# Light-activated Ion Channels in Solitary Photoreceptors of the Scallop *Pecten irradians*

ENRICO NASI and MARIA DEL PILAR GOMEZ

From the Department of Physiology, Boston University School of Medicine, Boston, Massachusetts 02118; and Marine Biological Laboratory, Woods Hole, Massachusetts 02543

ABSTRACT Retinas from the scallop *Pecten irradians* were enzymatically dispersed, yielding a large number of isolated photoreceptors suitable for tight-seal recording. Whole-cell voltage clamp measurements demonstrated that the phototransducing machinery remained intact: quantum bumps could be elicited by dim illumination, while brighter flashes produced larger, smooth photocurrents. Single-channel currents specifically activated by light were recorded in cell-attached patches, and were almost exclusively confined to the rhabdomeric region. Their density is sufficiently high to account for the macroscopic photoresponse. Channel activation is graded with stimulus intensity in a range comparable to that of the whole-cell response, and can be recorded with illumination sufficiently dim to evoke only quantum bumps. Light-dependent channel openings are very brief, on average 1 ms or less at 20–22°C, apparently not because of blockage by extracellular divalent cations. The mean open time does not change substantially with stimulus intensity. In particular, since dwell times are in the millisecond range even with the dimmest lights, the channel closing rate does not appear to be the rate-limiting step for the decay kinetics of discrete waves. The latency of the first opening after light onset is inversely related to light intensity, and the envelope of channel activity resembles the time course of the whole-cell photocurrent. Unitary currents are inward at resting potential, and have a reversal voltage similar to that of the macroscopic light response. Voltage modulates the activity of light-sensitive channels by increasing the opening rate and also by lengthening the mean open times as the patch is depolarized. The unitary conductance of the predominant class of events is ~48 pS, but at least one additional category of smaller-amplitude openings was observed. The relative incidence of large and small events does not appear to be related in a simple way to the state of adaptation of the cell.

## INTRODUCTION

It has been established that gated ion channels underlie the generation of the light response in both vertebrate and invertebrate photoreceptors (Bacigalupo and Lisman, 1983; Matthews, 1987). While in amphibian rods and cones unitary currents

Address reprint requests to Dr. Enrico Nasi, Department of Physiology, Boston University School of Medicine, 80 E. Concord St., Boston, MA 02118.

J. GEN. PHYSIOL. © The Rockefeller University Press · 0022-1295/92/05/0747/23 $2.00
Volume 99 May 1992 747–769

can only be resolved in the absence of extracellular divalent cations (Haynes, Kay, and Yau, 1986; Zimmerman and Baylor, 1986; Matthews, 1987), the transduction channels of invertebrate visual cells can be examined under physiological conditions. However, rapid progress toward a detailed characterization of their properties has been hindered in part by the technical difficulties involved in obtaining glia-free cells suitable for patch clamp recording from the standard invertebrate eye preparations (Bacigalupo and Lisman, 1983). We have circumvented some of these problems by enzymatically isolating visual cells from the eye of the scallop. One of the remarkable features of its retina is the presence of two separate layers of photoreceptors (Dakin, 1910). The proximal layer is composed of rhabdomeric cells that depolarize in response to light (McReynolds and Gorman, 1970*a*), while cells in the distal retina possess modified ciliary appendages (Miller, 1958; Barber, Evans, and Land, 1967) and produce hyperpolarizing photoresponses (McReynolds and Gorman, 1970*a*). In both cases a light-induced increase in membrane conductance has been demonstrated (McReynolds and Gorman, 1970*b*), but with markedly different ion selectivity (Gorman and McReynolds, 1978). The apparent lack of interneurones and synaptic interconnections (Barber et al., 1967) indicates that these responses are true receptor potentials by primary sensory cells, making this organism particularly interesting for vision research. In this report we examine light-dependent, single-channel currents recorded from cell-attached patches in isolated photoreceptors of the rhabdomeric type. The properties of these channels closely resemble those of the cell's macroscopic light response. Patch clamp measurements in hyperpolarizing ciliary cells (Gomez and Nasi, 1991) will be described in a separate article.

Preliminary reports of these results have appeared previously (Nasi and Gomez, 1990, 1991).

## METHODS

### *Isolation of Photoreceptor Cells*

*Pecten irradians* were obtained from the Marine Biological Laboratory (Woods Hole, MA). A piece of mantle containing several eyes was pinned to a Sylgard-coated Petri dish on the stage of a stereomicroscope illuminated with dim red light ($\lambda > 650$ nm). A circular incision was made around the edge of the eye to remove the cornea, the lens, and the pigmented mantle. The exposed retina was carefully lifted from the argentea layer and severed from the optic nerve. Retinas were incubated in either Pronase (750–800 P.U.K./ml; Calbiochem Corp., La Jolla, CA) for 40–45 min at 22°C or papain (20 units/ml, preactivated with 0.6 mg/ml L-cystein; Sigma Chemical Co., St. Louis, MO) for 30–35 min. After washing in sea water supplemented with 3% fetal calf serum, they were gently triturated with a Pasteur pipette that had been fire-polished to an orifice diameter of 0.5 mm. An aliquot of the resulting cell suspension was transferred to the recording chamber. To facilitate cell adhesion, the chamber was pretreated with concanavalin A (5 mg/ml in 1 M NaCl) for 2 h, followed by washing with distilled water (Bader, MacLeish, and Schwartz, 1979). After plating, cells were continuously superfused at a rate of ~1 ml/min. All recordings were performed in artificial sea water (ASW) containing 480 mM NaCl, 10 mM KCl, 50 mM $MgCl_2$, 10 mM $CaCl_2$, 10 mM HEPES, and 5.5 mM D-glucose, pH 7.8.

*Recording and Stimulation*

The patch clamp amplifier, designed and constructed in our laboratory, had a conventional resistive feedback in the head-stage, which could be electronically switched between 100 MΩ and 50 GΩ. Currents were low-pass filtered with a Bessel 4-pole filter using a cutoff frequency ranging from 500 to 2,000 Hz. Records were usually digitized on-line at a 5–10-kHz sampling rate by an IBM AT computer equipped with an analog/digital interface board (model 2821; Data Translation, Marlboro, MA) and stored on Bernoulli cartridges (Iomega, South Roy, UT). Alternatively, signals were first recorded on a video-cassette recording system (PCM 601/SL; 2700; Sony) and subsequently transferred to the computer for analysis. Voltage and light stimuli were applied by a microprocessor-controlled programmable stimulator (Stim 6; Ionoptix, Milton, MA). Software was written to perform standard single-channel analysis tasks, including baseline drift removal, digital filtering, current–amplitude histograms, dwell-time distribution, and nonlinear multiexponential fitting to histograms of open and closed times. During experimental manipulations the cells were viewed with a Newvicon TV camera (Panasonic), using near infrared illumination ($\lambda > 715$ nm). Linear extrapolation of the spectral sensitivity data of McReynolds and Gorman (1970*b*), plotted on a wave-number scale, indicates that at 715 nm the cell's sensitivity should be reduced by ~4.5 log with respect to the peak (500 nm). We never observed excitation induced by the infrared light; as a precaution, however, after seal formation cells were dark adapted for several minutes before testing light responses.

Patch electrodes were fabricated with borosilicate capillary tubing (type 7052; Garner Glass, Claremont, CA) and fire polished immediately before use. For cell-attached recordings, electrodes were filled either with normal ASW or divalent-free solution (500 mM NaCl, 10 mM HEPES, and 1 mM EDTA, pH 7.8) and had a resistance ranging from 5 to 16 MΩ. During seal formation suction was applied by means of a vacuum regulator and monitored with a vacuum gauge, and was kept as low as possible (typically 0.5–2.0 cm $H_2O$) in an attempt to minimize gross distortions of the membrane patch, which may lead to anomalous behavior (see Discussion). Several tests of the physiological viability of isolated cells were made by whole-cell recording of membrane currents, filling the patch pipette with 200 mM KCl, 100 mM K-aspartate, 12 mM NaCl, 6 mM $MgCl_2$, 1 mM EGTA, and 300 mM sucrose, pH 7.3. The photoresponse, however, tended to run down rapidly; inclusion of ATP (6 mM) and GTP (100 μM) in the internal solution failed to alleviate the problem. As an alternative, the perforated-patch method (Horn and Marty, 1988) was also used. This technique is designed to circumvent both the potentially traumatic mechanical rupture of the membrane and washout problems. Electrodes for perforated-patch recording were front-filled with the standard intracellular solution, while the back-filling solution also included 0.1–0.2 mg/ml of the pore-forming antimycotic agents nystatin (Calbiochem Corp.) or amphotericin B (Sigma Chemical Co.; Rae, Cooper, Gates, and Watsky, 1991). Since the induced aqueous pores have an effective radius on the order of 4 Å, loss of larger molecules from the interior of the cell is prevented. Membrane perforation, monitored via the capacitative transients induced by repetitive voltage steps, was only obtained in a fraction of the attempts, perhaps because nystatin and amphotericin B can reliably induce permeabilization only if the membrane contains a significant amount of sterol (Andreoli and Monahan, 1968). When successful, stable access to the cell interior ($R < 30$ MΩ) required 15–45 min.

For optical stimulation, light from a 150-W tungsten–halogen light source was collected by a condenser lens and passed through a heat-absorbing filter (>95% rejection for $\lambda > 800$ nm; Melles-Griot, Irvine, CA). A solenoid-driven shutter (Uniblitz; Vincent Associates, Rochester, NY) was used to deliver flashes or steps of light. Controlled attenuation of the beam was provided by a set of calibrated neutral density filters that could be interposed in the light path.

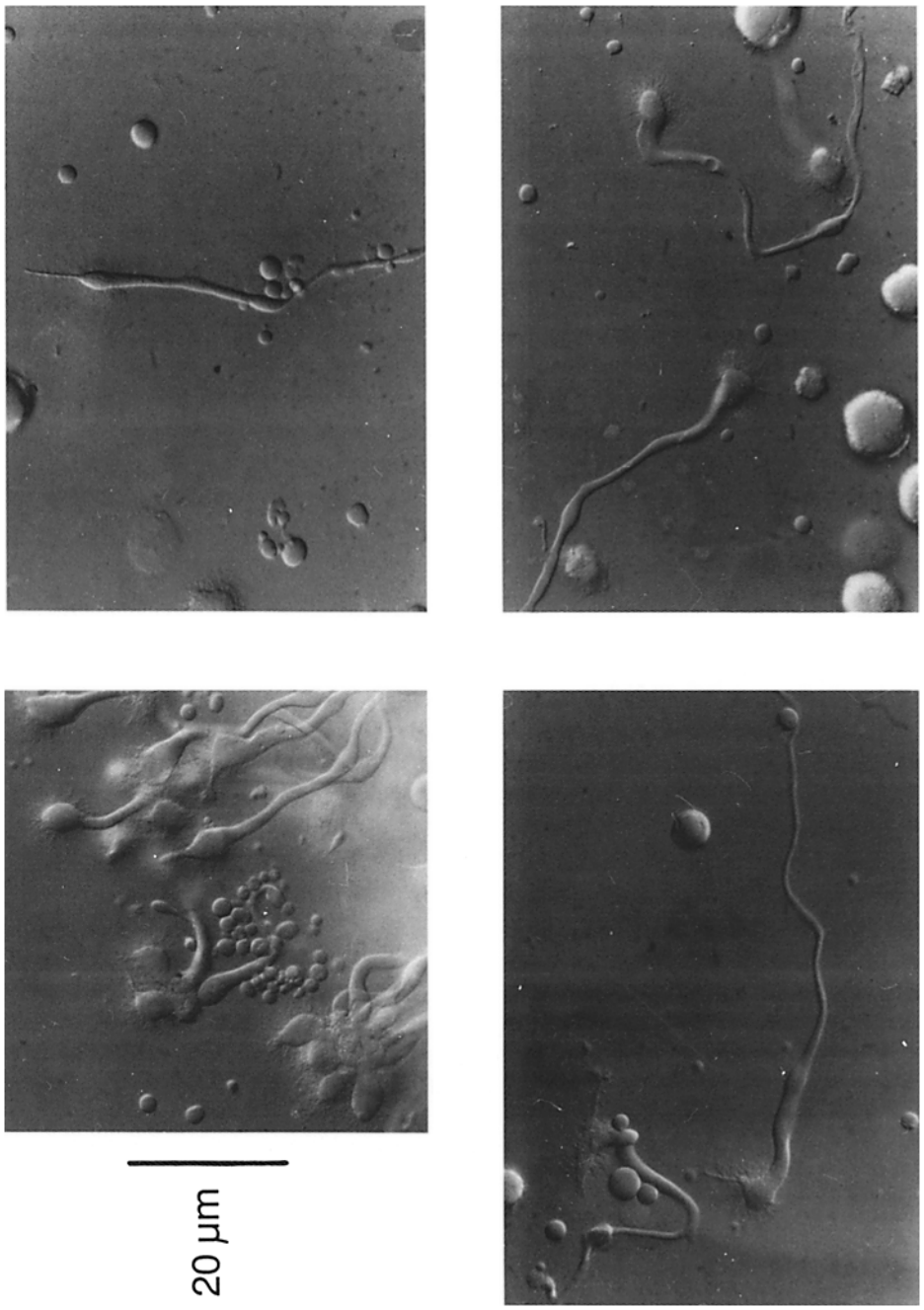


FIGURE 1.

A pin hole served as a field stop, restricting the illuminated region to a spot ~150 μm in diameter, thus avoiding exposure of all the cells in the chamber to light. The stimulating beam was combined with the microscope illuminator beam by means of a cube beam splitter. White light was used for stimulation unless otherwise indicated. The intensity of the unattenuated beam, measured with a calibrated radiometer (United Detector Technology, Hawthorne, CA), was 280 μW/cm$^2$. Light intensity is expressed as $\log_{10}(I/I_o)$, where $I_o$ is the unattenuated light intensity.

The protocol used to prepare isolated cells for scanning electron microscopy has been described previously (Nasi, 1991*a*).

## RESULTS

The enzymatic dissociation procedure we have developed routinely results in a large yield of solitary cells and small clusters of cells. The first micrograph in Fig. 1 shows a small piece of the proximal portion of the retina in the process of being dissociated. Numerous photoreceptors are visible, displaying a distinct microvilli-covered rhabdomeric lobe. Several examples of isolated cells are shown in the other micrographs. The cell body is smooth and elongated, ~20–25 μm long and 4–5 μm wide. A stretch of axon is usually retained, occasionally exceeding 200 μm in length. Vigorous mechanical trituration, on the other hand, typically causes cleavage of the axon near the hillock. Shorter axon stumps are advantageous to improve space clamp in whole-cell recording. Detailed features of the solitary cells can be appreciated in the scanning electron micrographs in Fig. 2. In particular, notice the presence of larger processes in one of the cells. Such structures, which Barber et al. (1967) identified as cilia, can also be discerned in the Nomarski micrographs of Fig. 1.

The functional viability of the preparation was tested by recording the macroscopic photocurrent under voltage clamp. Cells with short axons were chosen, with the aim of improving space clamp. Fig. 3 shows the responses of a rhabdomeric photoreceptor to flashes of light of increasing intensity. The membrane current was measured using the perforated-patch method, with the pipette sealed onto the somatic lobe and the holding potential ($V_h$) set at −50 mV. Similar photocurrents, up to 2.5 nA in amplitude, were observed in many other solitary rhabdomeric cells ($n$ = 12), indicating that their phototransducing machinery remains functional after isolation. The size and shape of the photoresponses measured with conventional whole-cell clamp and with the perforated-patch technique were comparable.

High-resistance seals could also be obtained on the villous membrane of the rhabdomeric lobe, presumably the light-sensitive region of the cell, and unitary current recordings were performed in cell-attached patches. Because during on-cell

Figure 1. (*opposite*) Nomarski micrographs of solitary rhabdomeric photoreceptors. The top left panel shows a cluster of cells in the process of separating from the retina during the dispersion procedure. The remaining photographs present examples of isolated cells displaying a prominent microvilli-covered lobe, with the occasional presence of a larger cilium-like structure (e.g., top right and bottom left panels). The slender cell body gradually tapers, giving rise to the axon.

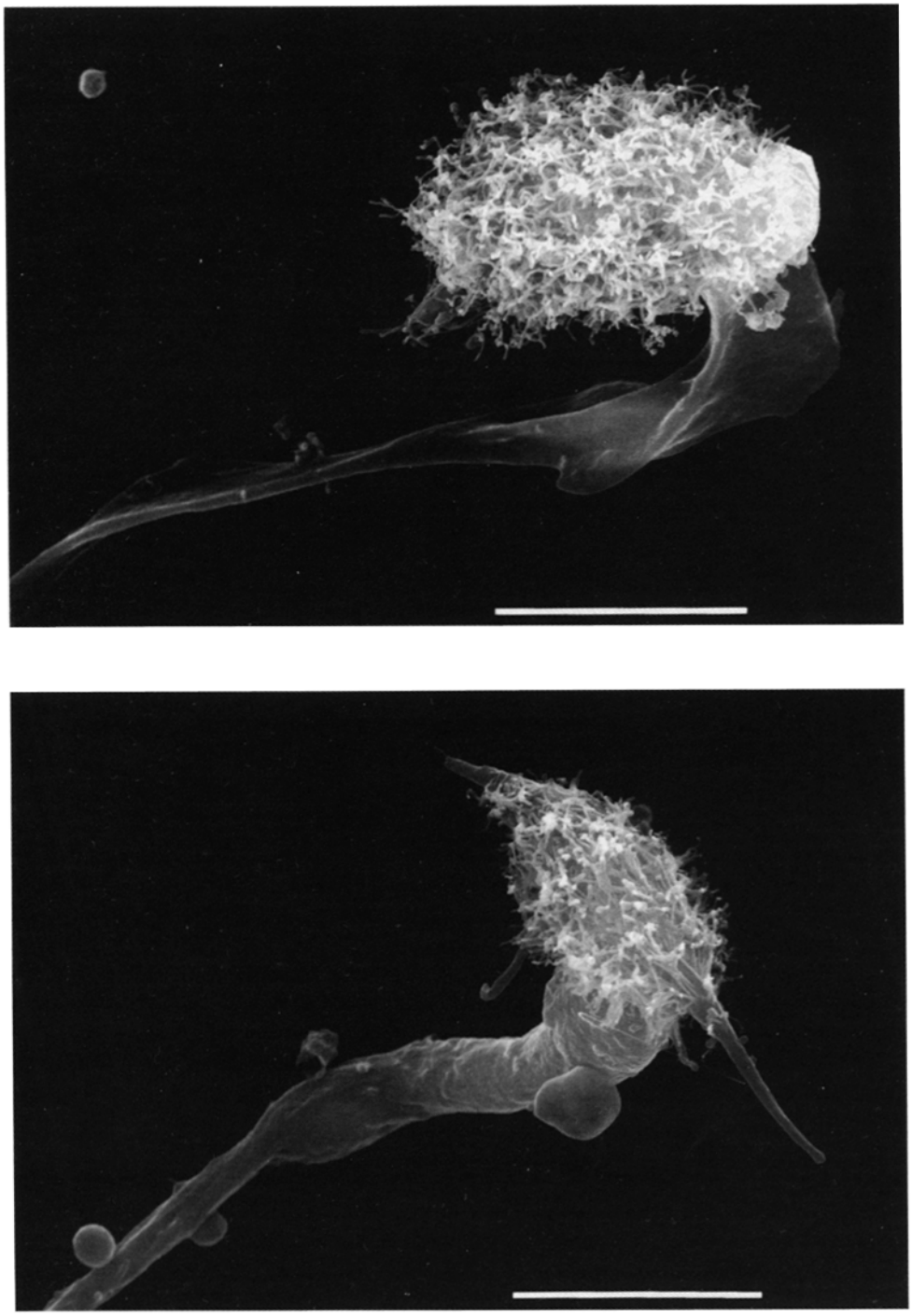

FIGURE 2. Scanning electron micrographs of two isolated rhabdomeric visual cells. The presence of a conspicuous protrusion, amidst the microvilli, can be clearly visualized in the photograph on the left. Calibration bars: 10 μm; SEM operating at 20,000 V, 3,500×.

patch recording the photoreceptors were not under voltage clamp, it was important to determine whether any observed channel activity was a direct consequence of photostimulation, or whether it resulted from light-induced changes in membrane voltage. A channel was therefore classified as light dependent only if in the dark it could not be activated by voltage stimulation over a wide range of depolarizing and hyperpolarizing potentials (usually spanning 90–120 mV). The top traces in Fig. 4 illustrate the lack of channel activity in a patch examined at various pipette potentials in the dark. Presentation of a sustained step of light with the pipette potential ($V_p$) set at +60 mV elicited a vigorous burst of very brief inward current excursions, which gradually subsided to a lower maintained rate (lower trace). The rectangular appearance of the unitary currents can be appreciated in the two expanded windows of recording at the bottom of the figure. These segments were selected from the late portion of the trace, when the membrane potential (and hence the driving force on the channel currents) is expected to be at steady state. Light-dependent channels were observed in > 100 patches.

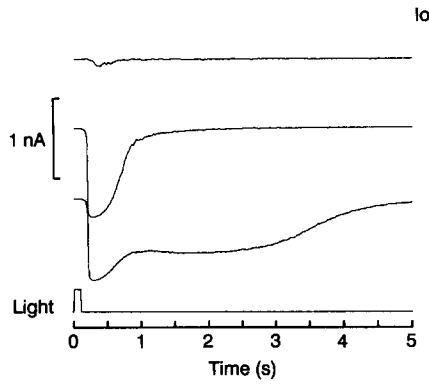


FIGURE 3. Photocurrents in an isolated rhabdomeric cell voltage clamped with the perforated-patch technique. The recording electrode was filled with an intracellular solution containing amphotericin B and sealed on the cell body. The holding potential was −50 mV and the series resistance was compensated. The relative intensity of the flash (100 ms in duration) is indicated near each trace.

Continuous illumination was used to examine the steady-state behavior of light-induced channel openings. In Fig. 5 *A* a patch that was unresponsive to membrane depolarization in the dark (*left*) exhibited prominent channel activity in the presence of a bright light (0 log). Two equi-spaced levels of open-channel current are visible, suggesting the presence of at least two light-dependent channels of the same type. Short portions of the record (marked by the bars and the letters *a* and *b*) are expanded in Fig. 5 *B*. The current–amplitude histogram for the entire record, presented in Fig. 5 *C*, exhibits a small but distinct hump around 2.9 pA, which corresponds to the main open-channel current level. A histogram of open times, binned at 0.5-ms resolution, was constructed after setting the transition threshold midway between the closed and open current levels. Multiple openings were excluded from the analysis. The open time distribution (Fig. 5 *D*) is satisfactorily described by a single exponential function. The first bin (0–0.5 ms) was excluded from the least-squares fitting because short events can escape detection owing to the limited bandwidth of the recording ($f_c$ = 1.5 kHz). The apparent mean open time was 0.98 ms, without correcting for missed events. Because of the presence of more than one channel, closed times were not analyzed.

It was of interest to determine whether the short-lived openings of light-sensitive channels may in part reflect blockage of the conduction pathway by divalent cations. Such a mechanism operates in the light-suppressible current of vertebrate rods (Haynes et al., 1986; Zimmerman and Baylor, 1986; Matthews, 1987). The trace in

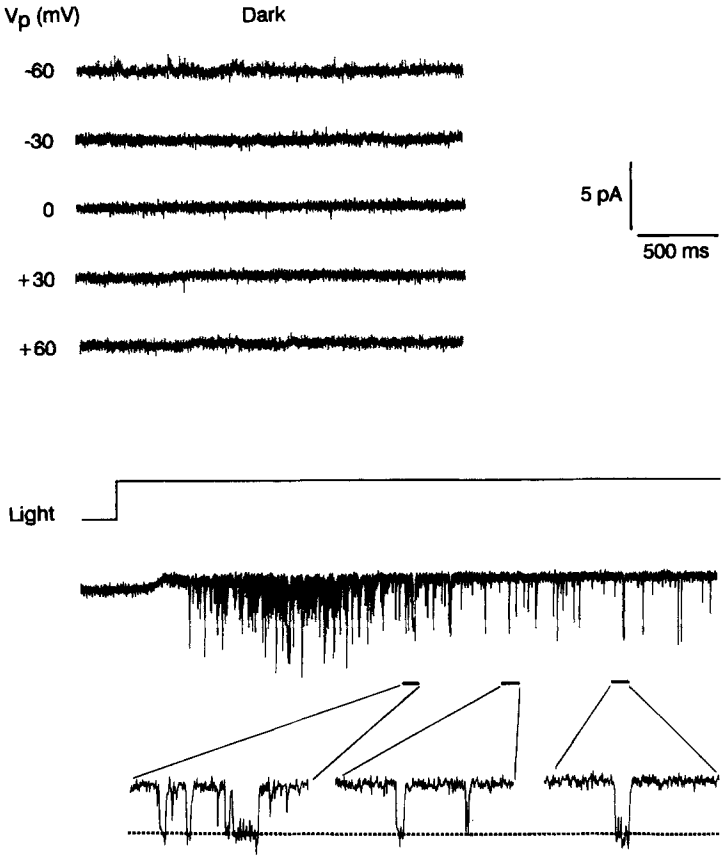


FIGURE 4. Example of light-dependent channels recorded in a cell-attached patch on the rhabdomeric lobe. The top traces illustrate the lack of channel activity in the dark as different potentials were applied to the patch electrode. Presentation of a sustained step of light ($\log(I/I_0) = -1.2$) at $V_p = +60$ mV elicits a burst of openings, which subside to a low steady state. Representative segments of recording, each 100 ms in duration, are shown on an expanded time scale at the bottom. Shortly after light onset a small glitch and a smooth upward deflection are visible in the current trace (see also subsequent figures). These are due to the cell's macroscopic photoresponse inducing capacitative and shunt currents (through the patch leakage and also through the seal resistance as an extracellular potential is generated).

Fig. 6 *A* was obtained by filling the recording electrode with a solution lacking Ca and Mg and containing 1 mM EDTA. Light-dependent unitary currents were similar to the ones observed in normal sea water, and consisted of very brief excursions to the open state, best appreciated in Fig. 6 *B*. The mean duration of the openings,

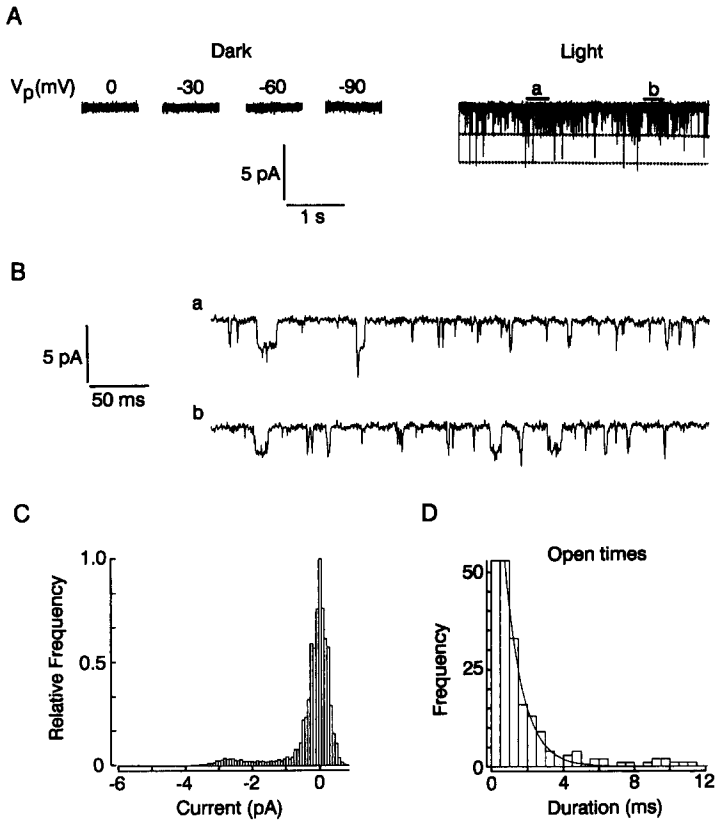


FIGURE 5. Kinetic properties of light-activated ion channels. (*A*) A patch in the rhabdomeric lobe of a cell was tested first with voltage stimulation in the dark, resulting in no channel openings (*left*). In the presence of continuous bright illumination (0 log) vigorous sustained activity is recorded, with a main open-channel state and occasional excursions to an amplitude level twice as large. (*B*) 20-fold time expansion of the record segments labeled *a* and *b* in panel *A*. At least one double transition can be discerned. Patch hyperpolarized by 30 mV. (*C*) Current–amplitude histogram for the entire stretch of recording (4,500 ms), showing a distinct open-channel current level at ~2.9 pA. (*D*) Distribution of open times obtained by setting a threshold criterion of 1.45 pA. The total number of events detected was 405 and the fractional open time was 0.084. The smooth curve is a single exponential function least-squares fitted with a Simplex algorithm, neglecting the first bin because of missed events. The estimated mean open time was 0.98 ms.

estimated from the time constant of a single-exponential function least-squares fitted to the open-time histogram, was 0.7 ms (Fig. 6 *C*), comparable to that found in the presence of divalent cations (e.g., Fig. 5 *D*). A similar pattern was observed in four other patches tested with divalent-free solution. We therefore conclude that the short dwell times in the conducting state probably reflect the intrinsic gating mechanism of the channel, rather than occlusion of the open channels by external calcium or magnesium.

Several requirements should be fulfilled for the observed light-induced unitary

currents to account for the macroscopic photocurrent. (*a*) Channel activity must be graded with light intensity. (*b*) The dynamic range should be similar to that of the whole-cell light response. (*c*) The time course of light-dependent channel activity should resemble the shape of the macroscopic photoresponse and exhibit a similar dependency on the intensity of the stimulus. (*d*) The reversal potential must agree with that of the light response. (*e*) The density and unitary conductance of the

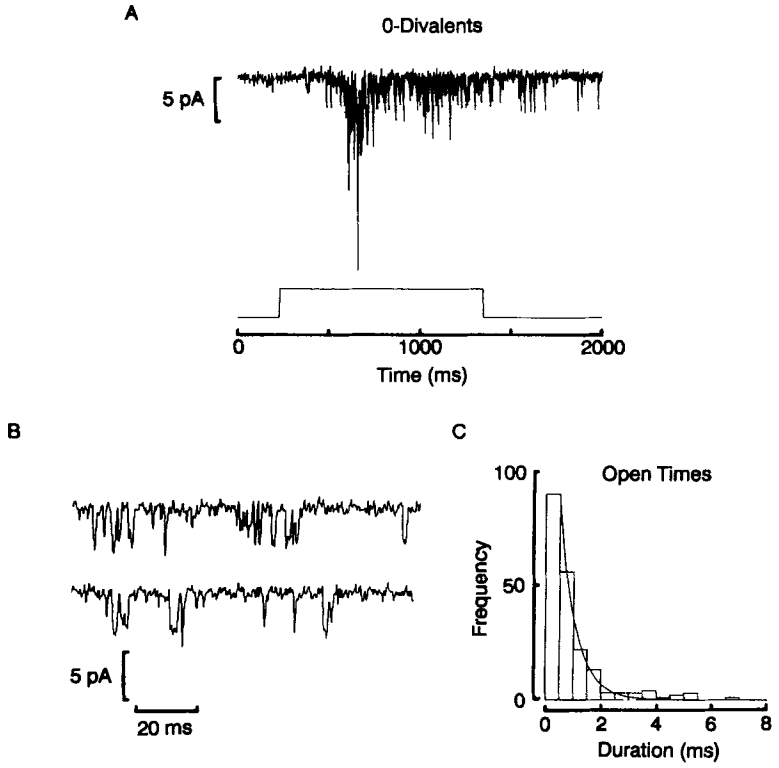


FIGURE 6. (*A*) Light-activated single-channel currents in the absence of external divalent cations. The patch electrode was filled with 500 mM NaCl, 10 mM HEPES, and 1 mM EDTA, pH 7.8. A 200-ms segment of the trace (1,560–1,760 ms) is displayed on an expanded time scale in *B*, and shows that channel openings are brief, similar to the ones recorded in normal sea water. (*C*) Open-time histogram constructed for the last 1-s period of recording, based on a total of 401 events (multiple-channel openings were discarded). The distribution was fitted with a single exponential function, yielding a mean open time of 0.7 ms ($V_p$ = +60 mV, relative light intensity −3.2 log).

light-activated channels should be compatible with the size of the macroscopic photocurrent.

The membrane voltage (or current) of most rhabdomeric cells exhibits discrete waves, the frequency of which is increased by dim continuous illumination (Yeandle, 1958). The inset at the top of Fig. 7 shows an example of macroscopic fluctuations elicited by a dim steady light (−5.6 log). The current recording was made under

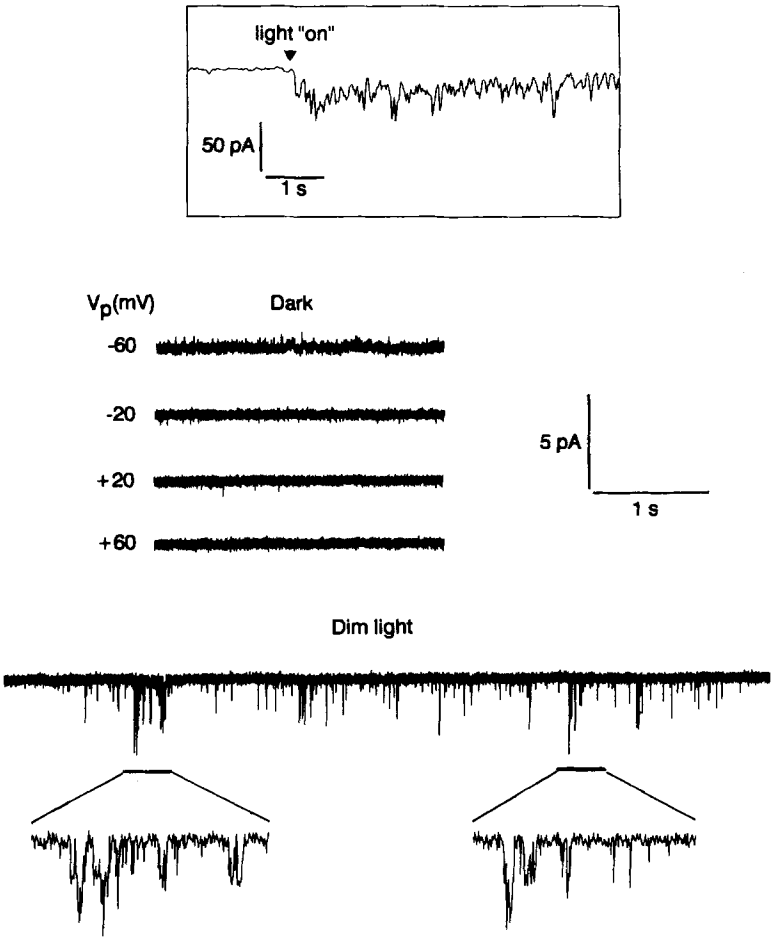


FIGURE 7. Bursts of light-dependent channels induced by dim illumination. (*Inset*) Discrete waves of macroscopic membrane current in response to dim, steady illumination (log ($I/I_0$) = −5.6). Perforated-patch whole-cell clamp recording using amphotericin B as the pore-forming agent; holding potential −50 mV. (*Middle*) Lack of activity in a patch of rhabdomeric membrane stimulated with voltage in the dark. (*Bottom*) Effect of continuous light (−5.6 log) in the same patch; $V_p$ = +30 mV; $f_c$ = 2 kHz). Light-activated channel events tend to group into bursts of similar duration to the whole-cell discrete waves (*top*), only considerably less frequent. The expanded traces show that each burst is composed of multiple brief openings.

voltage clamp using the perforated-patch technique. Since discrete waves are thought to be the elementary constituents of the macroscopic light response (Dodge, Knight, and Toyoda, 1968), it is expected that light-activated single-chanel currents should be visible at similarly low levels of light stimulation, insufficient to evoke a full-fledged, smooth photocurrent. Under such conditions their activity should tend to be clustered in bursts. Fig. 7 demonstrates that both predictions are borne out in recordings from patches of rhabdomeric membrane in *Pecten:* voltage stimulation

spanning a wide range of potentials failed to induce channel events in the dark (*middle*). Presentation of a sustained light of very low intensity (−5.6 log attenuation) induced bursts of channel activity (*bottom*), each with a duration comparable to the whole-cell quantum bumps measured under identical conditions. Notice the expanded traces, showing that each burst is composed of several brief openings, similar to those elicited by bright lights (e.g., Figs. 4 and 5 *B*). These observations were confirmed in four other cells.

Fig. 8 shows the effect of varying the intensity of brighter steps of light on the activity of light-dependent channels. The patch was held at $V_p$ = +20 mV, and 1-s stimuli were applied ~1 min apart with an attenuation of −4.1, −3.2, and −2.6 log, respectively. As the light is made brighter, the frequency of openings increases considerably, concomitant with a shortening of the latency of the first opening (arrows). Current–amplitude distribution histograms were constructed for the last 1,000-ms period of recording (indicated by the pairs of arrowheads above each trace). With the brightest stimulus (bottom trace) the presence of at least one discrete open-channel current level is clearly reflected in the current–amplitude histogram on the right (see arrow). Examination of individual events revealed no systematic differences in the mean open time as a function of light intensity: the average values for these recordings were 1.8 ms at −4.1 log, 1.0 ms at −3.2 log, and 1.3 ms at −2.6 log (multiple openings were excluded from the analysis). The early burst of light-evoked unitary currents typically saturates at intensities comparable to those required to saturate the peak amplitude of the cell's light response. Further increases in light intensity give rise to sustained channel activation, which can outlast by many seconds the duration of the flash (data not shown). This feature parallels the slow recovery of the macroscopic current after intense photostimulation (e.g., bottom trace in Fig. 3).

The peak amplitude of the whole-cell photocurrent (recorded at holding potentials similar to the resting $V_m$ of these cells) can exceed 2 nA. Considering the small size of the rhabdomeric lobe, where most of the phototransducing functions should be localized, a high density of light-dependent channels is expected in that region (see Discussion). In fact, the majority of patches contained at least two or three channels, even when relatively fine-tipped patch electrodes were used ($R$ > 15 MΩ). Moreover, in ~10–20% of the patches the number of channels was quite large, precluding resolution of individual openings and giving rise instead to pseudo-macroscopic photocurrents. An example is shown in Fig. 9. On occasion, these currents exceeded the compliance range of the amplifier (50 pA), driving it into saturation.

Light-dependent channels with similar characteristics were also occasionally observed in the somatic lobe of the cell (where gigaohm seals are obtained more easily), usually in the region adjacent to the rhabdomere. The density of channels in the somatic membrane appears to be substantially lower, as judged by the high incidence of silent patches. The presence of occasional light-sensitive channels in the cell body is not unexpected, because a very small macroscopic light response can sometimes be measured in cells that have lost their rhabdomeric lobe in the course of the dissociation (data not shown).

While voltage stimulation alone is ineffective in activating light-dependent channels, membrane potential can exert a distinct modulatory effect on their gating,

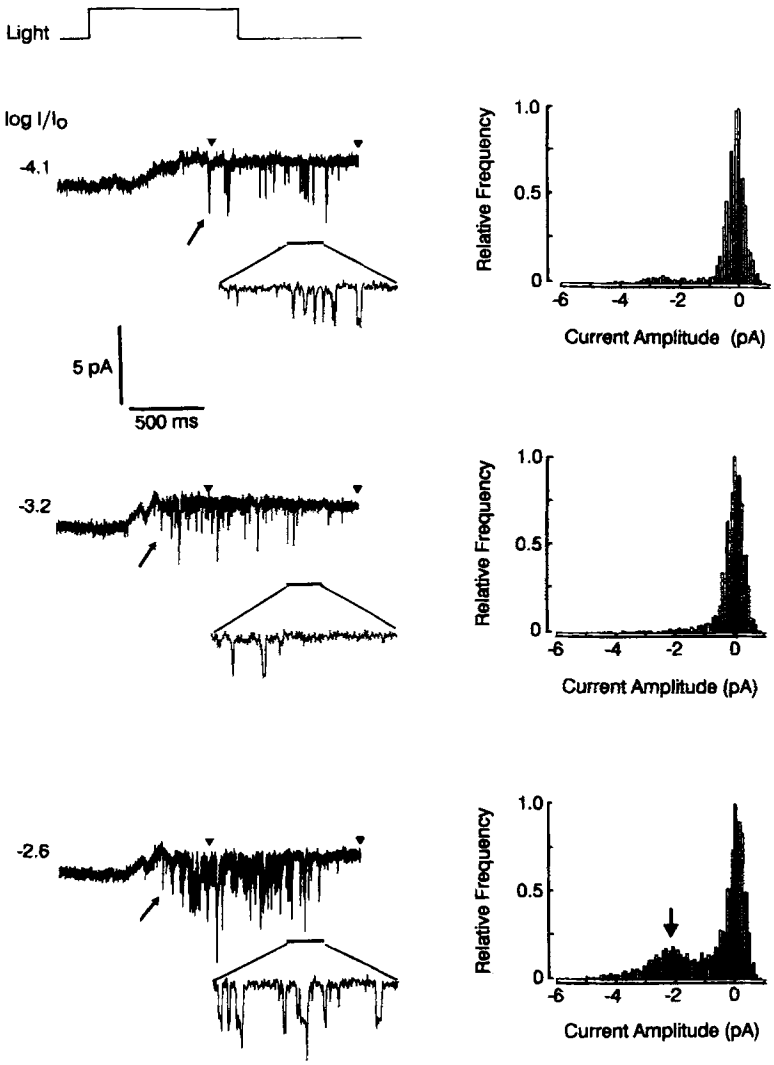


FIGURE 8. Effect of stimulus intensity on light-dependent channel activity. The patch was hyperpolarized by 20 mV, and the arrows in each record point to the first channel event occurring after stimulus onset. Relative light intensity indicated near each trace. Current–amplitude histograms constructed for the last 1-s period of recording are presented on the right.

which manifests itself as a pronounced decrease in channel activity when the patch is hyperpolarized. The control trace (Fig. 10 *A*, *left*) was recorded during the application of a voltage ramp in the dark, spanning the range from +60 to −60 mV. Subsequent presentation of a −3.5 log continuous light elicited a large burst of channel openings (*right*). After channel activity subsided to a low, steady level the pipette potential was varied (Fig. 10 *B*). It is evident that hyperpolarization causes a

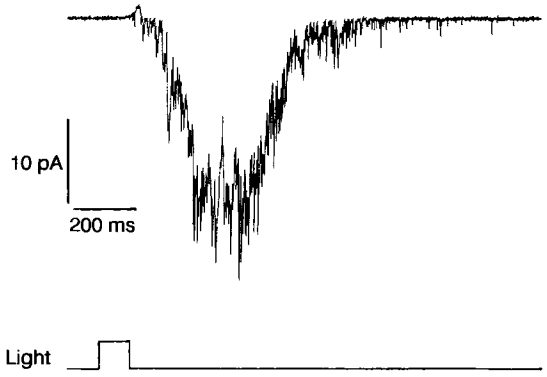


FIGURE 9. High density of light-sensitive channels in a patch of rhabdomeric membrane. A 100-ms step of bright light (−1.2 log) was delivered while the patch was hyperpolarized by 30 mV. A large burst of channel activity is observed as the stimulus is turned on, resembling a macroscopic photoresponse (peak amplitude ≈ 32 pA).

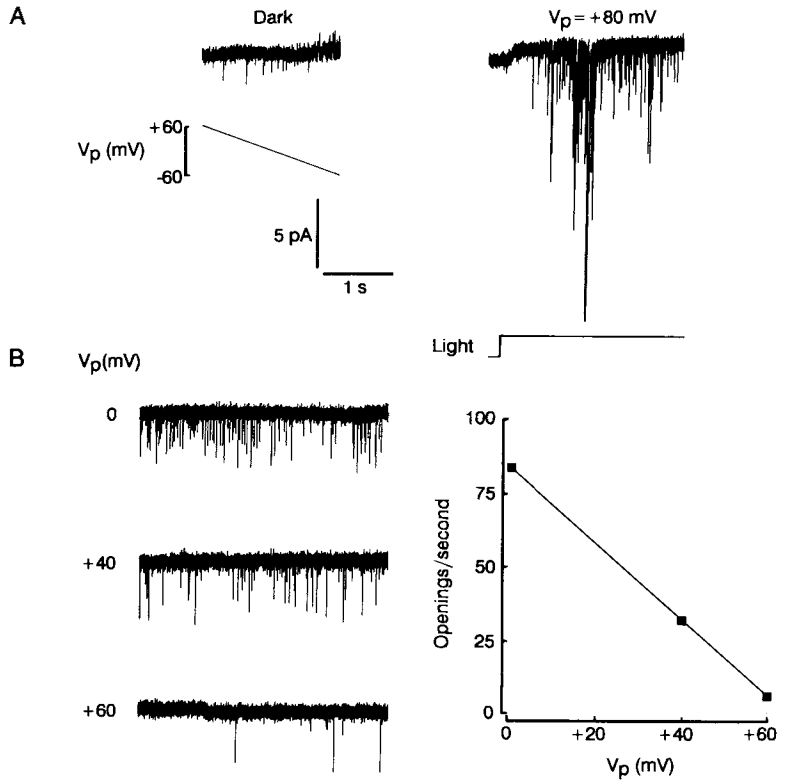


FIGURE 10. Effect of voltage on light-dependent channel gating. (*A*) Demonstration of light-induced activation of ion channels by application of a voltage ramp in the dark (*left*) and then a sustained step of light (−3.5 log) from a steady electrode potential of +80 mV (*right*). The few residual events visible in the ramp trace are due to a previously delivered light flash. No effect of voltage per se is observed. After the initial burst elicited by light, channel activity stabilized at a low level and several steps of voltage were applied in the presence of continuous illumination (*B*). The frequency of openings decreases progressively with hyperpolarization, but at each potential the activity rate is maintained. The frequency of events at the three voltages is plotted on the right as a function of the applied voltage.

noticeable decrease in the frequency of openings. The plot on the right illustrates the dependency of the rate of channel events on the imposed patch potential. The distribution of open times was little affected by voltage changes within this range, suggesting that the main consequence of hyperpolarization is a decrease in the opening rate, rather than a progressive shortening of channel openings, with the consequent increase in the number of events that escape detection at the bandwidth of the recording. An effect of voltage on open times is noticeable, however, with large depolarizations beyond the reversal potential of light-sensitive channels. In Fig. 11 a patch from a different cell was stimulated with a step of light (−1.2 log), first at $V_p$ = +50 mV and later at $V_p$ = −100 mV. In addition to the much increased frequency of openings at depolarized patch potentials, the expanded traces qualitatively illustrate the fact that open states tend to be significantly longer, an effect first reported by Bacigalupo, Chinn, and Lisman (1987) in *Limulus* ventral nerve cells.

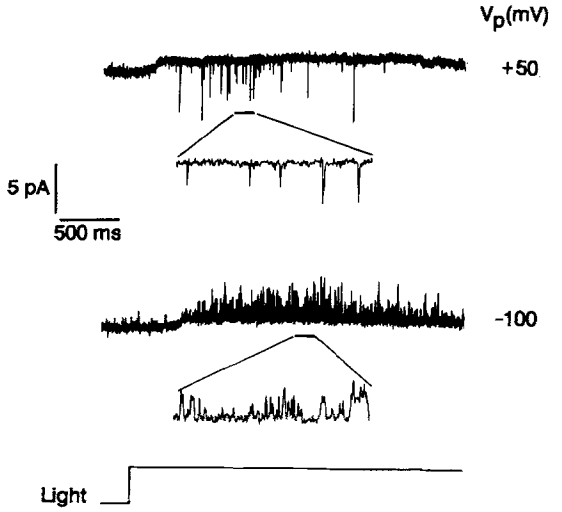


FIGURE 11. Effect of depolarization beyond the reversal voltage of light-dependent channels. A sustained step of light (−1.2 log) was delivered while the patch was hyperpolarized by 50 mV (*top*). After a few minutes of dark adaptation, the patch was depolarized by 100 mV and an identical light stimulus was applied again. In addition to the increased frequency of channel events, the lengthening of the open times can also be appreciated in the two expanded windows of recording.

The open-state conductance of the light-dependent channels was determined by presenting brief steps of light while the patch electrode was maintained at different voltages. Only "late" openings (occurring no less than 1,000 ms after light termination) were used, since while the cell undergoes the phasic light-induced depolarization (McReynolds and Gorman, 1970*a*) the driving force transiently changes. No obvious qualitative differences were otherwise evident between the early and the late events (but see Discussion). In Fig. 12 unitary currents reverse sign at a pipette command potential around −50 mV. Since the resting potential in *Pecten* rhabdomeric cells is about −40 mV (McReynolds and Gorman, 1970*a*), this reversal voltage corresponds to approximately +10 mV across the patch (assuming that membrane repolarization is practically complete by that time), in good agreement with the reversal of the macroscopic voltage photoresponse measured in the intact proximal retina (McReynolds and Gorman, 1970*b*). The *I-V* plot shows that the dependence of open-channel current on voltage is linear, with a slope conductance of 49 pS. Similar values were obtained in three other patches (mean = 46.5 pS).

In all recordings a single open-channel current level is predominant, and overlapping events usually have an amplitude that is a multiple of the unitary current. However, other current levels are also occasionally seen, albeit with considerably lower incidence. Fig. 13 shows a recording in which a voltage ramp was delivered first in the dark (top trace) and subsequently in the presence of continuous light, which

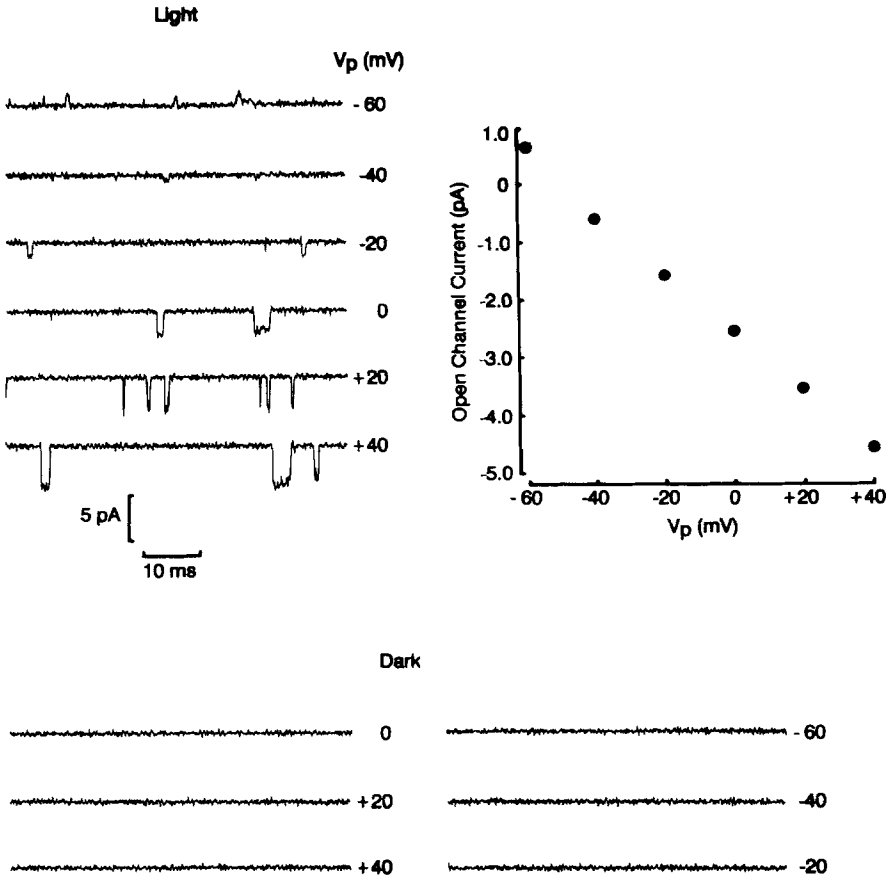


FIGURE 12. Unitary conductance of the light-sensitive channels in rhabdomeric cells. Channels were activated by a step of light while the pipette was held at different voltages (*top left*). Events occurring at least 1 s after the termination of the stimulus were used to determine the current–voltage relation, plotted on the right. The single-channel conductance for this patch was 49 pS. The traces at the bottom show that in the absence of photostimulation no channel openings were observed regardless of the patch voltage.

elicited substantial channel activation. The amplitude of the events decreases progressively as the patch is gradually depolarized, and unitary currents invert at an electrode potential of about −54 mV, similar to measurements performed with steady voltages (e.g., Fig. 12). In the central portion of the trace several small-amplitude channel openings can be seen, interspersed with the larger, more characteristic

light-induced events. These can be better appreciated in the expanded segment at the bottom of the figure: the continuous line fitted to the large events has a slope of 50 pS, while the dashed line superimposed on the smaller openings corresponds to a conductance of ~17 pS. While the number of events in this recording is insufficient to permit any quantitative analysis, it is perhaps noteworthy that the few double openings that occur are heterogeneous (namely, large–small). Two channels of the same type, each capable of switching randomly to either a large- or a small-conductance state, should give rise also to large–large and small–small overlapping openings. The observed pattern is more consistent with the presence of two different channels of unequal conductance; alternatively, one could envision a more complex

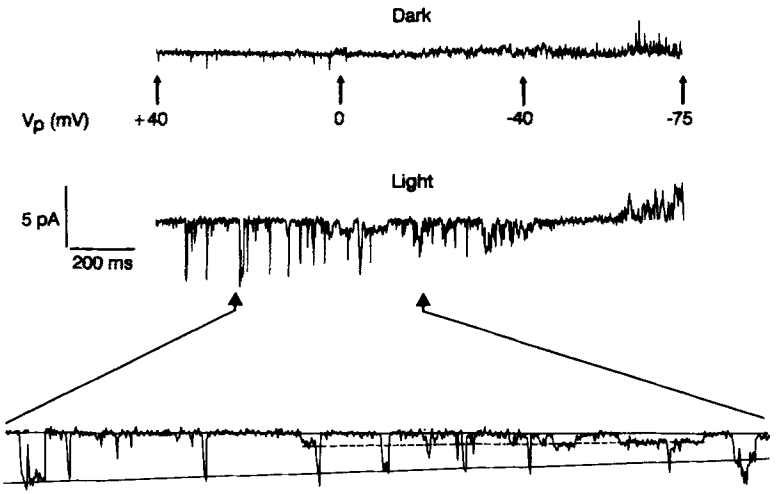


FIGURE 13. Small-amplitude channel openings induced by light. A ramp of voltage (72 mV/s) was applied to a patch in the dark (*top*) and in the presence of steady light (520 nm, 0.57 $\mu W \cdot cm^{-2}$, i.e., ~$1.5 \times 10^{12}$ photons $cm^{-2} \cdot s^{-1}$; *middle trace*). In addition to the most common class of unitary currents, excursions to a smaller conductance level can be observed in the middle portion of the trace. In the bottom part of the figure a segment of the recording was expanded, and straight lines with slopes of 50 and 17 pS were fitted to the large and the small events, respectively.

scheme involving slowly changing gating modes (Johnson, Bacigalupo, Vergara, and Lisman, 1991). Unfortunately, while small-amplitude, light-induced openings were observed on many occasions, the presence of multiple channels in those patches precluded statistical analysis. Future experiments will address this issue.

In *Limulus*, small, light-dependent channel openings have also been described (Bacigalupo and Lisman, 1983; Nagy 1990; Nagy and Stieve, 1990). Johnson et al. (1991) reported that the incidence of small events tends to become higher sometime after light onset, and suggested that transition from large to small events could contribute to the decreased amplitude of the photocurrent during the slow phase of light adaptation. A quantitative analysis of such apparent nonstationary behavior is difficult, particularly without voltage-clamping the cell (see Discussion). A corollary

idea, however, can be examined qualitatively: if the state of light adaptation is associated with the occurrence of small, rather than large channel openings, stimulation of a dark-adapted cell should yield a lower proportion of small events, as compared with a similar response elicited in the presence of background adaptation. Fig. 14 shows the results of such an experiment. Voltage stimulation applied to the patch in the dark revealed no channels, whereas presentation of a moderately bright step of light elicited a distinct response. Shortly afterwards a dim background light was turned on, followed by the delivery of another step of white light. The intensity of the second stimulus was increased to insure responsiveness in spite of light adaptation. It proved difficult to accurately match the responses evoked by the two test

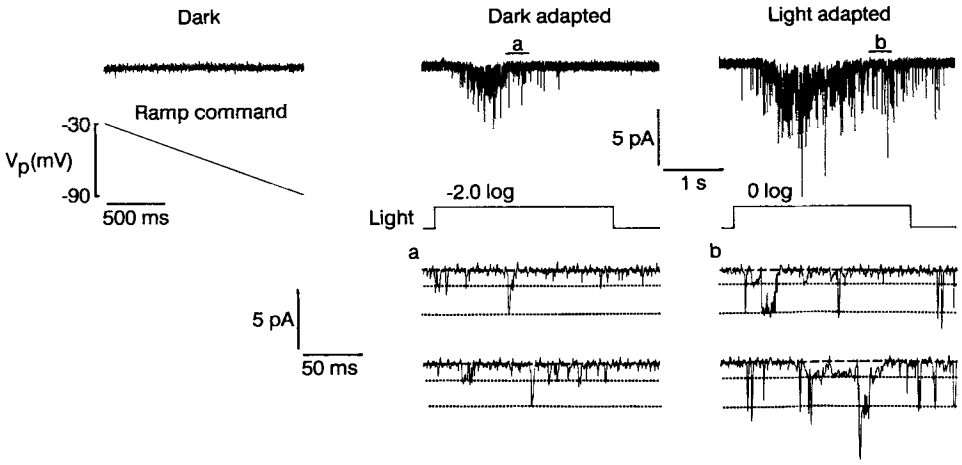


FIGURE 14. Effect of an adapting background stimulus on channel activity elicited by a step of light. The lack of responsiveness to changes in membrane potential alone was assessed first by administering a voltage ramp in the dark (*left*). A moderately bright light step was then applied, eliciting substantial activity of light-sensitive channels (*middle*). Subsequently, after a period of dark adaptation, a steady background stimulus (520 nm, 0.57 $\mu W \cdot cm^{-2}$) was turned on. When the channel activity subsided to a near-zero level, a second step of white light was delivered, the intensity of which was increased by 2 log units in order to elicit a second vigorous response (*right*). Channel openings recorded under dark-adapted conditions and in the presence of the adapting background light reveal a comparable mixture of large and small openings, as illustrated by the expanded traces at the bottom.

stimuli. Nevertheless, the overall similarity is evident. The expanded traces (*bottom*) illustrate the fact that both small and large events are present in each trace, with no obvious pattern of difference. A similar experiment was performed in a total of four cells, with comparable results.

## DISCUSSION

We have succeeded in obtaining viable solitary visual cells from the eye of *Pecten,* and have performed cell-attached measurements of light-activated unitary currents. Channels were found almost exclusively in the rhabdomeric lobe, confirming that the

phototransducing function is to a large extent spatially segregated. Their functional properties leave little doubt that they underlie the generation of the photoresponse. An attractive feature of enzymatically isolated molluscan photoreceptor preparations is the large yield of viable cells suitable for tight-seal patch electrode recording (Nasi, 1991*a*, *b*, *c*) without requiring mechanical stripping of surrounding glia or sonication.

A salient feature of the present recordings concerns the light sensitivity and dynamic range of the light-activated channels. In previous reports on *Limulus* ventral photoreceptors bright lights were used to evoke channel activity in a range that exceeds the typical saturation level for the macroscopic photoresponse (Bacigalupo and Lisman, 1983; Bacigalupo et al., 1987; Nagy, 1990; Nagy and Stieve, 1990). It was therefore of interest to ascertain light responsiveness of patches within the normal operating range of dark-adapted cells. The results obtained demonstrate that in *Pecten* photoreceptors light-dependent channel activity is graded with light intensity at stimulus levels comparable to the dynamic range of the whole-cell photocurrent. Light sensitivity appeared to extend to very low levels of illumination which typically cause the cell to produce quantum bumps. Under such circumstances channel openings clustered in brief bursts. These bursts are considerably more infrequent than the total rate of discrete waves produced by the cell. The discrepancy may be explained by the fact that quantum bumps are responses to single absorbed photons (Fuortes and Yeandle, 1964) and the associated changes in membrane conductance are confined to the region near the isomerized rhodopsin molecule (Fein and Charlton, 1975), so that only those fluctuations originating in the vicinity of the electrode may be recorded. The possibility of measuring unitary currents at very low light levels has an interesting implication. Bacigalupo and Lisman (1983) observed that the open times of light-sensitive channels in *Limulus* were short, and concluded that the rate-limiting step in the relaxation kinetics of quantum bumps must be the time course of the internal transmitter activity, rather than the channel closing rate. Such an inference was criticized by Dirnberger, Keiper, Schnakenberg, and Stieve (1985) because the light stimuli used in those measurements were very bright, and light adaptation accelerates the time course of discrete waves (Wong, Knight, and Dodge, 1982). As a consequence, it could not be ruled out that the mean duration of channel openings at low stimulus intensities may be compatible with the decay time constant of the quantum bumps generated under dark-adapted conditions. The present results indicate that even with extremely dim illumination the openings of light-sensitive channels are in the millisecond time scale, and therefore their closing rate cannot account for the relaxation of discrete waves.

The envelope of the channel openings is reminiscent of the time course of the macroscopic response, and the latency to the first opening is comparable to that of the photocurrent. Long latencies (400 ms or more) were sometimes seen, particularly when seal formation required relatively strong suction ($>5$ cm $H_2O$). These may be due to diffusional constraints imposed on the internal transmitter, since a "bleb" of membrane has been shown to be drawn into the recording pipette during seal formation (Hamill, Marty, Neher, Sakman, and Sigworth, 1981), sometimes for distances reaching 100 μm (Ruknudin, Song, and Sachs, 1991; Sokab and Sachs, 1991). For this reason, the negative pressure during seal formation was always monitored and kept as low as possible.

The incidence of light-sensitive channels in the rhabdomeric membrane is quite high: rarely did a patch fail to respond to light stimulation, and frequently too many channels were present to permit resolution of unitary currents. The limiting step for recording light-dependent unitary currents, therefore, was obtaining tight seals on the villous lobe of the cell (20–50% of the attempts). While such high density of light-activated channels contrasts with the lower yield reported in *Limulus* ventral photoreceptors (< 10% of the patches; Bacigalupo and Lisman, 1983; Bacigalupo et al., 1987), it should not be surprising in view of the small size of the phototransducing region of these cells, relative to the amplitude of the whole-cell photocurrent (> 2 nA at the peak). To derive a lower-bound estimate of the average number of light-sensitive channels expected in a patch, the rhabdomeric lobe can be approximated as a sphere ~5 μm in diameter, from which the microvilli emanate. A pipette with a tip orifice of 0.5 μm in diameter would subtend ~0.25% of the total surface (assuming that infoldings and microvilli are equally represented in the area under the electrode as in the rest of the rhabdomere). If the light-sensitive conductance is spatially uniformly distributed, one would expect up to 5 pA of current in the patch in response to a saturating stimulus. Unitary currents on the order of 2–3 pA are typical at the usual recording potentials, and with bright lights a reasonable value for the fractional open time is 0.1 (e.g., Fig. 5). As a result, it can be estimated that as many as 15–20 channels may be active in the small membrane area spanned by the electrode tip.

The predominant unitary conductance is rather similar to that reported in *Limulus* by Bacigalupo and Lisman (1983) and is perhaps representative of other rhabdomeric photoreceptors. The apparent constancy of mean open-time duration with stimulating light of different intensities is consistent with a gating scheme involving a positive internal transmitter (Colquhoun and Hawkes, 1981; Bodoia and Detwiler, 1984). The presence of additional classes of events, however, suggests that the mechanisms of visual excitation may involve further complexities. In *Limulus*, Nagy and Stieve (1990) and Nagy (1990) reported up to three different sizes of unitary currents activated by light, and suggested—on the basis of their activation latencies—that separate classes of ion channels may exist, gated by different internal transmitter systems. Johnson et al. (1991) provided evidence favoring the interpretation of a single class of ion channel proteins possessing subconductance states, and speculated that transition between different "modes" of gating involving the large vs. the small conductance state may be responsible at least in part for the decay of the photocurrent during the late phase of light adaptation. Some of the present results (see Fig. 14) do not lend support to such notion, at least in its simplest form, because large and small events occur with similar relative frequency whether a light response is evoked from dark-adapted or light-adapted conditions. Detailed examination of light-activated, single-channel currents, however, is hindered by one limitation, which is common to all the studies reported so far: cell-attached recordings are performed in photoreceptors that are not held under voltage clamp. Shortly after stimulus onset the cell membrane potential rapidly undergoes a large excursion, which can be on the order of 50 mV or more. This has two unfortunate consequences: (*a*) the driving force transiently changes in a substantial way, altering the amplitude of channel events and making unambiguous identification of different sizes of events difficult;

and (*b*) voltage per se may be responsible for fostering the appearance of one or another type of opening during different phases of the response. Under these circumstances, only the activity recorded either during the plateau phase of the response or after membrane repolarization can be analyzed with confidence. It is therefore not surprising that virtually all the information gathered so far on light-sensitive channels in invertebrates concerns the steady-state events. It may be premature, however, to assume that the molecular mechanisms underlying the generation of the early transient are qualitatively similar to those responsible for the late phase of the light response. In voltage-clamped *Lima* rhabdomeric photoreceptors the early component can be clearly separated, on the basis of its higher light sensitivity, greater susceptibility to light adaptation, and more positive reversal potential, from the late component of the photocurrent which appears at higher stimulus intensities (Nasi, 1991*c*). In *Limulus* ventral nerve cells the early transient of the light response is adversely affected by treatments that interfere with $PIP_2$ hydrolysis, $IP_3$-mediated Ca-release, and intracellular rises of calcium, while the plateau phase in response to more intense light steps is unaffected (Frank and Fein, 1991). Those authors have therefore suggested that the $IP_3$ cascade may underlie the generation of the initial part of the photoresponse, whereas the late component may be activated by a separate biochemical pathway. Simultaneous cell-attached patch recording and whole-cell voltage clamp will be required to permit a close examination of ion channels during the full course of the light response, in order to clarify whether separate effectors or activator systems may be implicated.

We wish to express our gratitude to Dr. Gregor Jones and Dr. John Lisman for critically reading the manuscript, and to the staff of the Marine Biological Laboratory, where the experiments were conducted.

This work was supported by NIH grant RO1 EY07559.

*Original version received 1 October 1991 and accepted version received 23 January 1992.*

## REFERENCES

Andreoli, T. E., and M. Monahan. 1968. The interaction of polyene antibiotics with thin lipid membranes. *Journal of General Physiology.* 52:300–325.

Bacigalupo, J., K. Chinn, and J. Lisman. 1987. Ion channels activated by light in *Limulus* ventral photoreceptors. *Journal of General Physiology.* 87:73–89.

Bacigalupo, J., and J. E. Lisman. 1983. Single-channel currents activated by light in *Limulus* ventral photoreceptors. *Nature.* 304:268–270.

Bader, C. R., P. R. MacLeish, and E. A. Schwartz. 1979. A voltage-clamp study of the light response in solitary rods of the tiger salamander. *Journal of Physiology.* 296:1–26.

Barber, V. C., E. M. Evans, and M. F. Land. 1967. The fine structure of the eye of the mollusk *Pecten maximus*. *Zeitschrift für Zellforschung und Mikroskopische Anatomie.* 76:295–312.

Bodoia, R. D., and P. B. Detwiler. 1984. Patch-clamp recordings of the light-sensitive dark noise in retinal rods from the lizard and frog. *Journal of Physiology.* 367:183–216.

Colquhoun, D., and A. G. Hawkes. 1981. On the stochastic properties of single ion channels. *Proceedings of the Royal Society of London B.* 211:205–235.

Dakin, W. J. 1910. The eye of *Pecten*. *Quarterly Journal of Microscopical Science.* 55:49–112.

Dirnberger, G., W. Keiper, J. Schnakenberg, and H. Stieve. 1985. Comparison of time constants of single channel patches, quantum bumps, and noise analysis in *Limulus* ventral photoreceptors. *Journal of Membrane Biology.* 83:39–43.

Dodge, F. A. B., B. W. Knight, and J. Toyoda. 1968. Voltage noise in *Limulus* photoreceptors. *Science.* 160:88–90.

Fein, A., and J. S. Charlton. 1975. Local membrane current in *Limulus* photoreceptors. *Nature.* 258:250–252.

Frank, T. M., and A. Fein. 1991. The role of inositol phosphate cascade in visual excitation of invertebrate microvillar photoreceptors. *Journal of General Physiology.* 97:697–723.

Fuortes, M. G. F., and S. Yeandle. 1964. Probability of occurrence of discrete potential waves in the eye of *Limulus*. *Journal of General Physiology.* 47:443–462.

Gomez, M., and E. Nasi. 1991. Whole-cell and on-cell patch recordings of the light-sensitive conductance in hyperpolarizing invertebrate photoreceptors. *Biophysical Journal.* 59:530*a*. (Abstr.)

Gorman, A. L. F., and J. S. McReynolds. 1978. Ionic effects on the membrane potential of the hyperpolarizing photoreceptors in scallop retina. *Journal of Physiology.* 275:345–355.

Hamill, O. P., A. Marty, E. Neher, B. Sakman, and F. J. Sigworth. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Archiv.* 391:85–100.

Haynes, L. W., A. R. Kay, and K.-W. Yau. 1986. Single cyclic GMP-activated channel activity in excised patches of rod outer segment membrane. *Nature.* 321:66–70.

Holz, R., and A. Finkelsten. 1970. The water and nonelectrolyte permeability induced in thin lipid membranes by the polyene antibiotics nystatin and amphotericin B. *Journal of General Physiology.* 56:125–145.

Horn, R., and A. Marty. 1988. Muscarinic activation of ionic currents measured by a new whole-cell recording method. *Journal of General Physiology.* 92:145–159.

Johnson, E. C., J. Bacigalupo, C. Vergara, and J. E. Lisman. 1991. Multiple conductance states of the light-activated channel of *Limulus* ventral photoreceptors. Alteration of conductance state during light. *Journal of General Physiology.* 97:1187–1205.

Matthews, G. 1987. Single-channel recordings demonstrate that cGMP opens the light-sensitive ion channel of the rod photoreceptor. *Proceedings of the National Academy of Sciences, U.S.A.* 84:299–302.

McReynolds, J. S., and A. L. F. Gorman. 1970*a*. Photoreceptor potentials of opposite polarities in the eye of the scallop, *Pecten irradians*. *Journal of General Physiology.* 56:376–391.

McReynolds, J. S., and A. L. F. Gorman. 1970*b*. Membrane conductances and spectral sensitivities of *Pecten* photoreceptors. *Journal of General Physiology.* 56:392–406.

Miller, W. H. 1958. Derivatives of cilia in the distal sense cells of the retina of *Pecten*. *Journal of Biophysical and Biochemical Cytology.* 4:227–228.

Nagy, K. 1990. Kinetic properties of single-ion channels activated by light in *Limulus* ventral nerve photoreceptors. *European Biophysics Journal.* 19:47–54.

Nagy, K., and H. Stieve. 1990. Light activated single channels currents in *Limulus* ventral nerve photoreceptors. *European Biophysics Journal.* 18:221–224.

Nasi, E. 1991*a*. Electrophysiological properties of isolated photoreceptors from the eye of *Lima scabra*. *Journal of General Physiology.* 97:17–34.

Nasi, E. 1991*b*. Whole-cell clamp of dissociated photoreceptors from the eye of *Lima scabra*. *Journal of General Physiology.* 97:35–54.

Nasi, E. 1991*c*. Two light-dependent conductances in the membrane of *Lima* photoreceptor cells. *Journal of General Physiology.* 97:55–72.

Nasi, E., and M. Gomez. 1990. Light-dependent ion channels in dissociated photoreceptors of the scallop. *Biophysical Journal.* 57:372*a*. (Abstr.)

Nasi, E., and M. Gomez. 1991. Light-activated channels in scallop photoreceptors: recordings from cell-attached and perfused excised patches. *Biophysical Journal.* 59:540*a*. (Abstr.)

Rae, J., K. Cooper, P. Gates, and M. Watsky. 1991. Low access resistance perforated patch recordings using amphotericin B. *Journal of Neuroscience Methods.* 37:15–26.

Ruknudin, A., M. J. Song, and F. Sachs. 1991. The ultrastructure of patch-clamped membranes: a study using high-voltage electron microscopy. *Journal of Cell Biology.* 112:125–143.

Sokab, M., and F. Sachs. 1991. Quantitative video microscopy of patch clamped membranes: stress, strain, capacitance, and stretch channel activation. *Biophysical Journal.* 59:722–728.

Wong, F., B. Knight, and F. A. Dodge. 1982. Adapting bump model for ventral photoreceptors of *Limulus*. *Journal of General Physiology.* 79:1089–1113.

Yeandle, S. 1958. Evidence of quantized slow potentials in the eye of *Limulus*. *American Journal of Ophthalmology.* 46:82–87.

Zimmerman, A. L., and D. A. Baylor. 1986. Cyclic GMP-sensitive conductance of retinal rods consists of aqueous pores. *Nature.* 321:70–72.