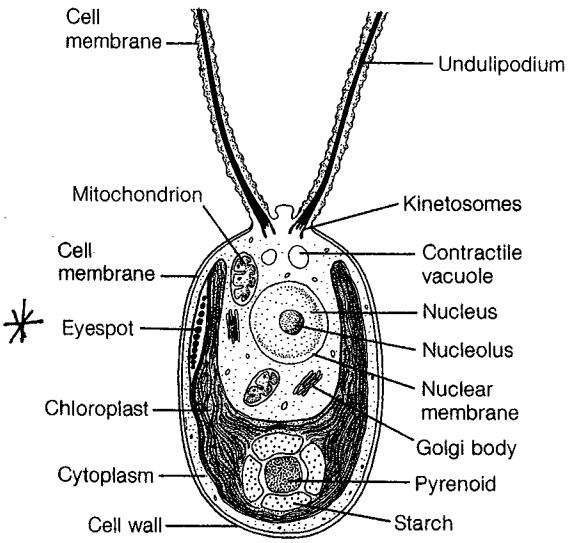
Algal Vision

CASE STUDY: light-activated channels.

OVERVIEW: Although it is easy to assume the ion channels gated by light would be a key component of vision in 'higher' organisms such as animals, the fact is that many lower eukaryotes have a crucial need to use light: to sense optimum conditions in a hostile environment.

Thus, photosynthetic organisms will migrate to regions of optimal light intensity on the basis of primitive light-sensing systems.

Of these, there are members of the Chlorophyta which have an identifiable eyespot.
One of these is Chlamydomonas.



Chlamydomonas sp.

B *Chlamydomonas* is similar in structure to the zoospores of *Acetabularia*. [Drawings by L. Meszoly.]

SOURCE: Margulis & Schwartz. 1997. Five Kingdoms. WH Freeman & Co.

page 1A of _

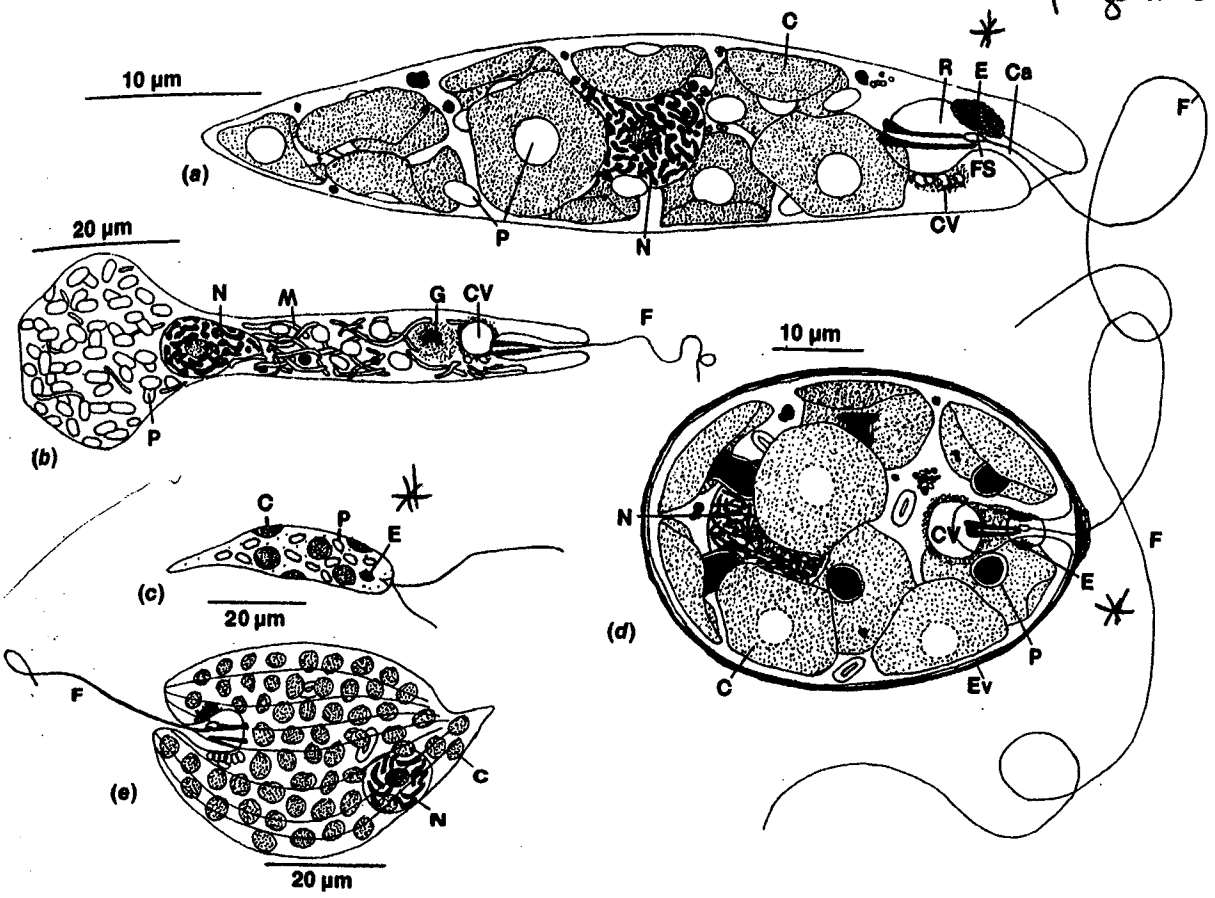


Fig. 4-6. *(a) Euglena gracilis. (b) Astasia klebsii. (c) Eutreptiella marina. (d) Trachelomonas grandis. (e) Phacus triqueter.* (C) Chloroplast; (Ca) canal; (CV) contractile vacuole; (E) eyespot; (Ev) envelope; (F) emergent flagellum; (FS) flagellar swelling; (M) mitochondrion; (N) nucleus; (P) paramylon grains or paramylon sheath around chloroplast; (R) reservoir. (After Leedale, 1967.)

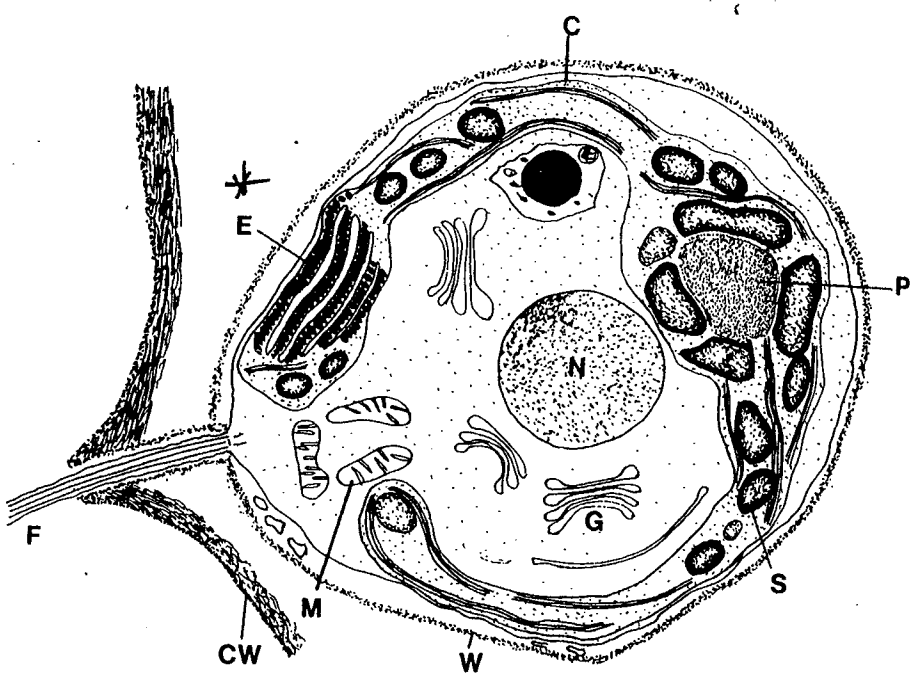
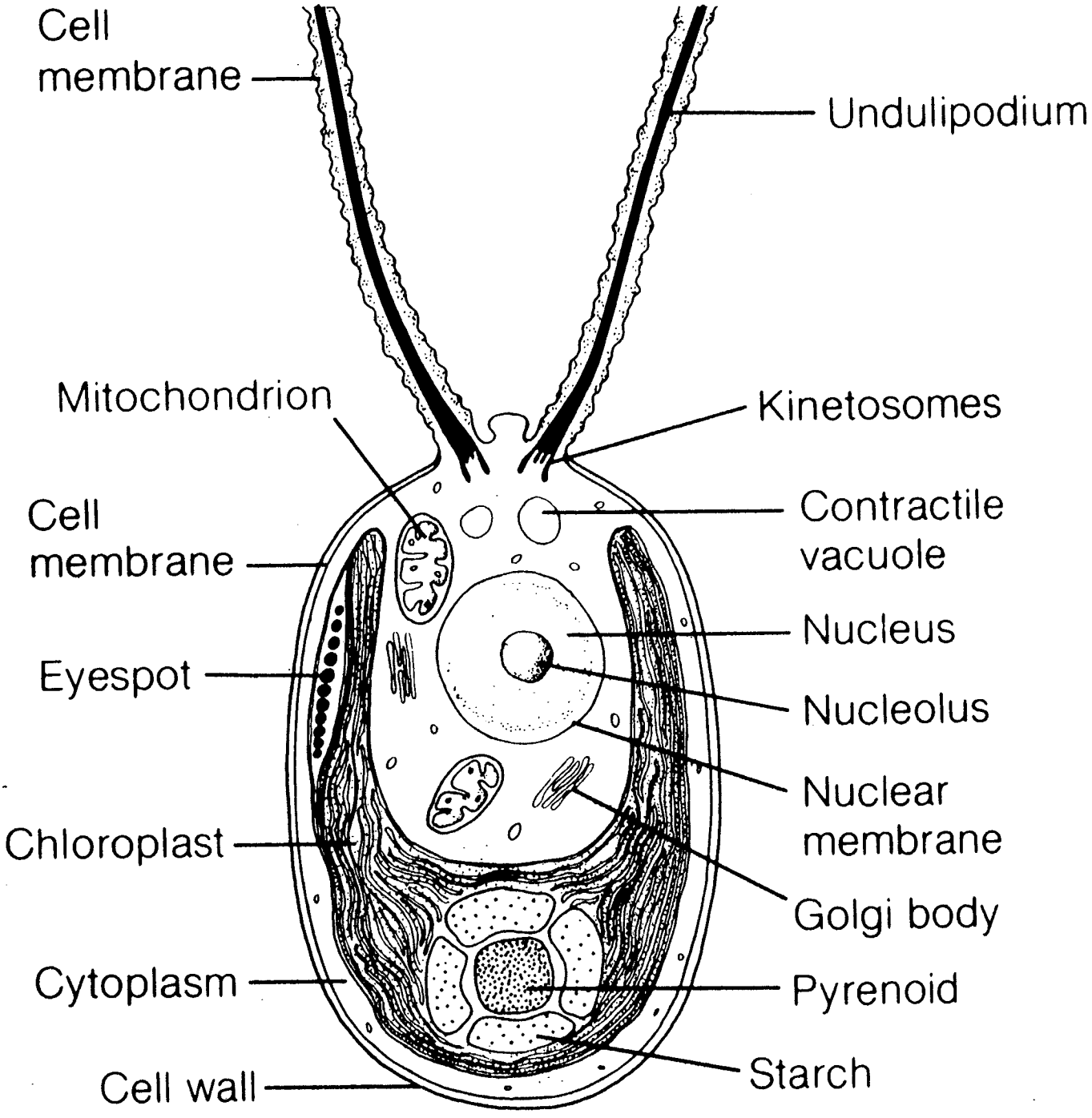


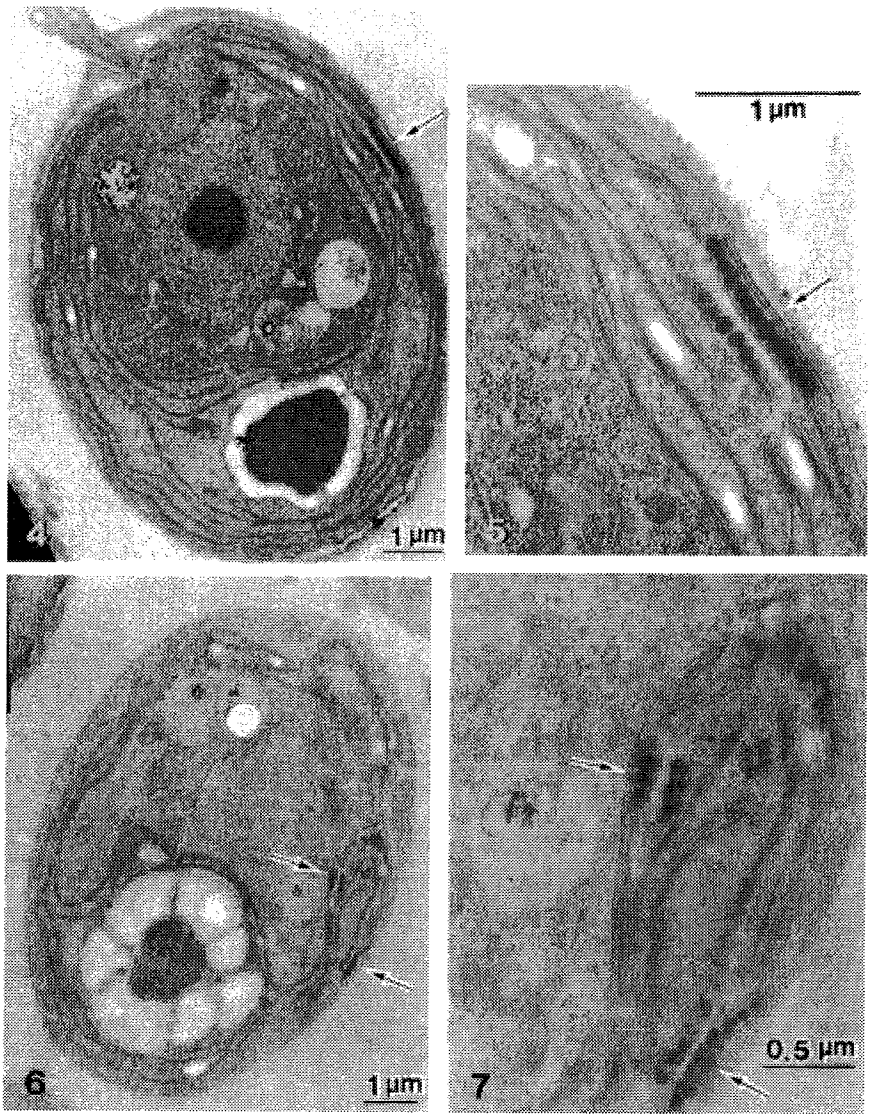
Fig. 15-1. Semidiagrammatic drawing of a cell in a *Volvox* vegetative colony. The colony wall (CW) is distinct from the cell wall (W). (C) Chloroplast; (E) eyespot; (F) flagellum; (G) Golgi; (M) mitochondrion; (N) nucleus; (P) pyrenoid; (S) starch. (Adapted from Pickett-Heaps, 1970.)

SOURCE: RE Lee 1980. Phycology. Cam Uni Press.



Chlamydomonas sp.

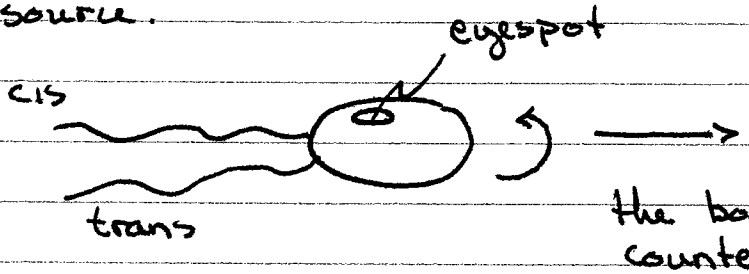
Figs 4–7. Thin-section electron micrographs of Chlamydomonas reinhardtii. 4,5. A wild-type cell. 6 ,7. A mes-10 cell. 5,7. The high magnification view around the eyespots in each 4 and 6. Arrows show the eyespots. Mes-10 has two eyespots in a cell, and an eyespot faced to the nucleus.



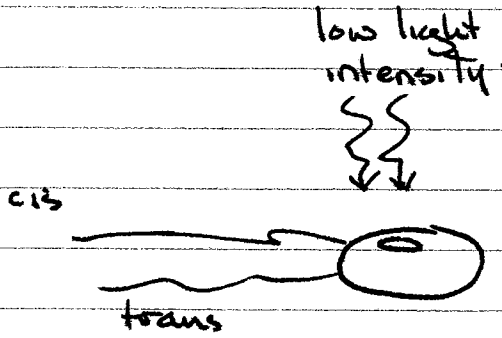
Nakamura, Shogo, Ogihara, Haruo, Jinbo, Kinue, Tateishi, Midori, Takahashi, Tetsuo, Yoshimura, Kenjiro, Kubota, Mamoru, Watanabe, Masakatsu & Nakamura, Soichi. (2001) Chlamydomonas reinhardtii Dangeard (Chlamydomonadales, Chlorophyceae) mutant with multiple eyespots. Phycological Research 49 (2), 115-121.

In chlorophyceae algae, there are two responses to light.

At low light intensity, the algae exhibit positive phototaxis, swimming towards the light source.



the body rotates counter clockwise around the swimming axis: ~ 20-40° per flagellar beat.



cis flagella beats at lower frequency, causing the swimming axis to shift, aiming towards the light.

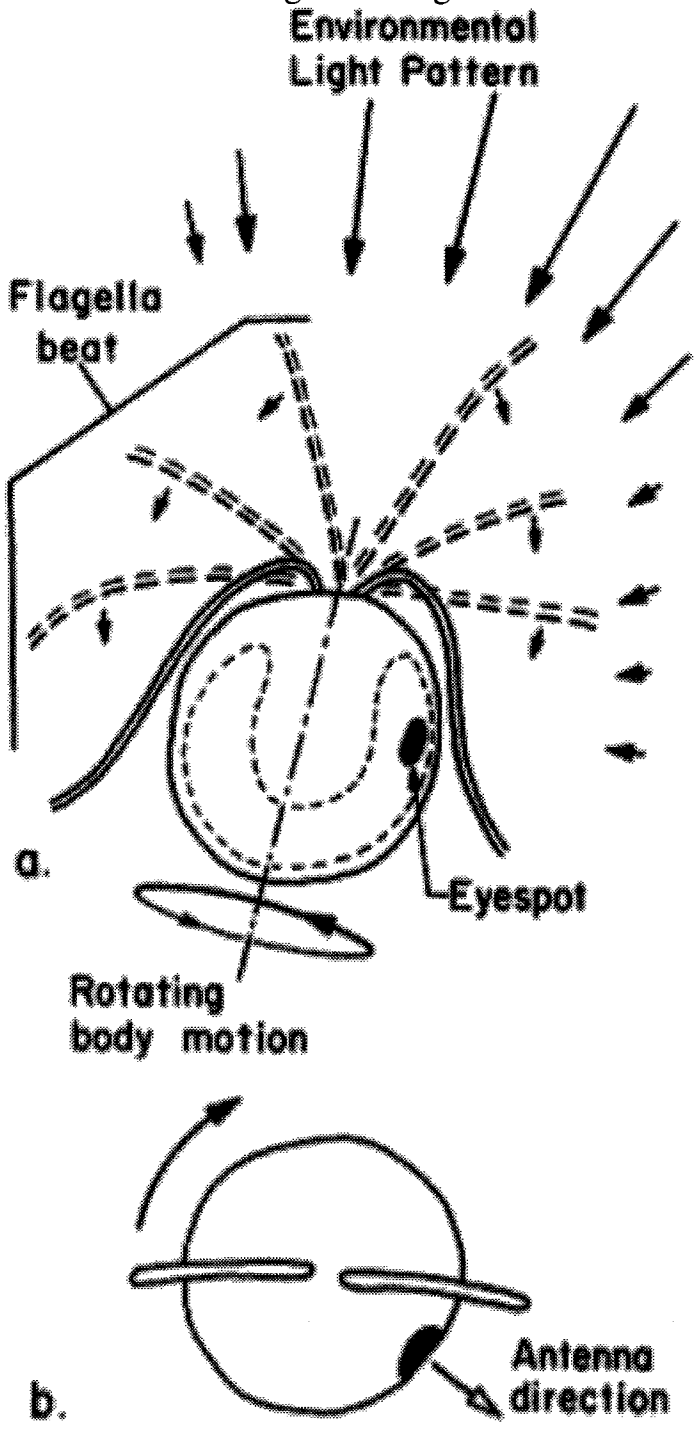
Joanna

At high light intensity, the cells shift from forward swimming (120 μm sec^{-1}) to backward swimming (20 μm sec^{-1}). That is, negative phototaxis.

positive phototactic
The mechanism is a bit different in multi-cellular algae, such as Volvox, in which multiple cells are involved in motility, so cells on one side are inhibited.

Hegemann, P. (1997) Vision in microalgae. Planta 203 265-274.

FIG. 2. Design principles of phototaxis in Chlamydomonas. (a) Side view of cell; (b) end view. The incident light pattern is indicated by solid arrows. The eyespot, which lies inside the chloroplast (dashed line), forms part of the antenna. Rotation of the cell causes the antenna to scan the incident light. This produces a signal that controls the flagellar beat (see Fig. 3). The antenna direction (open arrow) is normal to the cell surface. The antenna is most sensitive to light coming from this direction. Successive positions of the flagella during the power stroke are shown. Flagellar motion causes the cell to translate with the flagellar end forward and to rotate in the left-hand sense.



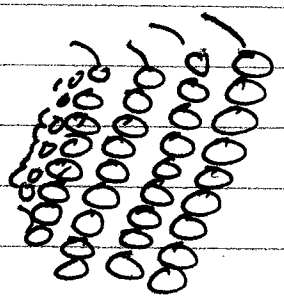
KENNETH W. FOSTER AND ROBERT D. SMYTH (1980) Light Antennas in Phototactic Algae. MICROBIOLOGICAL REVIEWS 44:572–630.

The eye spot is small, about 1 um in diameter. The optical "lens" is made of lipid globules which are carotenoid rich.

There are mutants of Chlamydomonas which lack the eyespot. They still perceive light, but do not orient themselves as well in light compared to wildtype*. So, the eyespot may function as a primitive lense, but is not the photoreceptor.

The light sensitivity is fairly broad, 450-550 nm and exhibits a peak at about 500 nm. This is at a wavelength longer than peak absorption by chlorophyll or carotenoids+.

The structure of the eyespot are disks of pigment globules: usually four layers

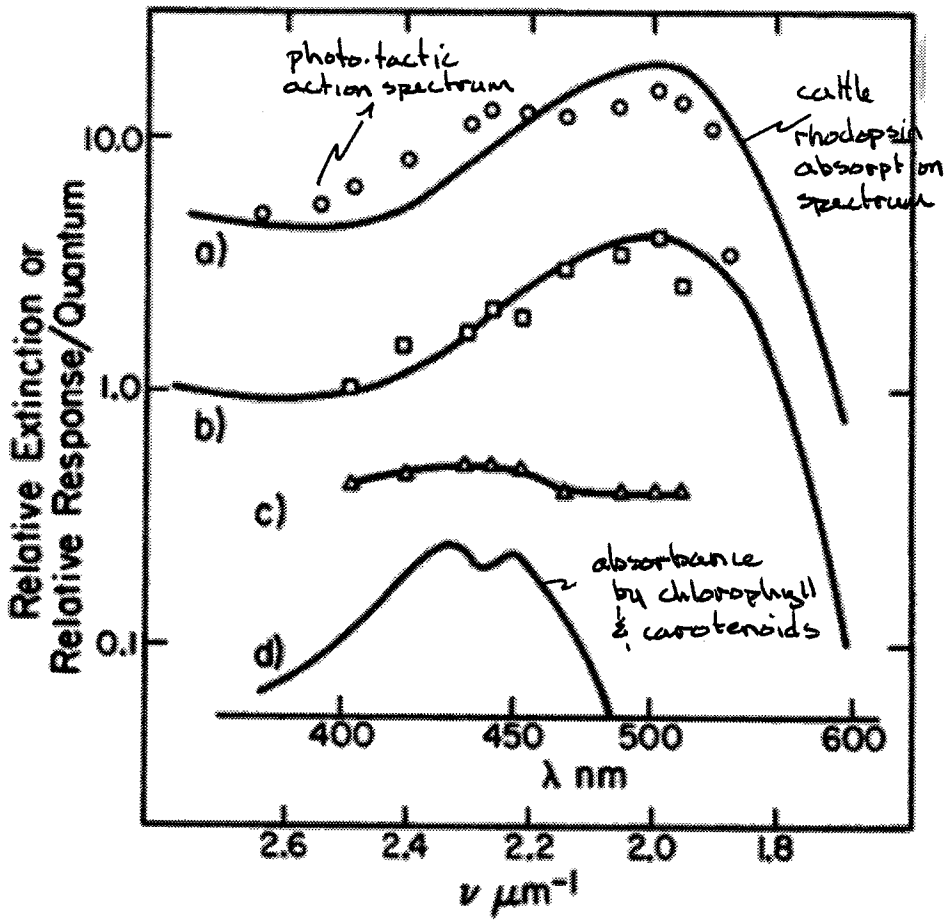


In Chlamydomonas, they are within the chloroplast, but tightly appressed against the plasma membrane.

* op. cit. Lee, RE 1980 Phycology. Cambr. Univ Press

+ Foster, KW and RD Smyth 1980 Light antennas in phototactic algae. Microbiol. Rev. 44: 572-630.

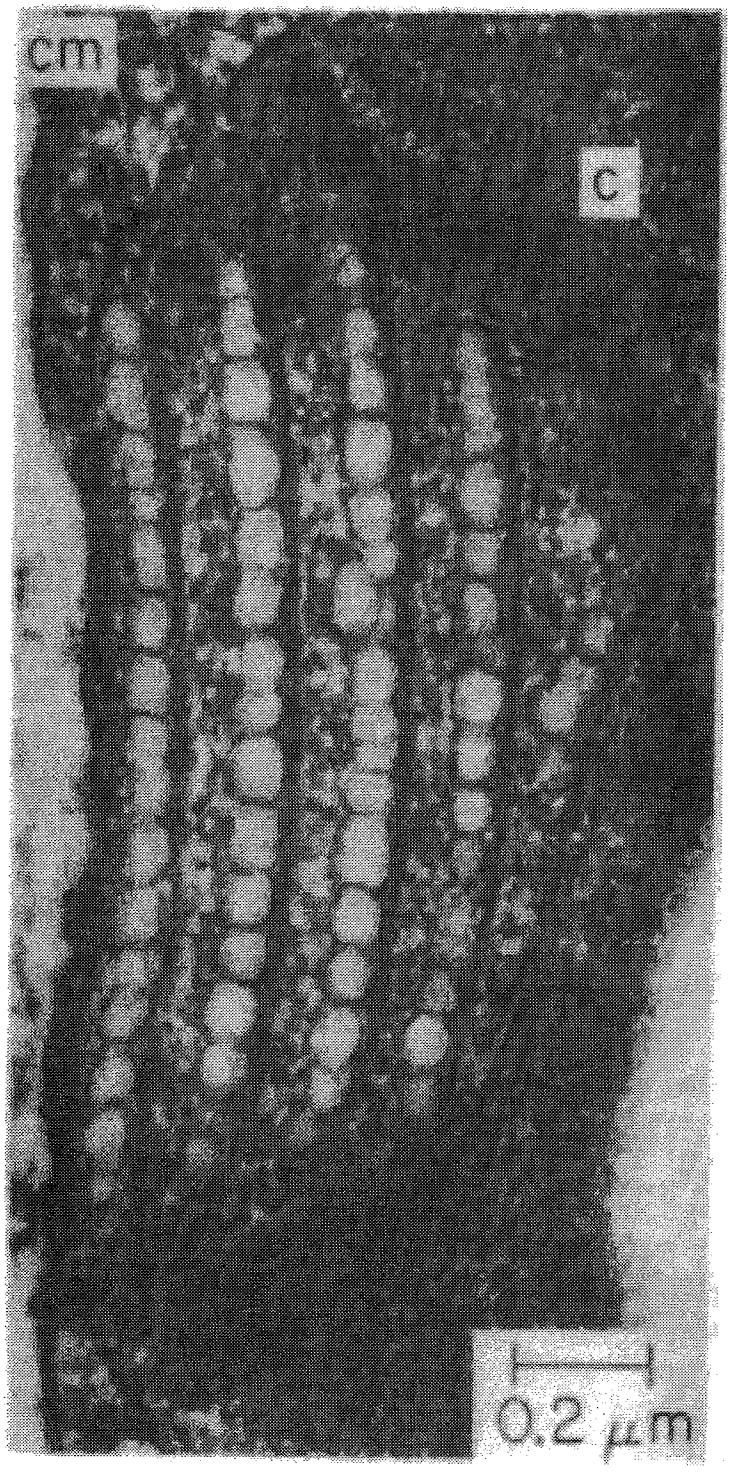
FIG. 17. Chlamydomonas spectra. (a) Action spectrum for phototactic aggregation. (b) Reciprocals of intercept intensities from linear plots of response versus log intensity. By theory these intercepts should be proportional to the absorption of the photoreceptor pigment. Solid lines (a) and (b) are absorption spectra of cattle rhodopsin (242). (c) Slope of lines obtained by plotting response versus log intensity at each wave-length. By theory the slopes should be proportional to the extinction spectrum of the screen. (d) Absorption spectrum of bulk pigments calculated from absorption spectra of chlorophyll a and b (216) and beta-carotene (240) and concentrations in cell (201). The maximum of (c) coincides with the peak absorption of the bulk pigments in (d). Chlamydomonas data are from Nultsch et al. (201).



nota bene: rhodopsin-like action spectra have a λ_{max} of 530 nm.

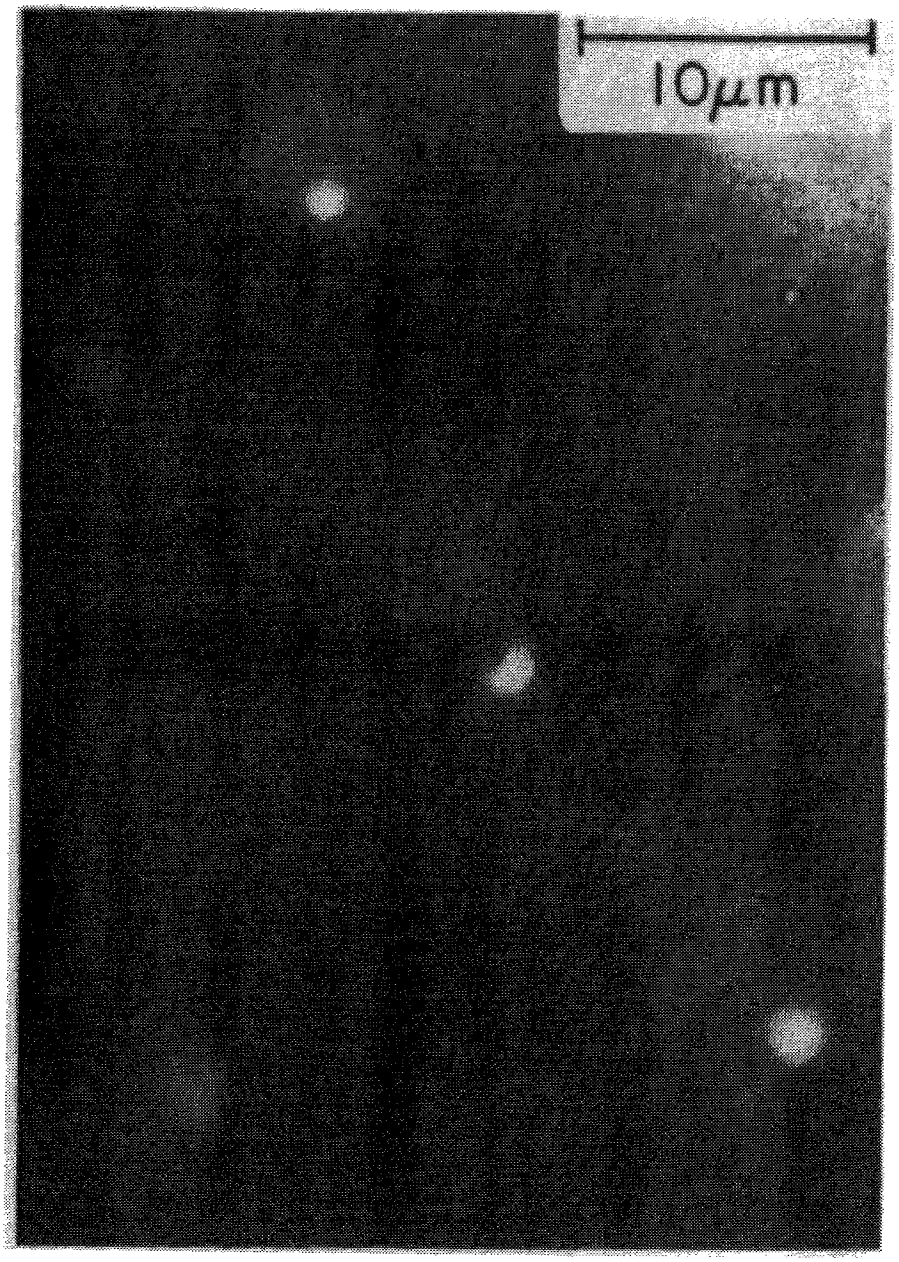
KENNETH W. FOSTER AND ROBERT D. SMYTH (1980) Light Antennas in Phototactic Algae. MICROBIOLOGICAL REVIEWS 44:572–630.

FIG. 7. Section through the eyespot of the chlorophycean Chlamydomonas reinhardtii showing the four layers of pigment globules making up the eyespot; each layer is covered on its inner face by a thylakoid double membrane. Abbreviations: cm, cell membrane; c, chloroplast. (Electron micrograph by L. Andrew Staehelin using a spray freezing technique [148].)



KENNETH W. FOSTER AND ROBERT D. SMYTH (1980) Light Antennas in Phototactic Algae. MICROBIOLOGICAL REVIEWS 44:572–630.

FIG. 6. Photograph of light reflected from the anterior surface of a colony of Volvox carteri f. weismannia taken with an epi-illuminated microscope. The bright spots (about 2.5 ,um across) are reflections from the eyespots. Each cell is visible by chloroplast fluorescence. (Photo by K. Foster and R. Birchem.)



KENNETH W. FOSTER AND ROBERT D. SMYTH (1980) Light Antennas in Phototactic Algae. MICROBIOLOGICAL REVIEWS 44:572–630.

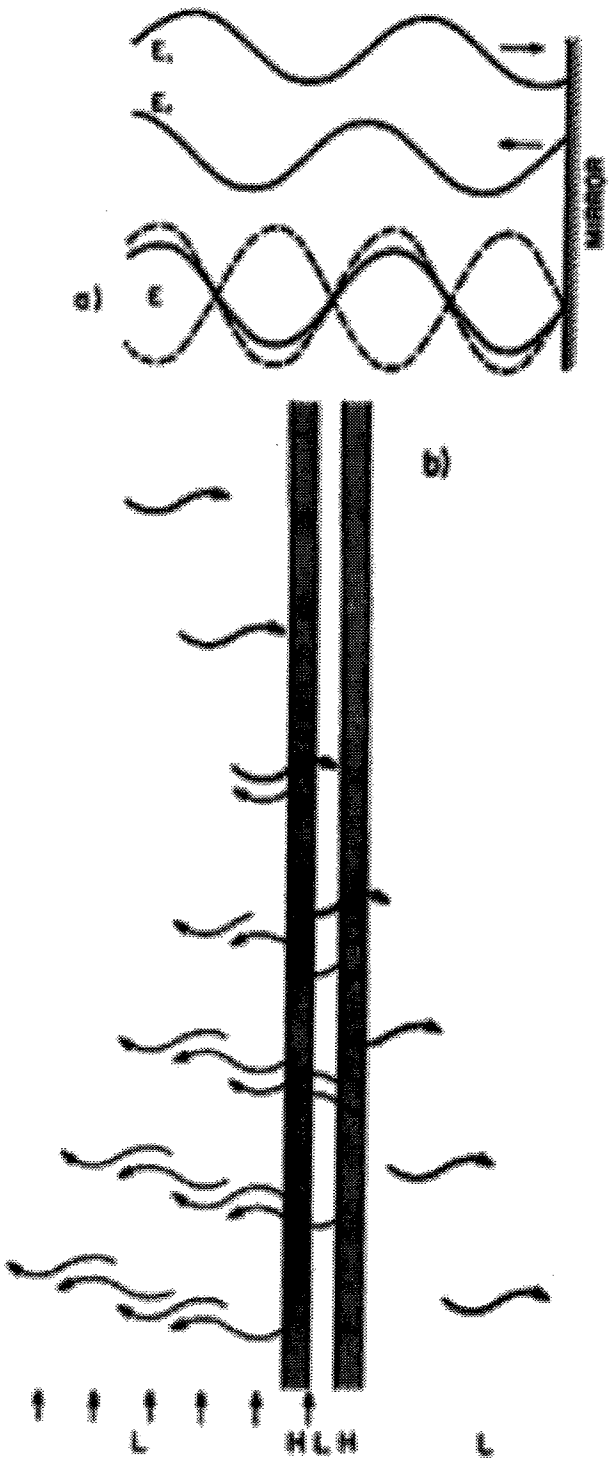
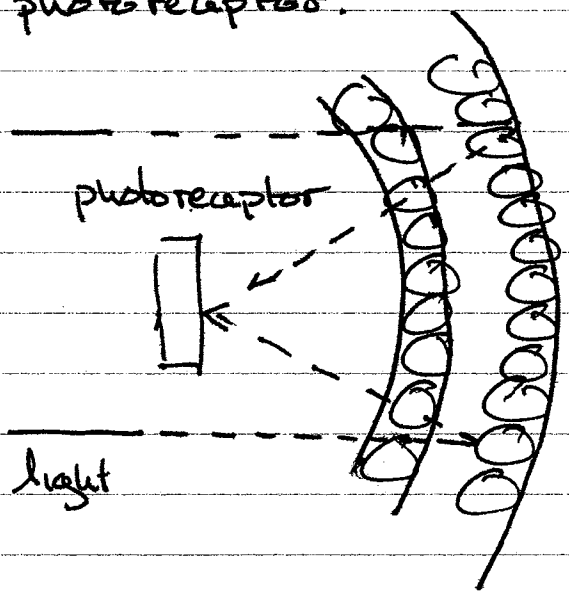


FIG. 4. Reflection. (a) Perfect mirror. Plot of incident wave, Ei, coming from the left, reflected wave, Er, going to the left, and the sum E = Ei + Er. The summed wave is a standing wave; the dashed lines show its extrema. (b) Interference reflector. Plot of a segment of a wave one wavelength long at seven successive instants of time. During the time interval between plots the wave advances half a wavelength. The wave is incident on a stack of transparent alternating high (H)- and low (L)-refractive index layers which are one-quarter of a wavelength thick. The first reflections produced by the wave segment at each of the four interfaces are shown. The front of each wave is indicated by an arrow. Vertical arrows indicate the zones of maximum intensity (electric energy density).

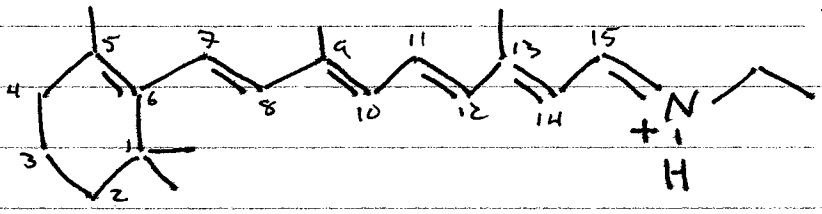
KENNETH W. FOSTER AND ROBERT D. SMYTH (1980) Light Antennas in Phototactic Algae. MICROBIOLOGICAL REVIEWS 44:572–630.

Ultrastructure, considerations of the physical aspects of light reflection suggest that the eyespot is a mirror, reflecting light onto some photoreceptor.



This gives the protist a very important advantage: information on the directional source of the impinging light.

Now, what about the photoreceptor? It's a rhodopsin-type system. Retinal-deficient Chlamydomonas mutants are "blind" lacking phototactic and photophobic responses. "Vision" is restored by supplying all-trans retinal.*

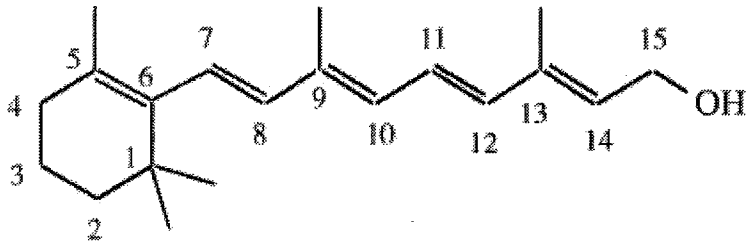


all-trans rhodopsin chromophore*.

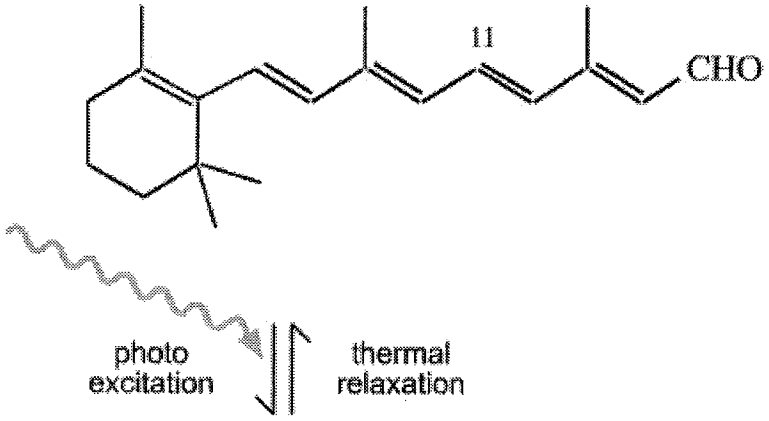
*Hegemann, P. (1997) Vision in microalgae. Planta 203: 265–274.

This is photo induced trans-to-cis isomerism exploited in the visual system where the conversion of all-trans-retinal to 11-cis-retanal is the main photon detector. With time, the 11-cis-retanal thermally relaxes back to the all-trans-retinal configuration. Retinal is derived from retinol, also known as vitamin A.

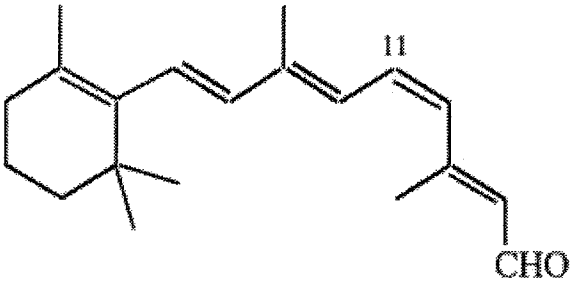
Retinol (vitamin A)



All *trans*-Retinal



11-*cis*-Retinal

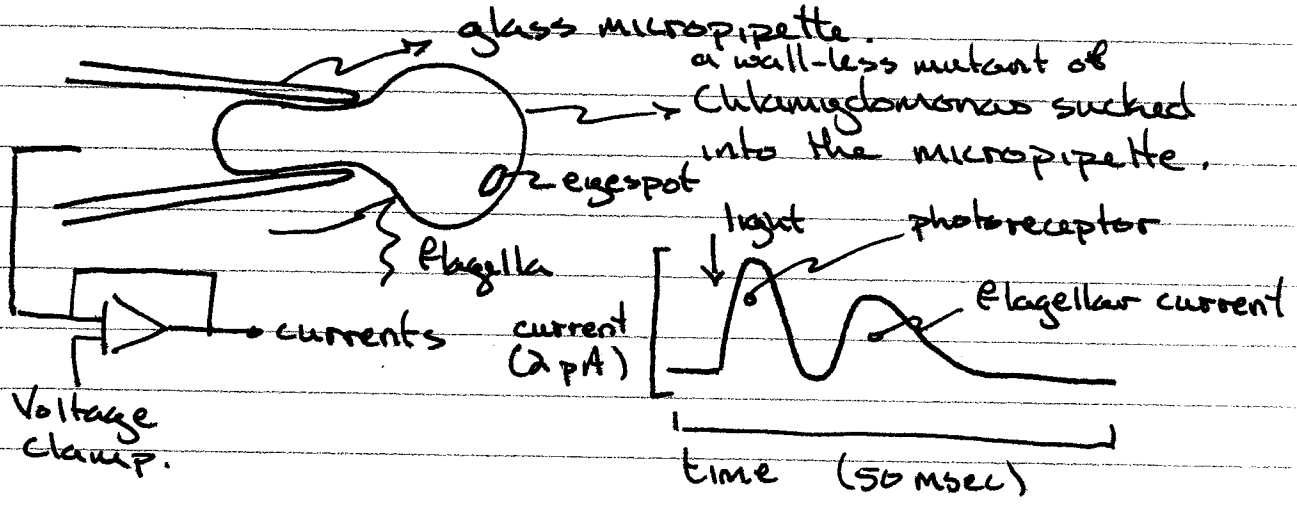


From the Chemogenesis Web Book. Photochemistry Section. By Mark R. Leach.

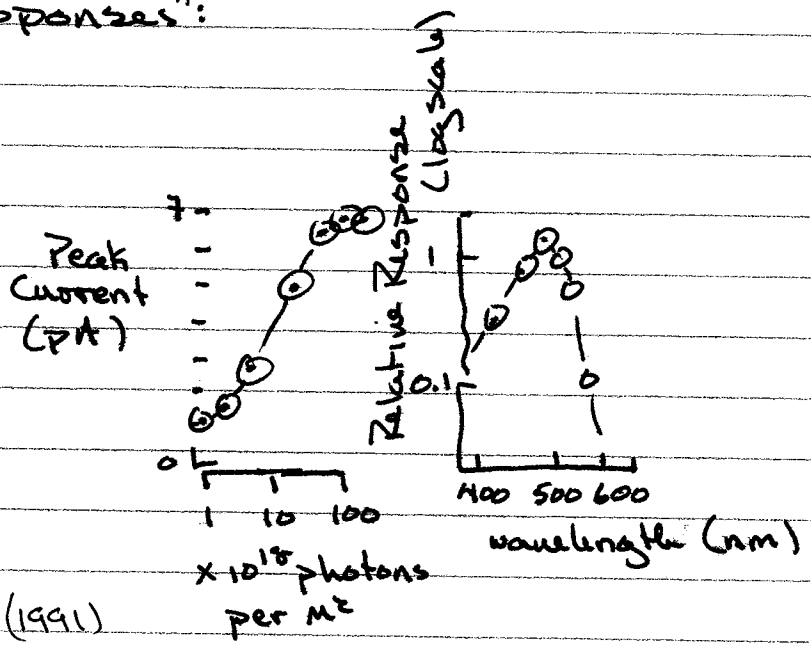
http://www.meta-synthesis.com/webbook/17_photo/photo.html

Photocurrents.

Light flashes elicit photocurrents in Chlamydomonas. These were measured using a variation of the patch-clamp technique:

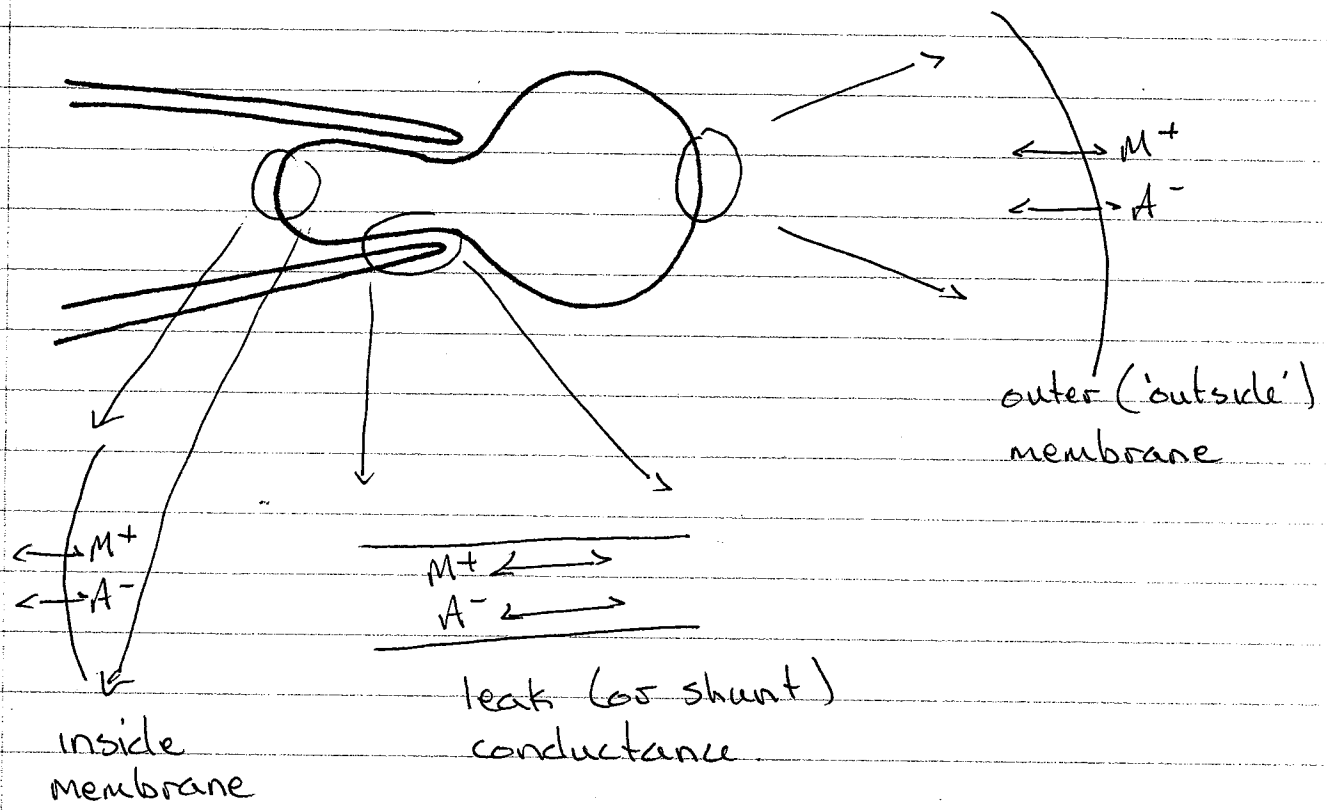


The basic result is that there are photocurrents: dependent upon light intensity, and with an action spectrum similar to phototactic and photophobic responses*:

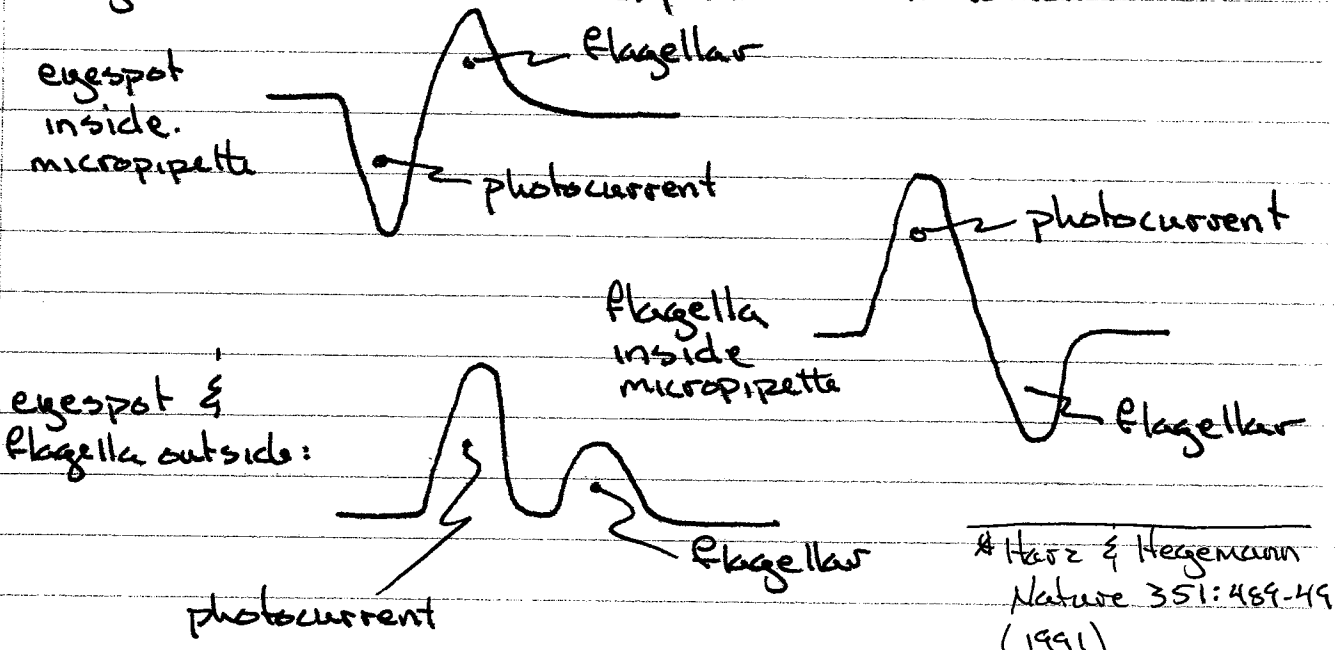


*Harz H & P Hegemann (1991) Rhodopsin-regulated calcium currents in Chlamydomonas. Nature 351: 489–491.

There are major complexities associated with the experimental protocol:



The attribution of currents to the eyespot and flagella was based on experiments in which*:



* Harz & Hegemann Nature 351: 489-491 (1991)

So, if there are photocurrents, attributed to the eyespot and the flagella, what ion(s) cause them?

To determine this, Harz & Hegemann examined the effect of removing Ca^{2+} from the external medium: In the configuration where both the eyespot and flagella were outside the micropipette, removal of Ca^{2+} caused the disappearance of both eyespot & flagellar photocurrents*.

Thus, a rhodopsin-mediated system relying upon Ca^{2+}-influx from the external medium to elicit the phototactic response.

The next step was to identify the transport mechanisms mediating ion influx and how they were regulated by light.

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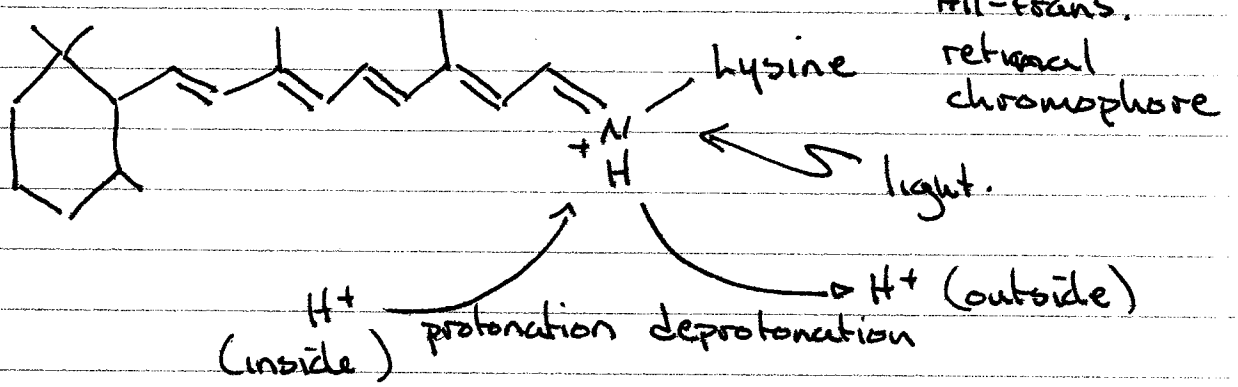
*Harz H & P Hegemann 1991 Rhodopsin-regulated calcium currents in Chlamydomonas. Nature 351: 489-491.

Digression: Bacteriorhodopsin.

Halobacterium halobium is a halo-phile (salt-loving) prokaryote which lives in saturated brines. It contains a purple membrane, isolated in sheets, which is comprised of only one protein species. The purple color is due to retinal, covalently linked to the protein. Thus, as with the retinal-containing protein rhodopsin, it was one of a family of proteins which 'sensed' light.

As it turns out, bacteriorhodopsin was a light-driven proton pump. The proton-pumping involved a light-mediated protonation/deprotonation of the Schiff base:



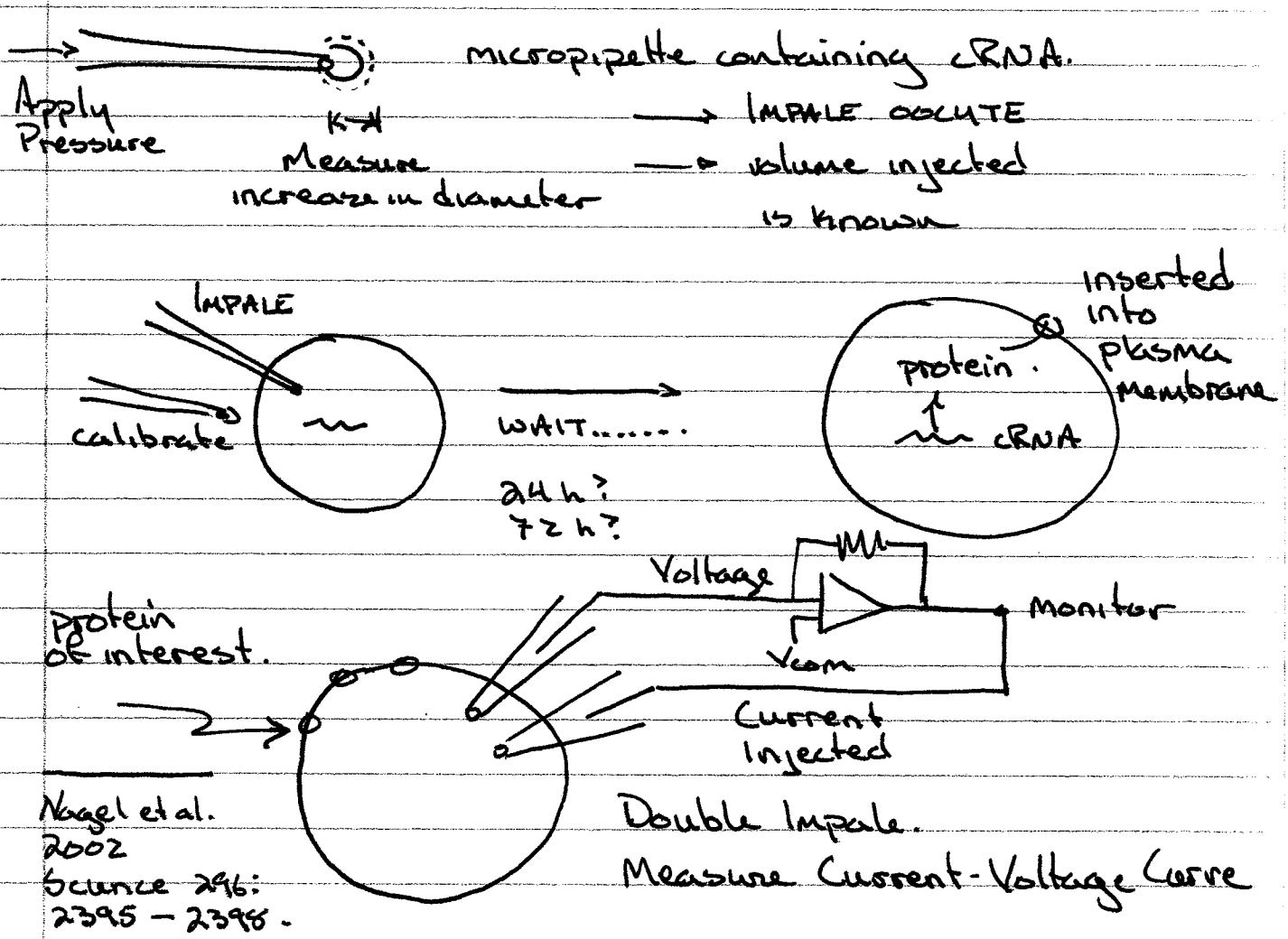
This became important, because a sequence from Chlamydomonas exhibited sequence similarity to the sequence in bacteriorhodopsin adjacent to the retinal-binding lysine.

Lauger P. 1991 Electrogenic Ion Pumps. Sinauer Associates, Sunderland MA. pp. 139–162

The identification of channelrhodopsin-1 began with a sequence similarity search of expressed sequence tag (EST) sequences to identify sequences related to bacterial rhodopsins, including bacteriorhodopsin.

The candidate sequence ('Chop-1') had seven hypothetical transmembrane sequences and a conserved region very similar to the retinal-binding sequence of bacteriorhodopsin.

To demonstrate the function of Chop-1, the cRNA was injected into Xenopus laevis oocytes in the presence of all trans retinal



Nagel et al.
2002
Science 296:
2395 – 2398.

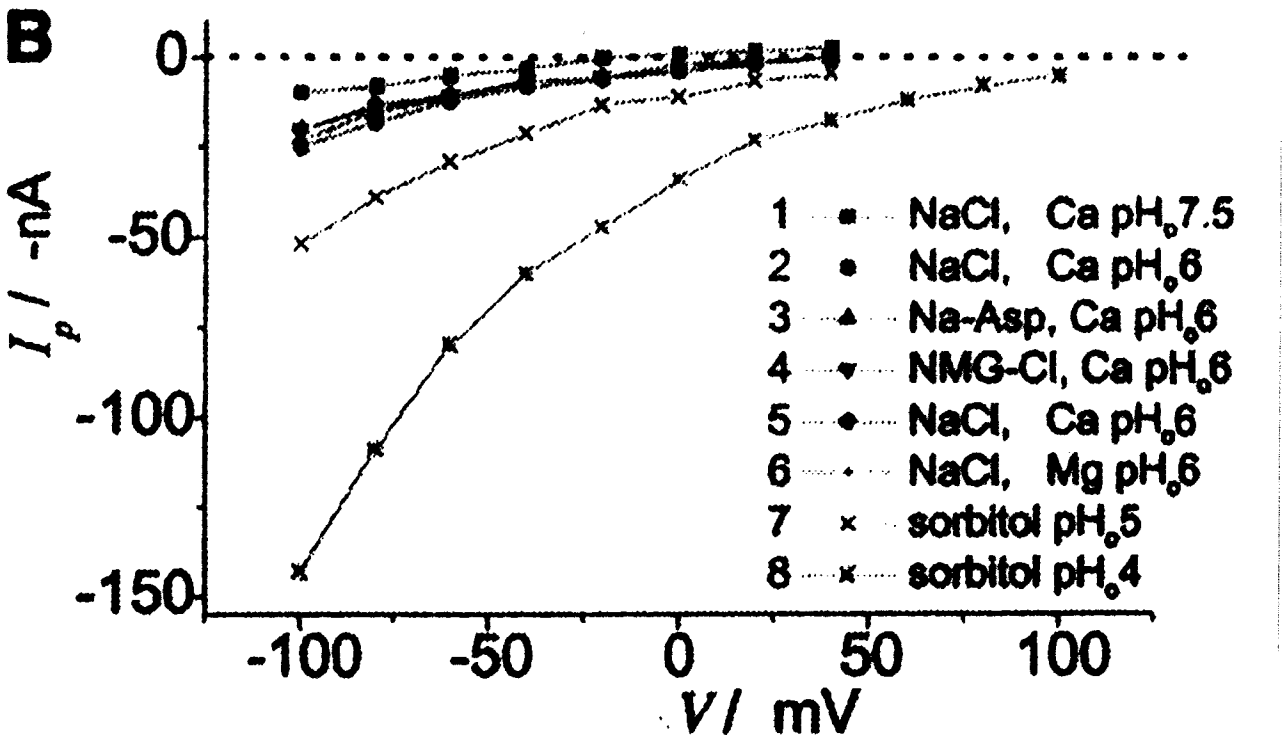
After expression of Chop1 (channelrhodopsin 1) in Xenopus oocytes, Nagel et al.* observed a photo-activated current. As a control, mock-injected oocytes lacked the photo-activated current. Green light was effective, red light was not.

To characterize the current, the oocyte was bathed with different solutions to distinguish between Na^+, Cl^-, Ca^{2+}, Mg^{2+} & H^+ flux through the channel. Maximal inward currents were observed at high $[H^+]_{external}$. They concluded that the channel is H^+-selective: the E_{H^+} predicted the reversal potential of the channel. The well-known bacteriorhodopsin is also H^+-selective (a H^+ pump) so this made some evolutionary sense.

The wavelength dependence of photo-activated current was similar to the wavelength dependence of the phototactic response, evidence the channel did play a role in the phototactic responses of Chlamydomonas.

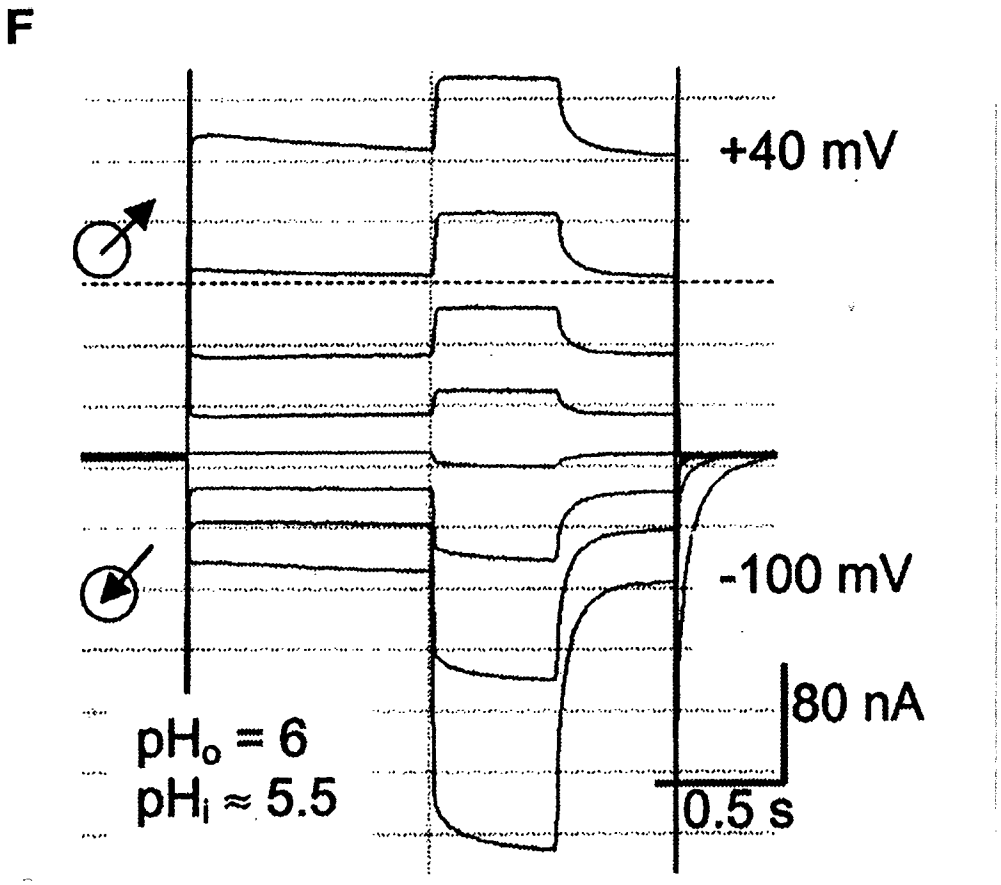
Nagel G, D. Ollig, M. Fuhrmann, S Kateriya, AM Musti, E Bamberg, & P Hegemann 2002 Channelrhodopsin-1: A light-gated proton channel in green algae. Science 296: 2395-2398

Voltage dependence of photocurrents. Solutions were buffered with 5 mM MOPS (pH = 7.5), MES (pH = 6), or citrate (pH = 5 and 4). Concentrations (in mM): **1** 100 NaCl, 2 $CaCl_2$, (pH = 7.5); **2** 100 NaCl, 2 $CaCl_2$, (pH = 6.0); **3** 100 Na-aspartate, 2 $CaCl_2$, (pH = 6.0); **4** 100 NMG-Cl, 2 $CaCl_2$, (pH = 6.0); **5** 100 NaCl, 2 $CaCl_2$, (pH = 6.0); **6** 100 NaCl, 2 EGTA, 2 $MgCl_2$, (pH = 6.0); **7** 200 sorbitol, 5 EGTA (pH = 5.0); **8** 200 sorbitol, 5 EGTA (pH = 4.0).



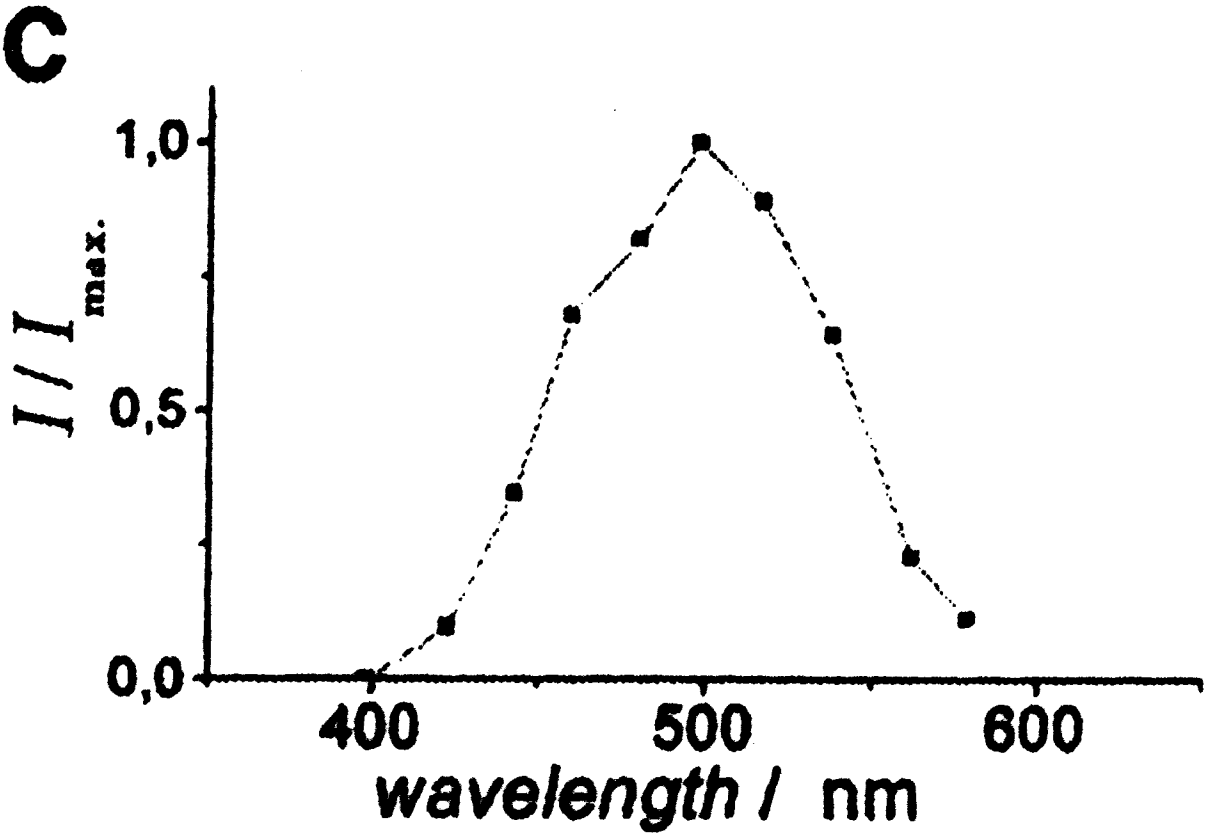
Nagel, G, D Ollig, M Fuhrmann, S Kateriya, AM Musti, E Bamberg and P Hegemann (2002) Channelrhodopsin-1: A light-gated proton channel in green algae. Science 296:2395–2398.

Current responses to voltage steps from V = –100 mV to +40 mV, followed by green light pulses. Bath solution: 100 mM Na-acetate, 5 mM Mes (pH adjusted to 6.0 with NaOH).



Nagel, G, D Ollig, M Fuhrmann, S Kateriya, AM Musti, E Bamberg and P Hegemann (2002) Channelrhodopsin-1: A light-gated proton channel in green algae. Science 296:2395–2398.

Wavelength dependence of the light-induced inward current at pH_o = 5.5 and –40 mV. The photocurrents were corrected for equal photon flux.



Nagel, G, D Ollig, M Fuhrmann, S Kateriya, AM Musti, E Bamberg and P Hegemann (2002) Channelrhodopsin-1: A light-gated proton channel in green algae. Science 296:2395–2398.

Subsequent to the discovery of the light-activated proton channel channelrhodopsin 1, the same research group (Nagel et al.*) identified a second photo-activated channel.

The same basic technique — heterologous expression of the poly-A mRNA in xenopus oocytes, followed by voltage-clamp — was used, as was the same basic technique — modification of the ionic species in the external solution — to characterize ion specificity of the channel.

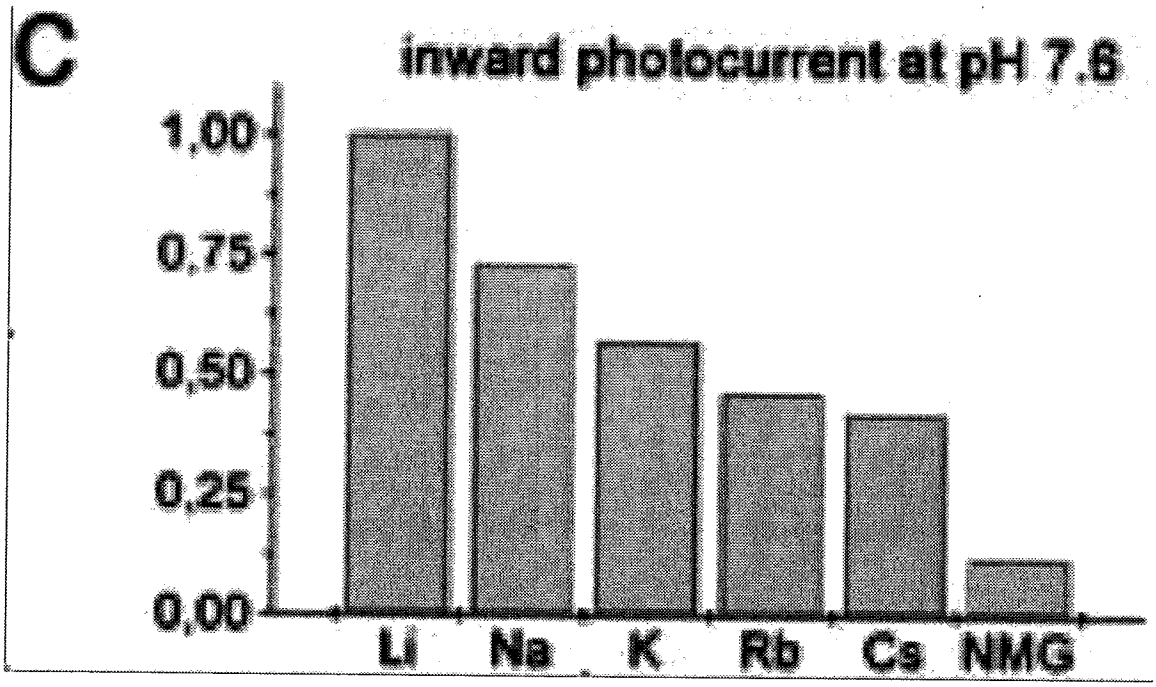
The channel exhibited a high permeability to H^+, but also conducted other cations:

	P_x / P_{Na}
H^+	$\sim 10^6$
guanidinium$^+$	13 ± 5
$CH_3NH_3^+$ (methylammonium)	6 ± 2
$(CH_3)_2NH_2^+$ (dimethylammonium)	4 ± 2
Li^+	2 ± 0.5
Na^+	1
K^+	0.5 ± 0.3
TMA^+ (tetramethylammonium)	< 0.06

nb. Anion insensitivity was demonstrated by replacing Cl^- with aspartate (which has no effect on current).

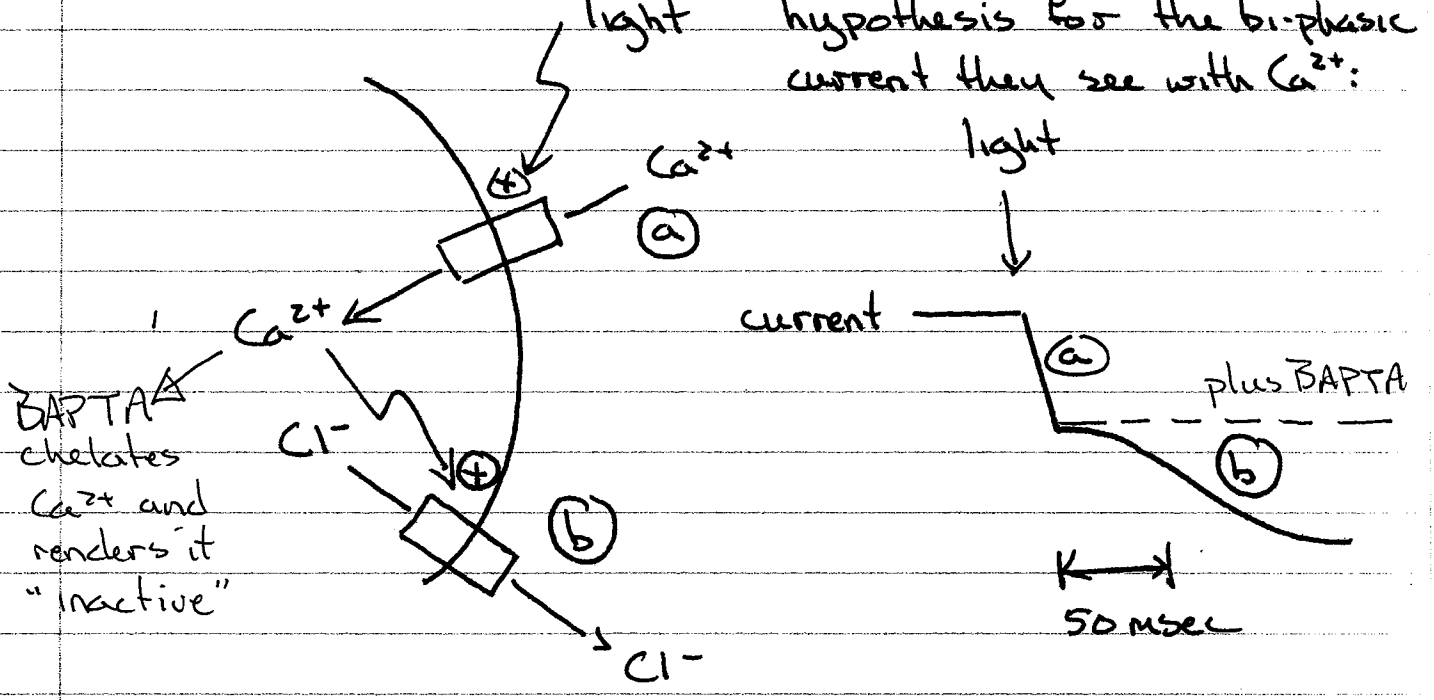
Nagel G, T Szellas, W Huhn, S Kateriya, N Adeishvili, P Berthold, D Ollig, P Hegemann & E Bamberg (2003) Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. Proc. Natl. Acad. Sci USA 100: 13940-13945.

(c) Normalized inward photocurrents at mM salt solutions of: LiCl, NaCl, KCl, RbCl, CsCl,and NMG-Cl, measured in the same oocyte. Currents are typical of those in four other experiments.



Georg Nagel, Tanjef Szellas, Wolfram Huhn, Suneel Kateriya, Nona Adeishvili, Peter Berthold, Doris Ollig, Peter Hegemann, and Ernst Bamberg (2003) Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. Proc. Natl. Acad. Sci. *USA* 100:13940–13945.

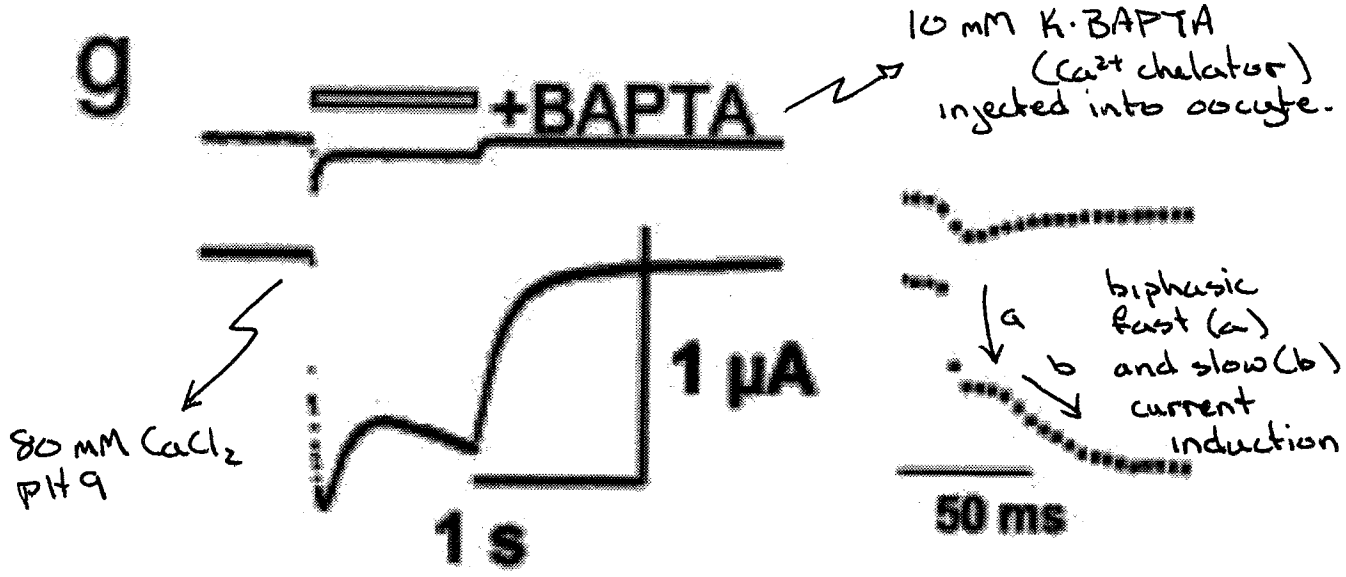
The channelrhodopsin-2 is also permeant to divalent cations ($Ca^{2+} > Sr^{2+} > Ba^{2+} >> Zn^{2+}$, Mg^{2+} (ca. 0)) but there is a complication with respect to Ca^{2+}, the most relevant divalent from a physiological point of view because of its role as a second messenger in the cytoplasm, and prevalence in the aqueous environment. They propose a working hypothesis for the bi-phasic current they see with Ca^{2+}:



and support the hypothesis with an experiment injecting the Ca^{2+}-chelator BAPTA into the cell, to minimize any increase in cytoplasmic Ca^{2+}: the effect is inhibition of the slow current.

Nagel et al. (2003) Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. Proc. Natl. Acad. Sci. USA 100: 13940–13945.

(g) Photocurrent in 80 mM $CaCl_2$, pH 9 at –100 mV (*Lower and Inset*, higher time resolution). Afterward, the oocyte was injected with 1,2-bis(2-aminophenoxy)ethane-N,N,N'-tetraacetate (BAPTA)(as K-salt) to a final concentration of 10 mM, and photocurrent was determined again (*Upper and Inset*). Currents are typical of those in four other experiments[1].



Georg Nagel, Tanjef Szellas, Wolfram Huhn, Suneel Kateriya, Nona Adeishvili, Peter Berthold, Doris Ollig, Peter Hegemann, and Ernst Bamberg (2003) Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. Proc. Natl. Acad. Sci. *USA* 100:13940–13945.

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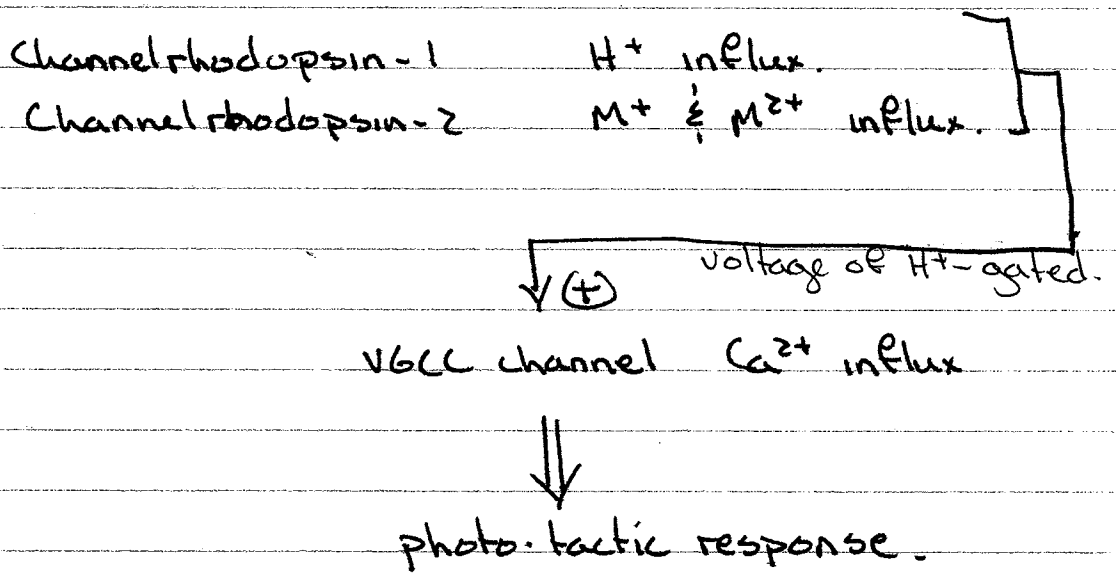
[1] The relative permeability of ChR2, as estimated from inward currents at –100 mV and 80 mM divalent cation, follows the sequence $Ca^{2+} > Sr^{2+} > Ba^{2+} >> Zn^{2+}, Mg^{2+}$ (*ca.* 0)

A recent model of algal 'vision' is presented by Nagel et al. (2004)* in which they discuss additional channels acting in the immediate photo-activated current response.

A very complex system clearly related to very archaic light-driven pumps in bacteria (bacteriorhodopsin of Halobacterium halobium)

There is little correspondendence to the signalling mechanism in animal vision (G-protein mediated).



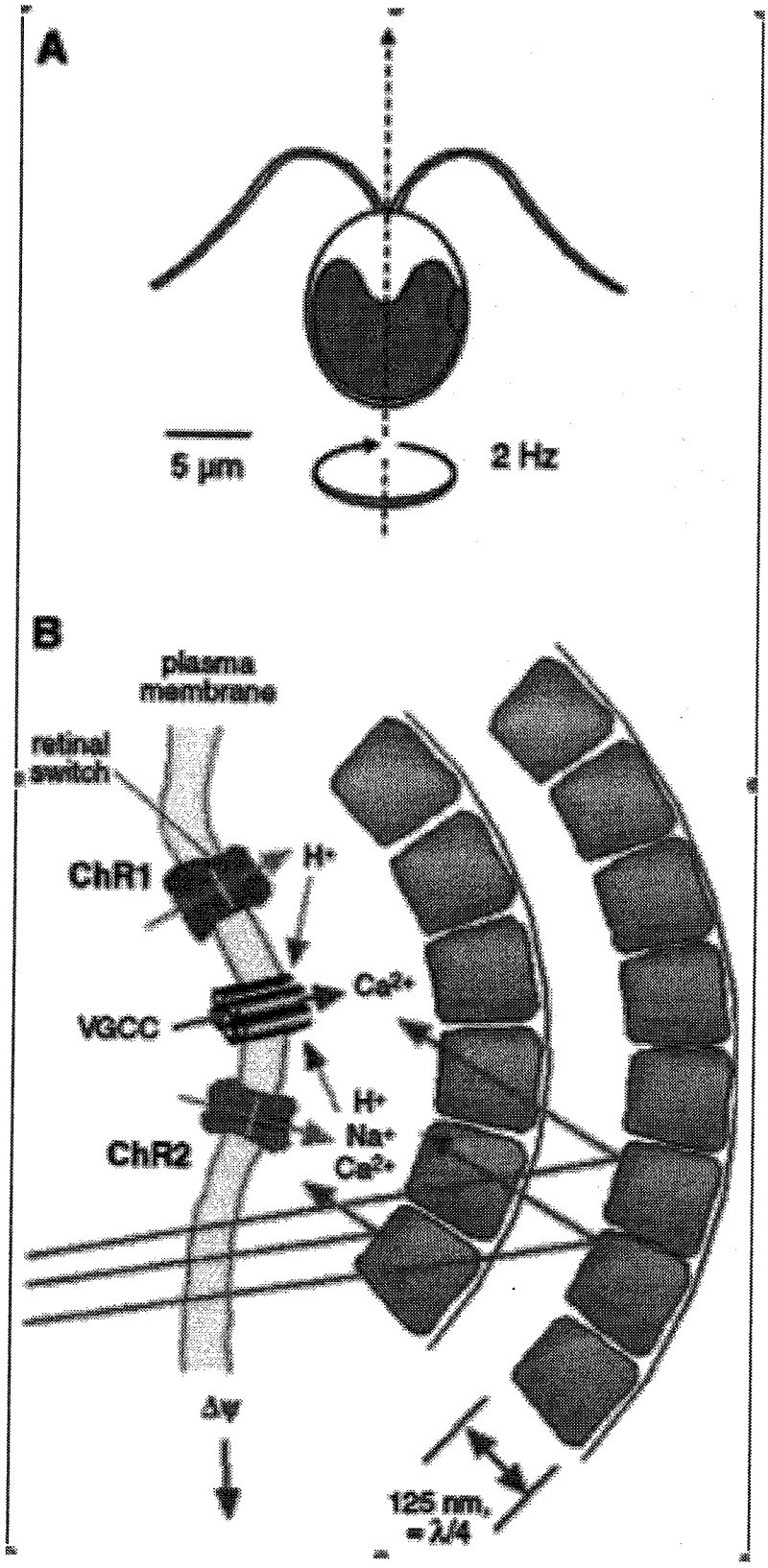


FIGURE 1. A: a Chlamydomonas cell with two flagella, a large chloroplast (green), and the yellow/orange eyespot. B: eye function under consideration of channelrhodopsin 1 (ChR1), channelrhodopsin 2 (ChR2), and a voltage- or H^+-gated Ca^{2+}channel (VGCC). The voltage change, the membrane and sensed by VGCCs in the flagellar membrane.

"Vision" in Single-Celled Algae. Suneel Kateriya, Georg Nagel, Ernst Bamberg, and Peter Hegemann. News Physiol Sci 19:133–137 [2004]

Photo-activated channels: The nano-biotechnological connection.

It may be possible to heterologously express one of the channelrhodopsins in a transgenic. For example mouse or some other model organism. The advantage is that light can be used, as required, to trigger channel activity in selected tissues or organs.

But, rather than using the Chlamydomonas channel, it is also possible to engineer other ion channels to be 'light-gated' by taking advantage of photo-isomerizations between cis and trans configurations*.

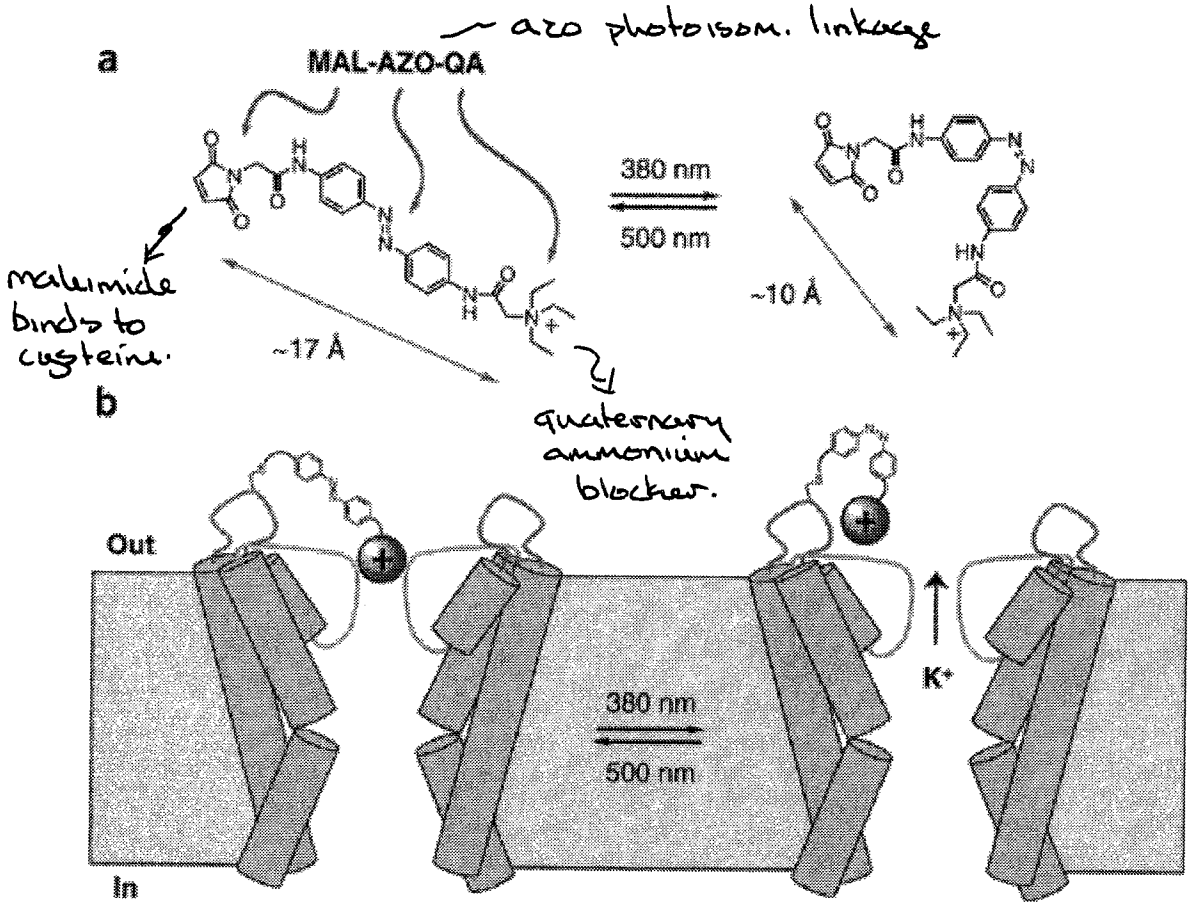
The shaker K^+ channel was the starting point. An amino acid, Glu422, is known to be about 15-18 Å from the pore, specifically a quaternary ammonium binding site that blocks channel activity. A cys was substituted for the glu.

MAh-AZO-QA

- maleimide to bind to the cysteine
- photoisomerizable linkage group.
- quaternary ammonium to block channel activity.

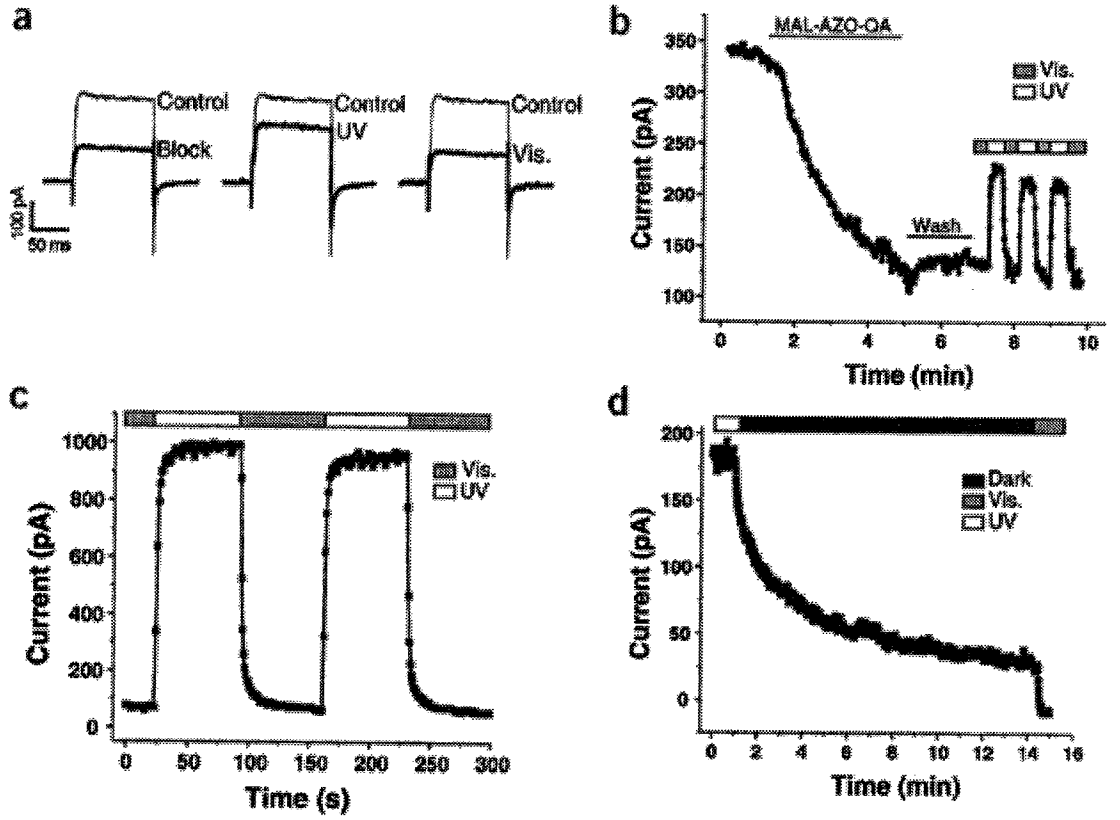
Banghart M, K Borges, E Isacoff, D Trauner & RH Kramer (2004) Light-activated ion channels for remote control of neuronal firing. Nature Neuroscience 7: 1381-1386.

Figure 1. Photoisomerization of MAL-AZ)-QA gates ionic currents through modified Shaker channels. **(a)** The rigid core of MAL-AZO-QA (between the alpha carbons flanking the azo moiety) changes by about 7Å upon isomerization. **(b)** MAL-AZO-QA blocks ion flow in the *trans* configuration but is too short to block effectively after photoisomerization to the *cis* configuration. Diagram shows a model of the inner helices of the Shaker K^+ channel, derived from the crystal structure of the bacterial K^+ channel MthK, with the dimensions of MAL-AZO-QA drawn roughly to scale.



Banghart M, Borges K, Isacoff E, Trauner D, Kramer RH. (2004) Light-activated ion channels for remote control of neuronal firing. Nat Neurosci. 2004 7:1381-1386.

Figure 2. Photocontrol of MAL-AZO-QA-modified Shaker channels in X. laevis oocytes. (**a**) Raw Shaker K^+ current traces recorded from an outside-out patch before and after treatment with MAL-AZO-QA. The top trace in each panel shows the current before MAL-AZO-QA application. Bottom traces represent current after 4-min application of 10 muM MAL-AZO-QA and 2-min washout (left trace), after 1-min exposure to ultraviolet (UV; 380 nm) light (middle trace) and after 1-min exposure to visible (Vis.; 500 nm) light (right trace). The patch was held at -90 mV and currents were elicited by 100-ms steps to -20 mV at 1 Hz. (**b**) K^+ current amplitudes from the same outside-out patch during perfusion with MAL-AZO-QA, during washout, and during alternating illumination with 380 and 500 nm light. (**c**) Inside-out patch from an oocyte treated with 100 muM MAL-AZO-QA for 30 min. The patch shows a large Shaker current in 380 nm light and almost complete block in 500 nm light. Pulse protocol same as above, except pulse duration was 30 ms. (**d**) Current block in the dark follows a biexponential time course with $tau_1 = 0.49$ min and $tau_2 = 4.79$ min.



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Proof of light regulation came from experiments in which the

- modified shaker K^+ channel expressed in oocytes.
- treatment with MAh-A20-QA.
- appearance of light regulation:

  UV: Activates

  Vis: Blocks.

The action spectra identified well-defined wavelengths (375 nm: Activates & 500 nm: Blocks) with no 'leakage'.

The final 'proof-of-concept' was expression in hippocampal pyramidal neurons. In which 500 nm & 390 nm light could be used to induce or silence AP trains, respectively.

Biotech Applications? Certainly an interesting research tool. Possibly a light-sensor. otherwise, enjoyable meddling with ~~&~~ nature....

Figure 3. Absorbance and action spectra of MAL-AZO-QA. (**a**) The ultraviolet and visible light spectrum of a MAL-AZO-QA-glutathione adduct (10 muM) in oocyte bath solution. To maximize the trans and cis isomers, the solution was exposed to visible and ultraviolet light, respectively, for 3 min. To generate the adduct, MAL-AZO-QA (1 M) was treated with reduced glutathione (1.5 M) for 12 h at 21 °C. (**b**) Unblocking of Shaker channels at different wavelengths. Currents are from an inside-out patch alternately exposed to various wavelengths between 300 and 480 nm to unblock the channels, and to 500 nm light to reblock the channels. (**c**) Reblocking of Shaker channels at different wavelengths. The time course of blocking at various wavelengths of visible light. Each trial is preceded by 1-min irradiation at 380 nm to unblock the channels. Traces are superimposed for comparison. Normalized current amplitudes were measured at 0.2 min after onset of blocking. (**d**) Action spectra for unblocking (left curve) and blocking (right curve) of Shaker K+ channels. Unblock (left axis): Current unblocked at each wavelength divided by current at 380 nm (n = 3-8 patches per wavelength). Currents were compared within each patch. Block (right axis): Fraction of normalized current blocked at 0.2 min after illumination with visible light (n = 2-7 patches per wavelength).

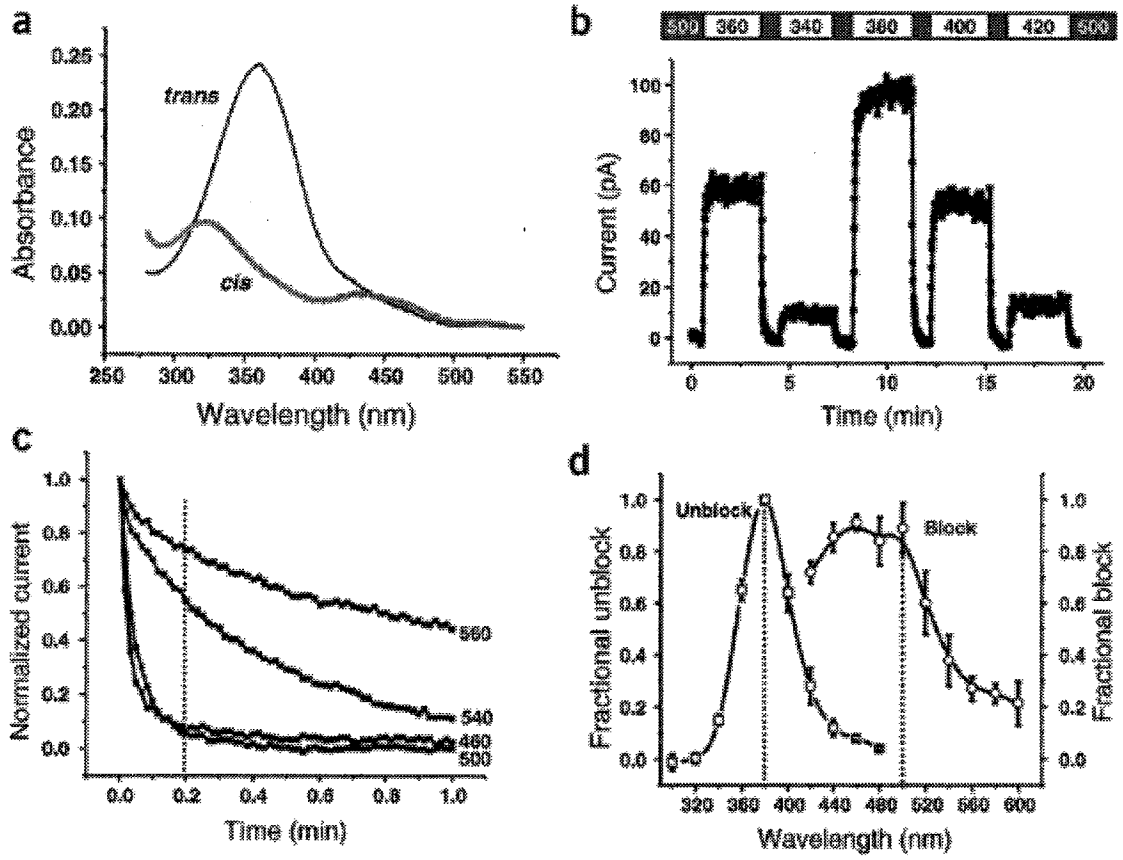

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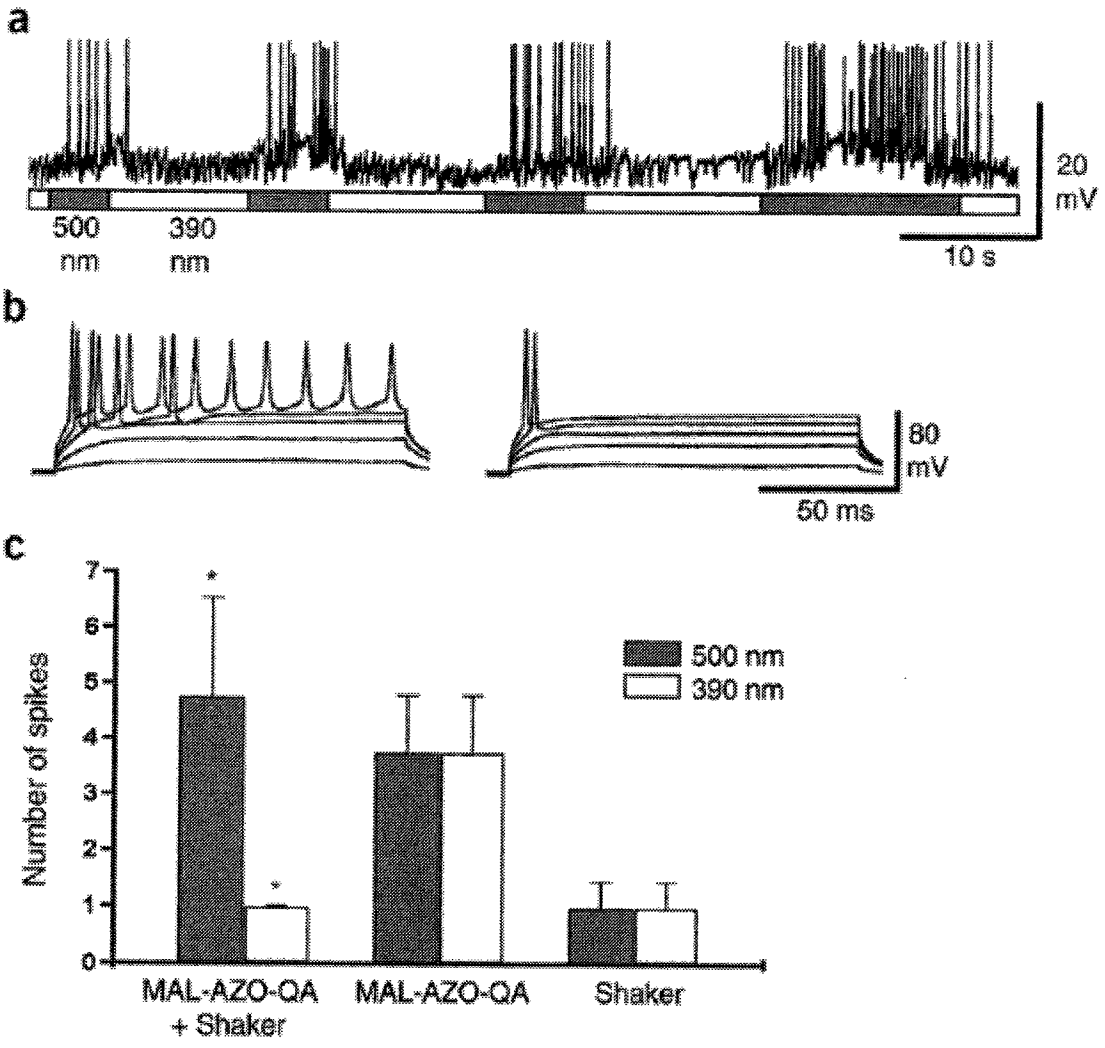
Figure 4. Expression of light-activated channels confers light sensitivity on hippocampal pyramidal neurons. (**a**) Spontaneous action potentials are silenced and revived by exposure to 390- and 500-nm light, respectively. A neuron that was transfected with the multiply mutated Shaker channel was treated for 15 min with MAL-AZO-QA before recording. The frequency of spontaneous synaptic potentials generated by untransfected presynaptic neurons is not affected by light. (**b**) Depolarizing current steps elicit repetitive firing in 500-nm light (left) but only single action potentials in 390-nm light (right). Neurons were held under current clamp at about -55 mV and were depolarized to about -15 mV. (**c**) Summary of repetitive firing data. Number of spikes resulting from a suprathreshold depolarization to -15 mV is significantly modulated by light in the multiply mutated Shaker-transfected neurons treated with MAL-AZO-QA (* $P < 0.01$). Neurons expressing the channel without MAL-AZO-QA treatment or treated with MAL-AZO-QA without channel expression were unaffected by light.



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