoid membranes with layers of carotenoid rich globules and photoreceptor molecules in the membranes between these globules (Figure 3a). *Chlamydomonas* has a flavin-associated blue-sensitive chromophore,<sup>6</sup> highly sensitive to blue light at a wavelength of 440 nm, intimately linked to its circadian clock. Although this algal species is essentially a plant, genetic analysis of the chromophore shows what has previously been considered an animal chromophore CRY2.<sup>7</sup> Indeed CRY1 and CRY2 have been detected in light-sensitive ganglion cells in the human retina responsible for pupillary light reactivity and setting of photocycles.<sup>8</sup>

How nice it would be to see these two organisms as fore-runners of on the one hand the rhodopsin-based photodetection systems of mammalian rod and cone photoreceptors and on the other precursors of the more recently discovered blue-wavelength detection systems in light-sensitive ganglion cells. Sadly, evolution is never as simple as that. For a start each of these organisms has multiple light detecting molecules. *Euglena* orientates itself towards light using a rhodopsin photopigment, but also has a blue-light receptor.<sup>9</sup> The photo-reorientation of

the organism away from light is modulated through increased levels of cyclic AMP produced by a blue-light-activated adenylyl cyclase (PAC).<sup>10,11</sup> Similarly, whereas negative phototaxis in *Chlamydomonas* is modulated by PAC, photoattraction occurs through the action of two sensory rhodopsins CSRA and CSRB discovered by Sineschekov *et al* in 2002,<sup>12</sup> also called channel-rhodopsins, ChR1 and ChR2 by Nagel's group,<sup>13</sup> and termed Acop1 by Susuki *et al*.<sup>14</sup> The two photoreceptor proteins have different absorption profiles with CSRA absorbing predominantly at blue-green wavelengths and promoting a photophobic response in response to strong illumination while CSRB absorbs at shorter wavelengths and leads to phototaxis at weaker light intensities.<sup>15</sup> There are around  $9 \times 10^4$  molecules of CSRA and  $1.5 \times 10^4$  molecules of CSRB in each cell.<sup>16</sup> ChR2 is a photoactivated cation channel but in addition acts as a light-driven proton pump. Although this double action might seem somewhat perverse it is not unique—other chloride channels also act as proton pumps, demonstrating their origin as  $\text{Cl}^-/\text{H}^+$  exchangers. But the ChRs do not exhibit homology with other ion channels but rather with rhodopsins. Indeed, these photoactive ion channels did not start their evolutionary existence in the *Euglenoids* and *Chlamydomonads* we have been discussing above. Such eukaryotes originated probably between 800



**Figure 3** Photoresponses in *Chlamydomonas reinhardtii*. The photoreceptive proteins reside in between thylakoid membranes (arrows) with carotenoid-rich granules (asterisks) between them. A photoelectric discharge is seen after a light impulse and this is maximal when correlating with the natural helical motion of the alga.

and 1200 million years ago,<sup>17</sup> but prokaryotes have been present in the fossil record around 1500 million years earlier.<sup>18</sup> Lynn Margulis presented the theory by which eukaryotes form through endophagy of prokaryote algae to produce chloroplasts and bacteria to yield mitochondria, both examples of endosymbiosis.<sup>19</sup> So we should look for the origin of these eukaryote photopigments in prokaryote bacteria.<sup>20</sup>

#### Photopigment origins

The photoresponsive prokaryotes to investigate are both those that are photosynthetic such as *Synechocystis* and those like *Halobacterium* that are not. *Halobacterium* species<sup>21,22</sup> have four photosensory proteins; Bacteriorhodopsin (BR) a proton pump, Halorhodopsin (HR), a light-gated chloride pump, and two sensory rhodopsins. All of these act as photoreceptors, but BR,

existing in much higher copy number than the other proteins, can act on its own as shown by producing blind mutants and recovering photosensitivity by reconstituting BR alone.<sup>23</sup> BR is a purple molecule, with an absorption maximum in green wavelengths, hyperpolarising the cell membrane at around 570 nm, whereas HR absorbs at green–yellow wavelengths, depolarising the membrane. The BR gene has limited sequence homology with other photoreceptive rhodopsins such as those in mammalian rod photoreceptors,<sup>20</sup> but does have the structural similarities of the seven transmembrane domains<sup>24</sup> and a protonated Lys-216 Schiff base where the prosthetic group of retinal binds. The big difference is what while eukaryote rhodopsins are associated with a G protein,<sup>25</sup> prokaryote rhodopsins are not. It had been considered that eukaryote and prokaryote rhodopsins were a prime example of evolutionary convergence,<sup>26</sup> but structural homologies have shown that these apparently quite



different amino-acid sequences are indeed formed through classic evolutionary divergence from a common ancestor.<sup>27,28</sup>

A completely different prokaryote class are cyanobacteria. They have number of photoreceptor molecules<sup>29</sup> from phytochromes like RcaE (the regulator of chromatic adaptation) in *Fremyella*, to Cph 1 a light-regulated biliprotein kinase absorbing in the far red. Blue-light photosensors with a conserved flavin-bound BLUF (blue light using FAD) domain illustrate the diversity of wavelengths absorbed by these photochromes. We like to think that as humans we have a highly developed sense of colour vision, but even these cyanobacteria are able to detect light of different wavelengths.<sup>30,31</sup> Why should these primitive organisms need such a complex system of chromatic detection? Cyanobacteria live in water columns, which at the surface are illuminated by light of a wide variety of wavelengths but where at depth blue light predominates. Thus cyanobacteria migrating up and down a water column experience a far greater range of background colour compared with their land-based plant relatives and differential sensitivity to a variety of wavelengths has evolved.

The problem here is that even as far back as the prokaryotes the complex seven transmembrane domain arrangement of opsin molecules seems to prevail without simpler photoreceptors existing concurrently. Darwin's original puzzle over ocular evolution seems still to be with us but now at a molecular level. Having said that investigation of opsin diversity sheds considerable light on the evolution of life once we get beyond the protist stage. As Eakin suggested over 50 years ago,<sup>32</sup> there are two evolutionary lines of photoreceptors, those involving animals with photosensitive cilia and those with rhabdomeres. The latter are the Protostomia including the arthropods, whereas the former are the Deuterostomes that include the vertebrates. They have different opsins (R and C) and different mechanisms of converting light signals to nerve impulses; C opsins functioning through a cyclic nucleotide pathway, whereas R opsins use phospholipase C for signal transduction. Two unusual organisms, *Amphioxus* and *Platynereis* have eyes using ciliary opsins and others with rhabdomeric opsins, putting them in an interesting transitional position between the arthropod rhabdomeric photoreceptors and the vertebrates ciliary photoreceptors. *Amphioxus*, the lancelet, found half buried in sand across the world is a protovertebrate, having a notochord but no true spine. It has rhabdomeric photoreceptors but also lateral eyes with ciliary photoreceptors.<sup>33</sup> The opsin in these cells is the *Amphioxus* homologue of melanopsin, coupled with Gq as are invertebrate rhodopsins.<sup>34</sup> *Platynereis* is a polychaete worm found living in marine kelp beds. Although clearly an invertebrate with eyes using rhabdomeric opsin, it also

has structures within the brain with a ciliary photoreceptor and vertebrate-type opsin,<sup>35</sup> specifically melanopsin, central to mammalian light-sensitive retinal ganglion cell function.<sup>36</sup> Although these provide remarkable examples of the potential transition between provision of the two types of photoreceptor, how are we to look further back to the very beginning of opsin evolution?

Perhaps we can go back to our pond and look for a more common creature, ironically one with no ocular structures at all, *Hydra* (Figure 1c).<sup>37</sup> And yet it has been known for decades that this relative of jellyfish clearly responds to light, particularly at blue wavelengths.<sup>38</sup> Its harpoon-like cnidophores are released in response to light through opsins and a cyclic-gated nucleotide channel.<sup>39,40</sup> Feuda sees these Cnidarians as a key to understanding the development of opsin-mediated vision.<sup>41</sup> *Hydra* not only has opsin genes but also ancestral paired homeobox domains Pax A and B<sup>42</sup> with the latter having a greater homology to Pax 6 the key master gene so well conserved through evolution from *Drosophila* to mammals.<sup>43</sup> But such control of eye development is only really needed when we have more than one cell making up the visual system. And to develop an imaging forming system requires a multicellular visual organ where photodetection and pigment shielding are functions of different cells as beautifully described by Arndt.<sup>44</sup> Discussion of such ocular evolution could have filled a whole volume<sup>45</sup> and this review has only skimmed the surface. Readers are directed to Ivan Schwab's magisterial volume 'Evolution's Witness: How Eyes Evolved' for further information.<sup>45</sup>

## Conflict of interest

The author declares no conflict of interest.

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