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Spontaneous Activity of the Light-Dependent Channel Irreversibly Induced in Excised Patches from *Limulus* Ventral Photoreceptors

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Summary. We have studied the properties of membrane patches excised from the transducing lobe of *Limulus* ventral photoreceptors. If patches are excised into an "internal" solution that resembles the ionic composition of the cytoplasm, channel activity is typically absent, but can be turned on by cyclic GMP (cGMP). In contrast, if patches are excised directly into sea water and subsequently examined in internal solution, they exhibit a high channel activity in the absence of any second messenger (spontaneous channel activity). Because these patches contained only light-dependent channels when examined before excision and because these spontaneous channels have properties in common with the light/cGMP-dependent channel, we believe that the spontaneously active channels represent light/cGMP-dependent channels that have been damaged by exposure to sea water, perhaps due to proteolysis activated by the high Ca^{2+} levels of the sea water. One type of the spontaneously active channel resembles the light/cGMP-dependent channel in open time, reversal potential, conductance states and voltage dependence. Application of micromolar Ca^{2+} to this channel produces a reversible decrease in the opening rate, indicating a high affinity binding site for Ca^{2+} on this channel. Another type of spontaneously active channel has a conductance state and reversal potential similar to the light/cGMP-dependent channel, but has apparently lost its dependence and sensitivity to Ca^{2+} and voltage.

Key Words *Limulus* photoreceptors · ionic channels · excised patches · spontaneous channel activity

Introduction

Limulus ventral photoreceptors have been an important preparation for the study of invertebrate phototransduction, amenable for electrophysiological recording of both macroscopic and single channel responses. Phototransduction takes place in the rhabdomeric lobe (R-lobe) of *Limulus* ventral photoreceptors (Stern et al., 1982; Payne et al., 1988). The membrane of the microvilli present in this lobe contains rhodopsin, which, when activated by light, initiates a cascade process involving second messengers (*see* Bacigalupo et al., 1990). Cyclic GMP appears to be the second messenger that directly opens the light-dependent channels (Bacigalupo et al., 1991). In dark-adapted conditions, thousands of these channels are opened after a photon activates a single rhodopsin molecule (Bacigalupo & Lisman, 1983).

The light-dependent channel is permeant to cations, and it has two major open conductance states, of 15 and 40 pS, both with mean open times of about 1 msec (Johnson et al., 1991). Although this channel cannot be gated by voltage in the dark, depolarization of the patch in the light profoundly affects its gating properties, greatly increasing its probability of being open (Bacigalupo, Chinn & Lisman, 1986; Johnson et al., 1991).

Membrane patches excised directly into internal solution typically remain silent. Perfusion of these patches with internal solution containing micromolar concentrations of cGMP reversibly activates the light-dependent channels (Bacigalupo et al., 1990; Bacigalupo et al., 1991). We now report that when patches from the R-lobe are excised under different experimental conditions, they present two different types of spontaneously active channels. Neither of these channels requires the presence of a second messenger to open. These patches exhibited only light-dependent channel activity prior to excision. Thus, both types of spontaneously active channels could be induced upon isolation of the patch. In the present work we describe the properties of these excision-induced events and compare them to light/cGMP-dependent channel events. Our results support the view that both types of excision-induced channels are altered forms of the light-dependent channel and that such alterations are somehow produced by the excision procedure. Furthermore, our results lead us to propose that the major and perhaps the only type of ionic channel present

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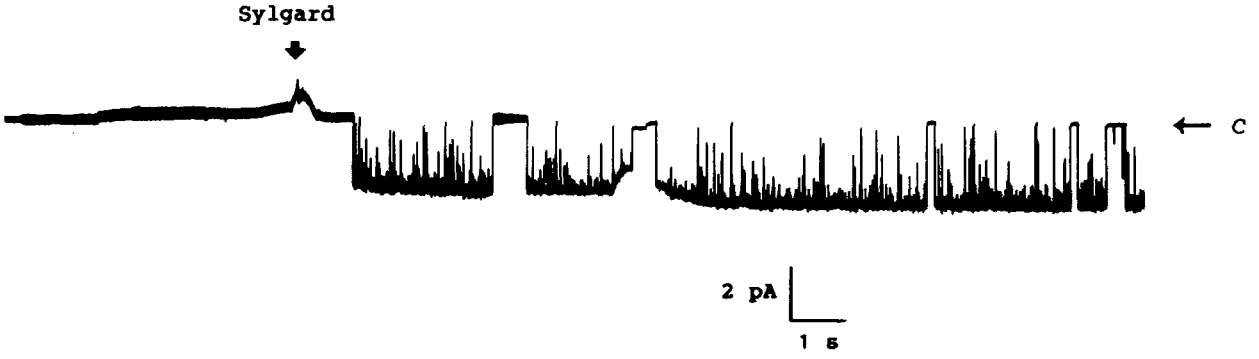

Fig. 1. S-channel activity induced upon touching the patch pipette on Sylgard. The arrow indicates the moment the pipette touched Sylgard at the bottom of the dish. The pipette voltage was maintained at +80 mV. The high current noise in the early part of this recording was induced by the manipulations done within the set-up during this operation. The arrow indicates the closed state of the channel.

on the microvillar membrane is the light-dependent channel; the other channel species found in the ventral photoreceptor (O'Day, Lisman & Goldring, 1982; for a review, *see* Fain & Lisman, 1981) must be chiefly confined to the nontransducing arhabdomeric lobe (A-lobe) of these cells.

Channel activity evoked by patch excision has been described previously in other systems (Chesnoy-Marchais, 1985; Strong et al., 1987*a,b*; Yazejian & Byerly, 1989; McClintock & Ache, 1990).

Some of the results reported in this work have been previously presented in preliminary form (Bacigalupo, Johnson & Lisman, 1987).

Materials and Methods

Ventral nerves obtained from the horseshoe crab *Limulus polyphemus* were treated with Pronase (Calbiochem, La Jolla, CA), according to procedures that are standard for this preparation, and glial cells and connective tissue surrounding individual photoreceptors were removed following the procedure developed by Stern et al. (1982). Patch clamp recordings were performed on the microvillar membrane of the light-sensitive lobe (rhabdomeric lobe). Data were digitized (Instrutech, Elmont, NY), stored in a video cassette recorder and analyzed using pClamp 5.5 (Axon Instruments). Details on the procedure for obtaining gigohm seals and on data analysis have been previously described (Johnson et al., 1991). The probability of being open was estimated as follows from a histogram of the raw digitized current data. The baseline noise was fit with a Gaussian; the points not accounted for by the baseline were integrated and divided by the charge that would have passed through a single continuously open 40-pS channel. This computed quantity is an estimate of the probability of being open multiplied by the number of channels in the patch (typically unknown).

We used artificial sea water (ASW) as external solution (in mM): 425 NaCl, 10 KCl, 10 $CaCl_2$, 22 $MgCl_2$, 26 $MgSO_4$, and 10 Tris at pH 7.8. Control internal solution (control IS) contained (in mM): 300 KCl, 5 bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetate (BAPTA; Molecular Probes), 2 $MgCl_2$, 300 sucrose, and we added $CaCl_2$ to obtain a pCa of 7.0; pH 7.0. Solution containing 10^{-5} M Ca^{2+} or higher did not contain BAPTA. All experiments were done at room temperature.

Patches were either excised into ASW and then transferred into internal solution or, alternatively, directly excised into internal solution. In the latter case, a jet of internal solution was delivered by pressure onto the photoreceptor during the excision of the membrane patch by means of a separate micropipette and the patch could then be exposed to ASW by moving the pipette away from the jet.

Results

Two Types of Spontaneous Channel Activity in Patches Excised from the Rhabdomeric Membrane

Cell-attached patches from the R-lobe membrane are typically silent in the dark (*see* below; Bacigalupo & Lisman, 1983; Bacigalupo et al., 1986; Johnson et al., 1991). When patches are excised in internal solution (IS) they no longer respond to light and are silent over a wide range of voltages, but they normally exhibit channel activity during exposure to IS containing micromolar levels of cGMP, as formerly reported. This cGMP-gated channel is the same as the light-dependent channel (Bacigalupo et al., 1991). This evidence strongly suggests that membrane patches from the R-lobe of ventral photoreceptors contain only light-dependent channels.

On some occasions, however, we observed that a stereotypical channel activity clearly distinguishable from that due to the light/cGMP-dependent channel developed spontaneously in our excised patches. This channel was characterized by having long closed times (the closed state is denoted by the arrows) followed by long openings (inward currents lasting tens of milliseconds on the average; *see* below) during which the channel frequently flickered between the open and closed states (Figs. 1 and 2).

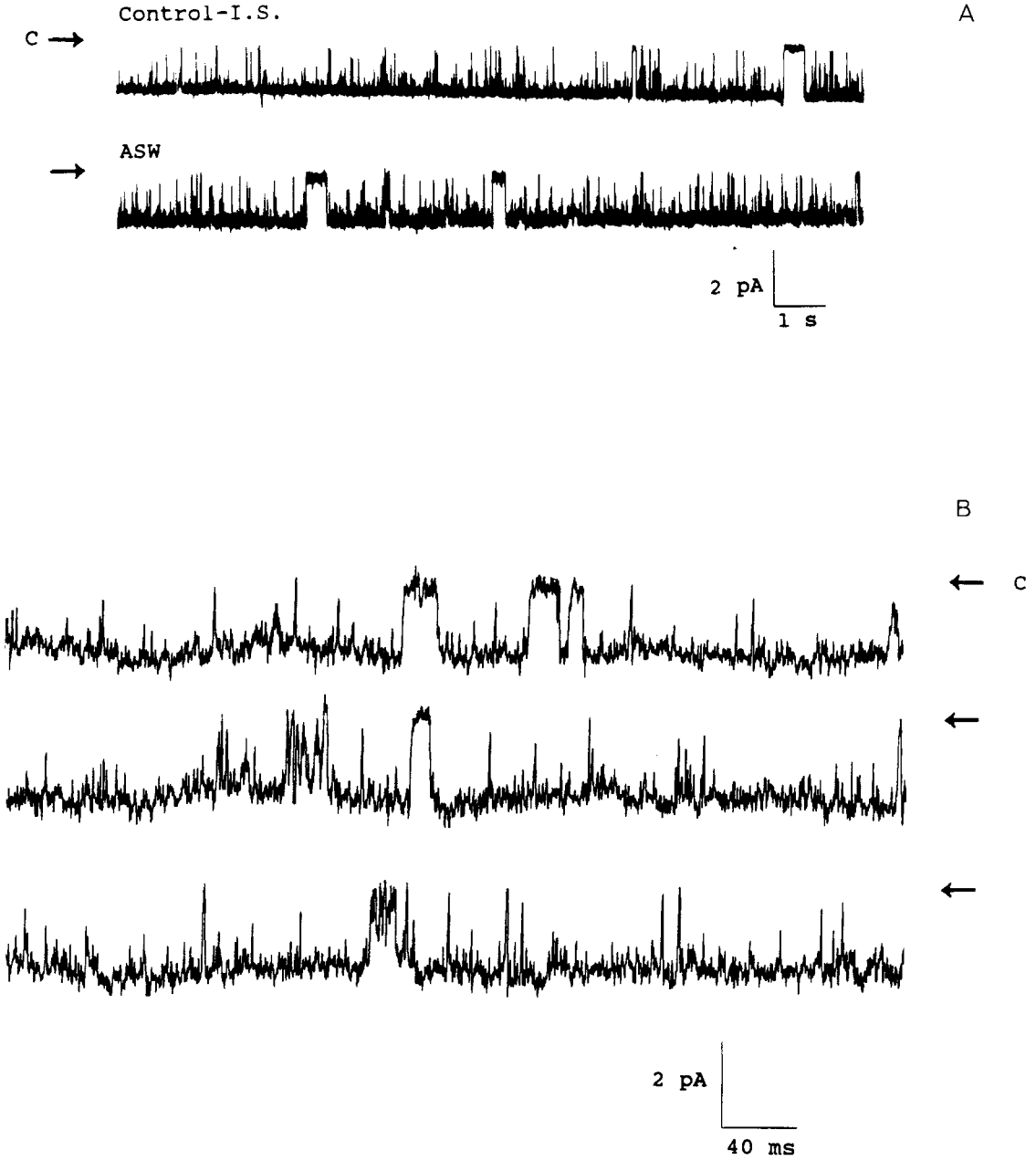

Fig. 2. (*A*) S-channel activity was the same in control-IS (top trace) as in ASW (bottom trace). Pipette potential: +50 mV. Arrows indicate the 0-current level (same patch as in Fig. 4). (*B*) S-channel current shown at a higher time scale. Bandwidth = 3,000 Hz. The arrow indicates the closed state of the channel.

This characteristic open-time kinetics is demonstrated in greater detail subsequently (*see* Fig. 7). We refer to this spontaneously active channel as the **S**-channel (for **slow** channel). It was seen following patch excision. However, in most cases we are uncertain how this activity was induced. Often it appeared to occur spontaneously, without performing any manipulations to the excised patch. But on other occasions, we were able to induce S-channel activity in an excised patch, as illustrated in Fig. 1. In this example, following excision of the patch into ASW there was no channel activity. After approximately 5 min, we gently and briefly touched the pipette tip against the Sylgard covering the bottom of the chamber. Shortly thereafter (~1 sec), S-channel activity developed. In some of these patches S-channel was induced following a very large voltage pulse (+100 mV or more) applied to the pipette, and in the rest of them the S-type activity developed without making any obvious change. It seems very likely

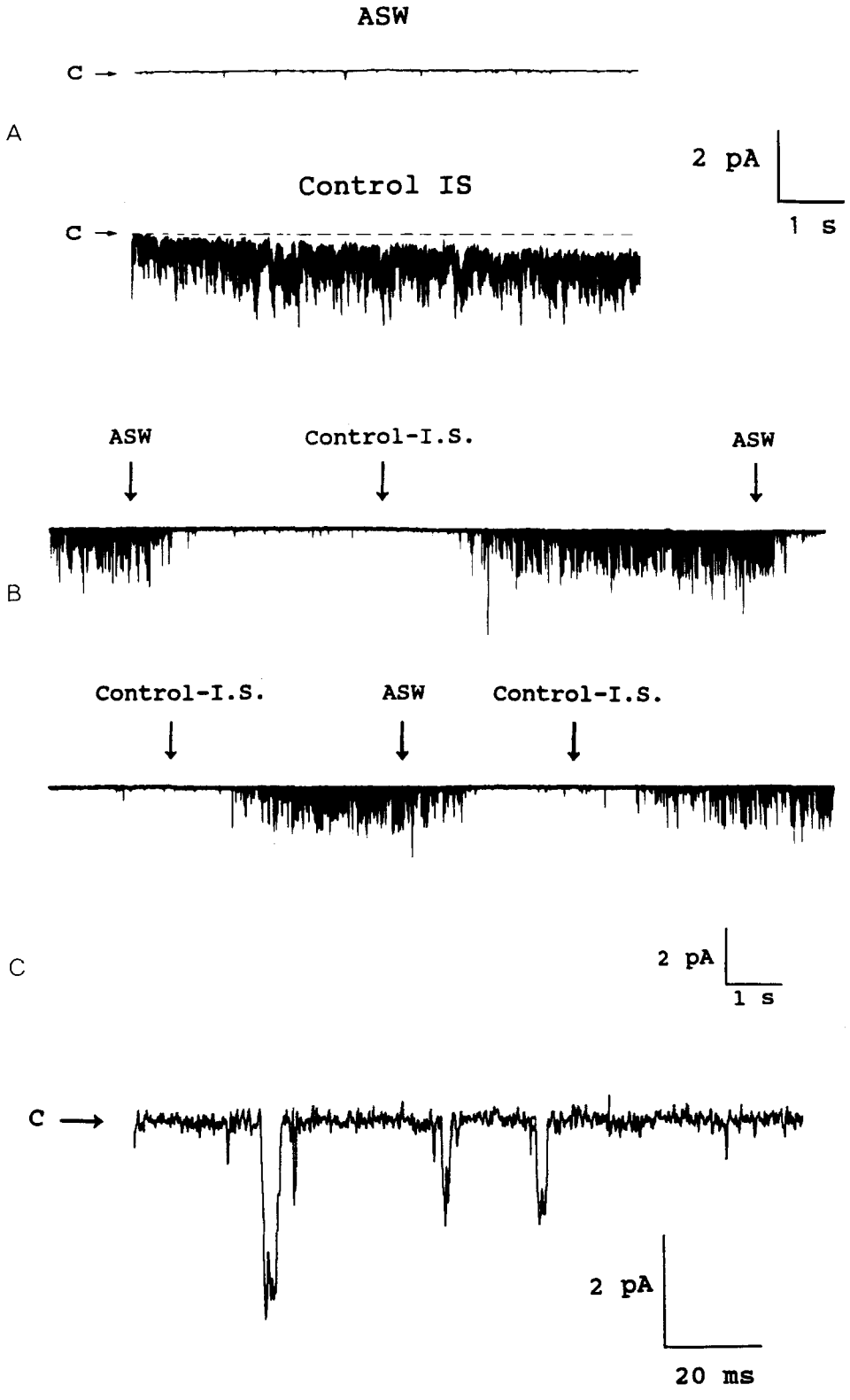

Fig. 3. Effect of ASW on the F-channel. (*A*) ASW suppressed the F-channel activity in an excised patch (top trace), but this channel was highly active in control-IS, with no second messengers added (bottom trace). This recording is representative of most patches in which an F-channel was expressed. (*B*) A different patch having a more moderate channel activity than the one shown in *A*. The channel activity could be reversibly shut down when the IS was replaced for ASW. A continuous current recording is shown. Pipette potential: +50 mV. (*C*) Continuous trace from the same patch as in *B*, showing F-channel activity at a faster time scale. Two current levels of different amplitude are resolved. Bandwidth = 3,000 Hz. The arrow indicates the closed state of the channel.

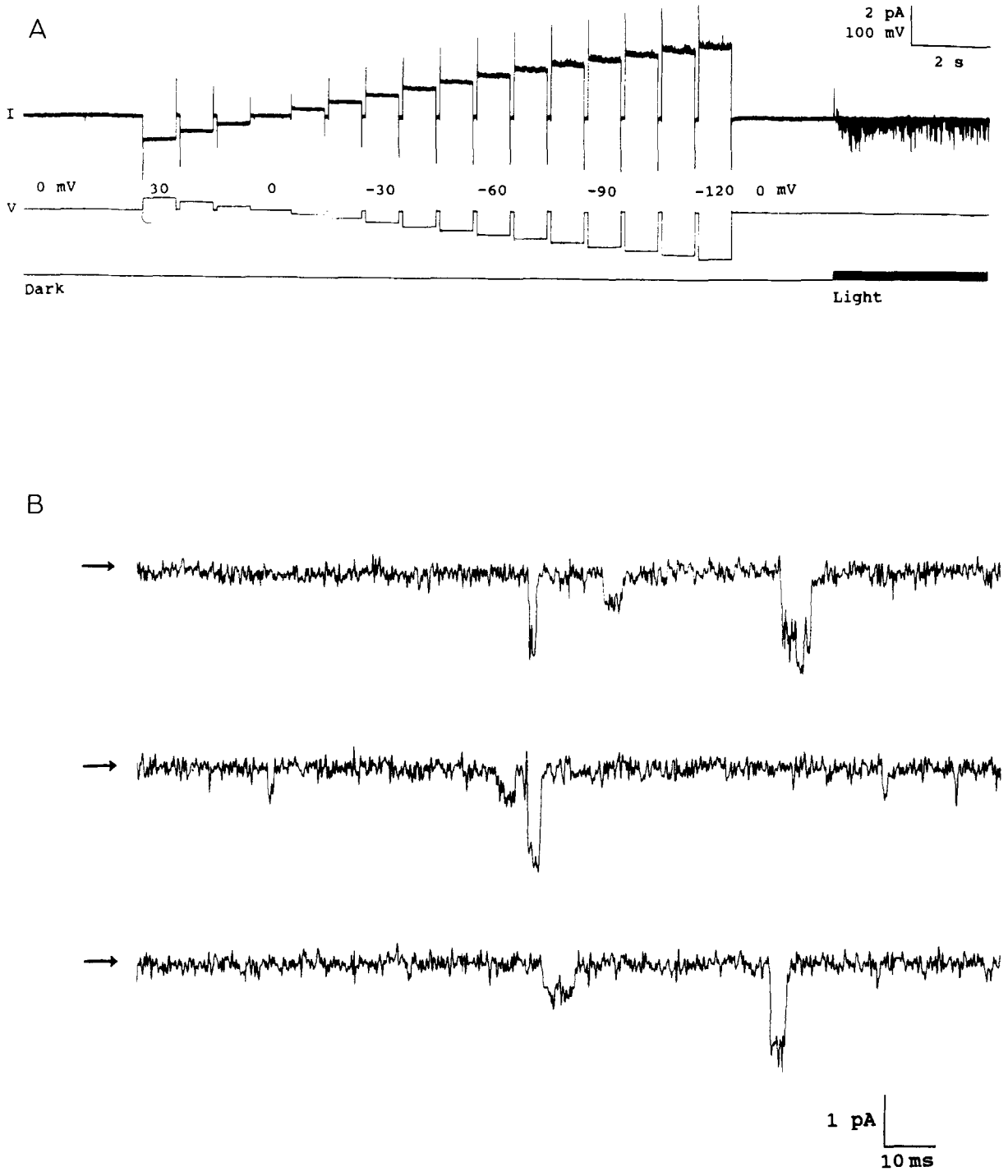

Fig. 4. Light-dependent activity induced by light and not by voltage (same patch as in Fig. 2). (*A*) A series of voltage steps in increments of 10 mV were delivered in the dark to the patch pipette over a wide voltage range. No channel activation was elicited by changing the membrane potential. The top trace corresponds to current and the bottom trace is the voltage monitor. Numbers on the voltage monitor are the voltage values applied within the patch pipette, therefore negative voltages depolarized the patch and 0 voltage corresponded to the photoreceptor resting potential (unknown). A light stimulus was applied to the cell a few seconds after the voltage-pulse protocol ended, eliciting channel activity. (*B*) The single-channel currents during illumination are presented here in more detail in an expanded time scale, in three continuous traces. Two size events can clearly be resolved. Bandwidth = 2,000 Hz. The arrow indicates the closed state of the channel.

that these patches had excised spontaneously and formed a closed vesicle, which was ruptured by voltage or spontaneously (*see* for example Sakman & Neher, 1983; Matthews & Watanabe, 1987). After it was induced, the S-channel activity did not depend on whether internal solution (IS) or ASW were used as the solution bathing the cytoplasmic side of the excised patches (Fig. 2*A*). Regardless of how the S-channel developed, its characteristics were the same. Figure 2*B* displays the S-channel at a fast-time scale.

On other occasions, a different type of spontaneous channel activity arose in patches that were transferred to control IS containing no second messengers, after they had been excised into ASW. These patches were silent in ASW, regardless of the pipette holding voltage, but in IS they presented high channel activity (Fig. 3*A*). This activity ceased if the patch was moved back to ASW or to IS with high free Ca^{2+} concentration (10^{-5} M), but it rapidly reversed upon return to the control-IS (Fig. 3*B*). This type of activity consisted of short inward current events under a pipette voltage of +50 mV (Fig. 3*C*). The magnitude and duration of these events quite closely resembled the light/cGMP-dependent events at comparable transmembrane potentials (see below). However, in contrast to the cGMP-dependent channel, this channel was active in control IS containing no cGMP. We called this spontaneously active channel the **fast** or **F**-channel based on the much shorter mean open time (*see* subsequent section, open time distribution: Fig. 9). The frequency of opening the F-channel was often much higher (5-100 times) than that of light-dependent events observed under bright illumination, comparable to that of the cGMP-dependent events at concentrations of cGMP of 100 μM and also higher than the cGMP-dependent activity usually observed at 10 μM cGMP (Bacigalupo et al., 1991).

In a total of 563 cell-attached patches that we studied from the R-lobe of *Limulus* ventral photoreceptors in physiological saline, we observed no voltage-gated channels at pipette potentials between +30 and −120 mV. Of these patches, 101 (18%) stayed silent with illumination and the remaining 462 (82%) exhibited channel activity during illumination. We excised 276 of the patches; 44 of these excised patches showed S-channel activity and 94 displayed F-channel activity. In all cases, the spontaneous activation was irreversible.

The observation that both types of spontaneous channel events typically developed in patches exhibiting solely light-dependent channel events previous to excision suggested that they are both altered forms of the light-dependent channel. Therefore, we examined the properties of both the S- and the F-channels and compared them with those of the light-dependent channel to establish whether they all correspond to the same channel type.

S-Type Spontaneously Active Channel

Before excision, we routinely examined the patch to establish what channels were present in it. In order to determine whether voltage-gated channels were present, the patch was depolarized in the dark prior to excision by stepping up the pipette voltage by as much as 120 mV. No channel activity was normally observed in our patches during hyperpolarization or depolarization in the dark. We then stimulated the cell with light to determine whether the patch exhibited light-dependent channel activity. After finishing this protocol, we excised the patch following one of the procedures described above. We compared the properties of the channels observed in the excised patch with those of the light-dependent channels observed in the same patch prior to excision.

Figure 4*A* shows a cell-attached patch, in which the current over a wide voltage range (pipette potential = +30 to −120 mV) was examined in the dark. The patch remained silent at all potentials. Channel activity was then induced by a light stimulus. Single-channel events of two sizes can be observed (Fig. 4*B*), as previously described (Johnson et al., 1991). These events correspond to the two major conductance states of the light-dependent channel. A probability density histogram of current amplitudes for this patch shows two peaks, at about 0.8 and 2.4 pA emerging from baseline (Fig. 5*A*). This indicates that there were two types of light-induced channel events in the patch. Current-voltage relations for both event types are displayed in Figure 5*B*. The slope conductances of the *I-V* curves revealed unitary conductance values of 14 and 41 pS (Fig. 5*B*) and currents for each event reversed at a pipette potential of 43 mV (patch potential = 43 mV depolarized from the photoreceptor membrane potential, which was not measured). The membrane potential of *Limulus* ventral photoreceptors under sustained bright illumination is between −30 and −40 mV, and therefore the reversal potential value is around 10 mV above zero (Bacigalupo & Lisman, 1983; Johnson et al., 1991).

After excision into internal solution, this patch developed the S-channel activity presented in Fig. 2. Figure 6*A* illustrates the probability density histogram of current amplitudes for this patch. The histo-

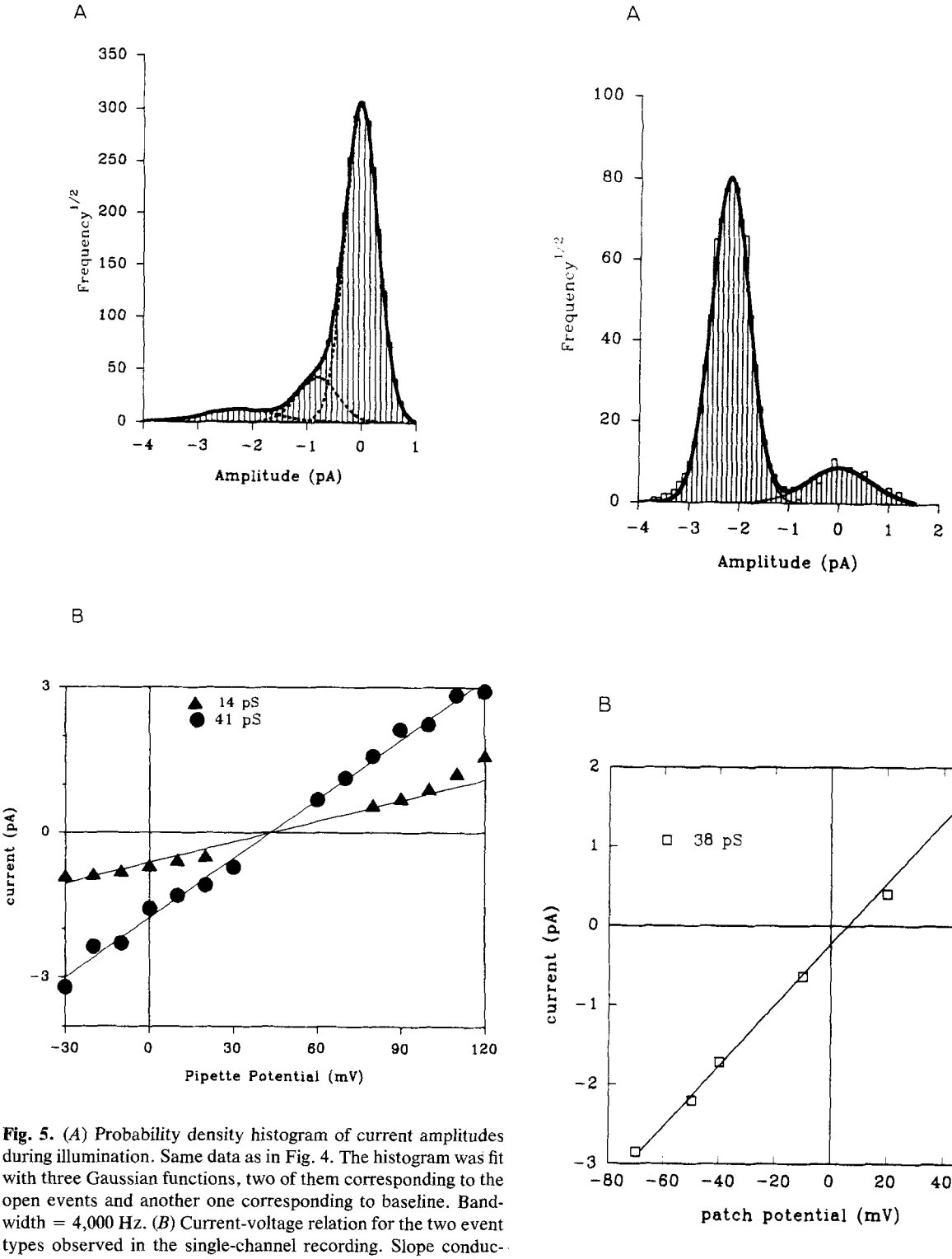

Fig. 5. (*A*) Probability density histogram of current amplitudes during illumination. Same data as in Fig. 4. The histogram was fit with three Gaussian functions, two of them corresponding to the open events and another one corresponding to baseline. Bandwidth = 4,000 Hz. (*B*) Current-voltage relation for the two event types observed in the single-channel recording. Slope conductances: 14 (▲) and 41 pS (●). The abscissa represents the voltage in the patch pipette. Therefore, when this potential is 0 mV the patch membrane is at the photoreceptor membrane potential. The actual value of the membrane potential is unknown, but it is likely between −30 and −40 mV and the reversal potential between +10 and +20 mV (Bacigalupo & Lisman, 1984; Johnson et al., 1991).

Fig. 6. (*A*) Probability density histogram of current amplitudes for the S-channel in same patch as in Fig. 2. Only one peak is observed for the open time of this channel. Pipette potential: +50 mV. Bandwidth = 4,000 Hz. (*B*) Current-voltage relation for the S-channel. A single curve of 38 pS was fit to the experimental points. The reversal potential for this channel is about 7 mV.

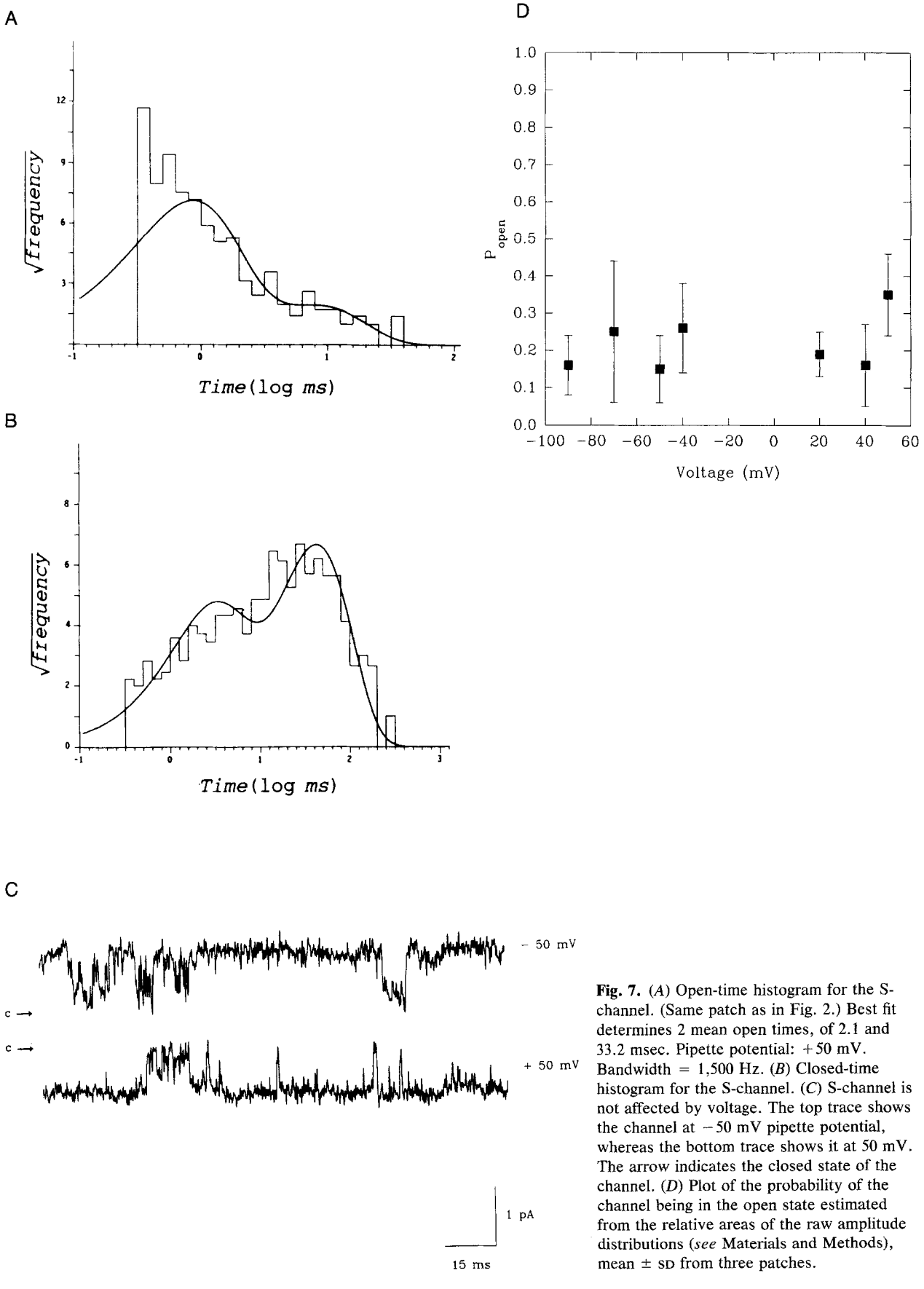

Fig. 7. (*A*) Open-time histogram for the S-channel. (Same patch as in Fig. 2.) Best fit determines 2 mean open times, of 2.1 and 33.2 msec. Pipette potential: +50 mV. Bandwidth = 1,500 Hz. (*B*) Closed-time histogram for the S-channel. (*C*) S-channel is not affected by voltage. The top trace shows the channel at −50 mV pipette potential, whereas the bottom trace shows it at 50 mV. The arrow indicates the closed state of the channel. (*D*) Plot of the probability of the channel being in the open state estimated from the relative areas of the raw amplitude distributions (*see* Materials and Methods), mean ± SD from three patches.

gram is well fit by two Gaussian functions, one corresponding to baseline and another having a peak at −2.2 pA, using a −50 mV holding potential. Figure 6*B* shows a current-voltage relation for the S-channel. Its slope conductance is 38 pS, quite close to the larger conductance state (41 pS) of the light-dependent channel. Unlike the light-dependent channel, however, the S-channel appears to have only a single conductance state. The reversal potential of about +7 mV is in the same range as that of the light/cGMP-dependent channel (Fig. 5*B*) and the macroscopic light-dependent current (Bacigalupo et al., 1986; Johnson et al., 1991; Bacigalupo et al., 1991).

The open time histogram is shown in Figure 7*A*. Two exponentials were needed to fit the data, yielding mean open times of 2.1 and 33.2 msec. The former value is close to the mean open time of the major subconductance states of the light-dependent channel (about 1 msec), whereas the latter value is one order of magnitude larger. Fig. 7*B* illustrates the closed-time histogram of the S-channel events. The S-channel is independent of voltage, as illustrated in Fig. 7*C*. This figure shows channel recordings from the same patch at two different patch potentials, −50 and +50 mV (pipette potentials +50 and −50 mV, respectively). We observed no obvious difference in channel kinetics within the voltage range we explored (150 mV), unlike the light/cGMP-dependent channel in cell-attached patches, whose open time is strongly voltage-dependent (*see* below; Bacigalupo et al., 1986; Johnson et al., 1991; Bacigalupo et al., 1991). In three patches examined under similar conditions, we observed that the probability of being open was independent of patch potential (Fig. 7*D*). On the other hand, the probability of being open of the light/cGMP-dependent channel increases substantially at positive patch potentials (Bacigalupo et al., 1991; Johnson et al., 1991).

Therefore, the S-channel has important characteristics in common with the light-dependent channel, as well as some major differences with it. Its unitary conductance is quite similar to the 40 pS conductance state of the light-dependent channel, while no significant activity of the small (14 pS) conductance state is observed. In addition, both channels have a very similar reversal potential. On the other hand, their kinetics is different and the S-channel lacks the characteristic voltage dependence of the light/cGMP-dependent channel. The S-channel was the only channel present in the excised membrane patches where it was observed, whereas prior to excision the only channel present in the patches was the light-dependent channel. These observations make it likely that both channels are different forms of the same protein.

F-Type Spontaneously Active Channel

Previous to excision, this patch displayed no channels other than the light-dependent channel (*not shown*). The F-channel exhibits important similarities as well as some differences with the light-dependent channel. F-channel activity is shown at high temporal resolution in Fig. 3*C*. Events of two amplitudes can be distinguished, which closely resemble those of the light-dependent channel. The probability density histogram of current amplitude for the F-channel events shows 2 peaks, at −4.0 and −1.5 pA, under a pipette potential of +80 mV (Fig. 8*A*). The current-voltage relations for both events are shown in Fig. 8*B*. The slope conductances are 15 and 41 pS, values virtually identical to those of the two major states of the light-dependent channel. In cell-attached patches, the two conductances have been shown to be subconductance states of a unique class of light-dependent channel. One evidence for this was the occurrence of transitions between such conductance levels (Johnson et al., 1991). We observed similar transitions in F-channel recordings (Fig. 8*C*).

The open-time distributions for both conductance levels can be fit well with one exponential (Fig. 9*A* and *B*), with mean open-time values of 1.1 and 0.9 msec for the 15 and the 41 pS states, respectively. These values are quite similar to those previously reported for the light-dependent channel (Johnson et al., 1991). The close-time distribution for the F-channel was fit using two time constants, same as for the light-dependent channel. The short time constant (0.4 msec) may reflect flickering of the channel to the close state (Fig. 9*C*). A distinguishing feature of the light/cGMP-dependent channel is the dramatic increase of its probability of being in the open state at strongly depolarizing voltages (Bacigalupo et al., 1986; Johnson et al., 1991; Bacigalupo et al., 1991). The spontaneously active F-channel in the same patch after excision presents a very similar voltage dependence (Fig. 9*D*). The channel spends a considerably longer time in the open state at depolarizing voltages than at hyperpolarizing voltages.

The similarity of the conductance and kinetic properties of the light/cGMP-dependent channel and the F-channel supports the view that the F-channel and the light/cGMP dependent channel correspond to the same channel protein, the F-channel being an altered form of the normal channel.

Internal Ca^{2+} Reduces F-Channel Activity

Calcium ions have an important role in *Limulus* phototransduction. They have been shown to be a mes-

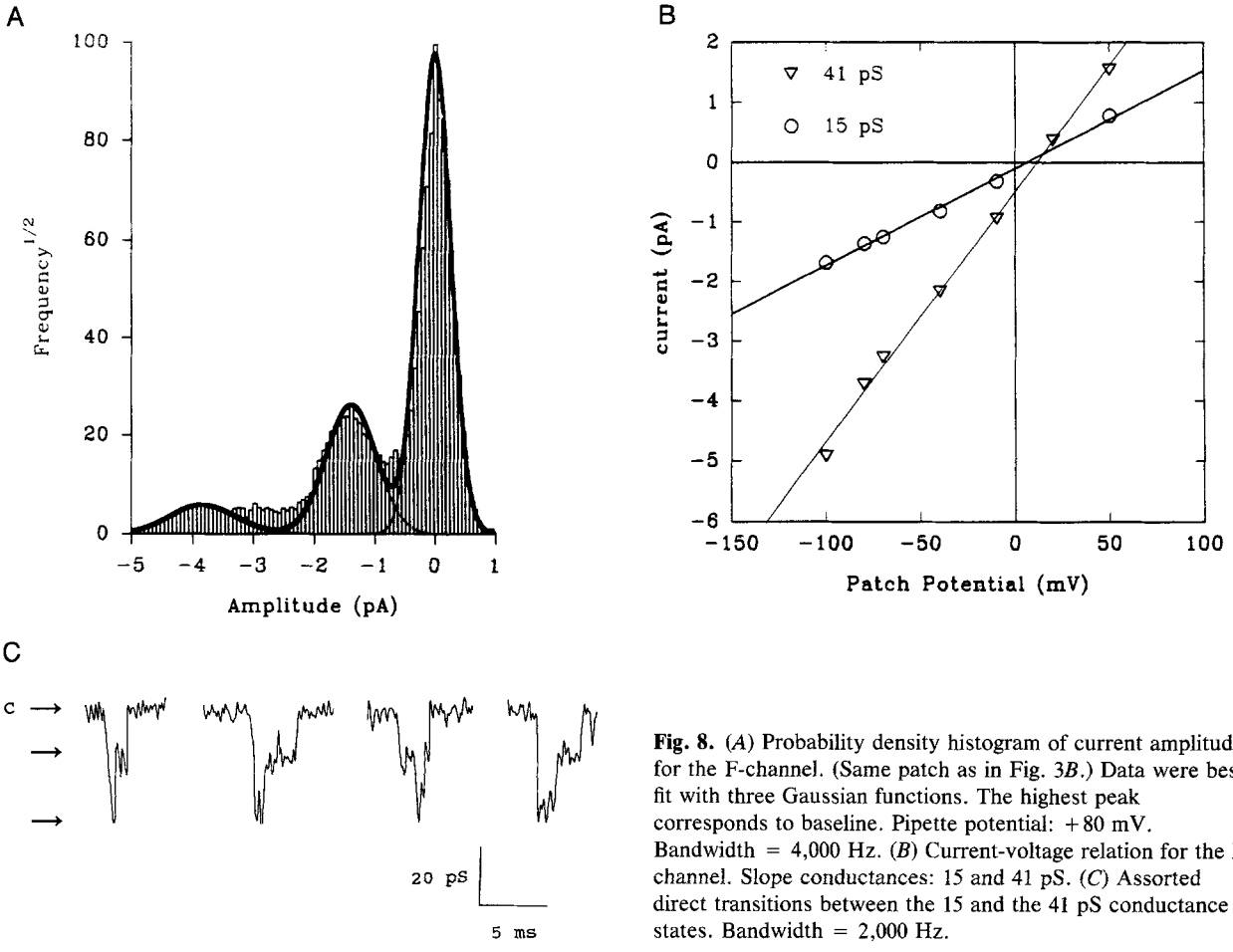

Fig. 8. (*A*) Probability density histogram of current amplitudes for the F-channel. (Same patch as in Fig. 3*B*.) Data were best fit with three Gaussian functions. The highest peak corresponds to baseline. Pipette potential: +80 mV. Bandwidth = 4,000 Hz. (*B*) Current-voltage relation for the F-channel. Slope conductances: 15 and 41 pS. (*C*) Assorted direct transitions between the 15 and the 41 pS conductance states. Bandwidth = 2,000 Hz.

senger in light adaptation and excitation (Lisman & Brown, 1971; Bolsover & Brown, 1985; Payne et al., 1988). One possible site of action of Ca^{2+} on the transduction cascade is the light-dependent channel. Since the F-channel appears to be a spontaneously active form of the light-dependent channel, we examined the effect of Ca^{2+} on the F-channel activity in order to evaluate the feasibility of a direct regulatory action of Ca^{2+} on the light-dependent channel. Figure 10 illustrates the effect of calcium on the spontaneously active channel in an excised patch. It shows records of the F-channel in IS with three different free Ca^{2+} concentrations: 0.1 μM, 10 μM and 1 mM. Raising Ca^{2+} concentration caused a pronounced reduction in channel activity. We observed very few events in IS containing 10 μM Ca^{2+}, a free Ca^{2+} level exceeded in the photoreceptor cytosol during bright illumination (Levy & Fein, 1985; O'Day & Gray-Keller, 1989). The bottom trace of the figure shows that high channel activity resumed when the patch was moved back to control IS demonstrating that the effect of Ca^{2+} was reversible. Similar results were observed in seven other experiments. These results show that the alteration of the channel by Ca^{2+} occurred within the Ca^{2+} concentration range of physiological significance (Levy & Fein, 1985; O'Day & Gray-Keller, 1989), consistent with a regulatory role for Ca^{2+} directly on the light-dependent channel.

Discussion

Patch clamp studies on the R-lobe membrane of *Limulus* ventral photoreceptors have shown that there is normally no channel activity in cell-attached patches in the dark and that light activates a single type of light-dependent channel (Bacigalupo & Lisman, 1983; Bacigalupo et al., 1986; Johnson et al., 1991). Exposure of the internal side of these patches to micromolar concentrations of cGMP, following excision into IS, could induce the activation of the light-dependent channels while Ca^{2+} could not (Bacigalupo et al., 1990; Bacigalupo et al., 1991). We report in the present paper that, after excision, a

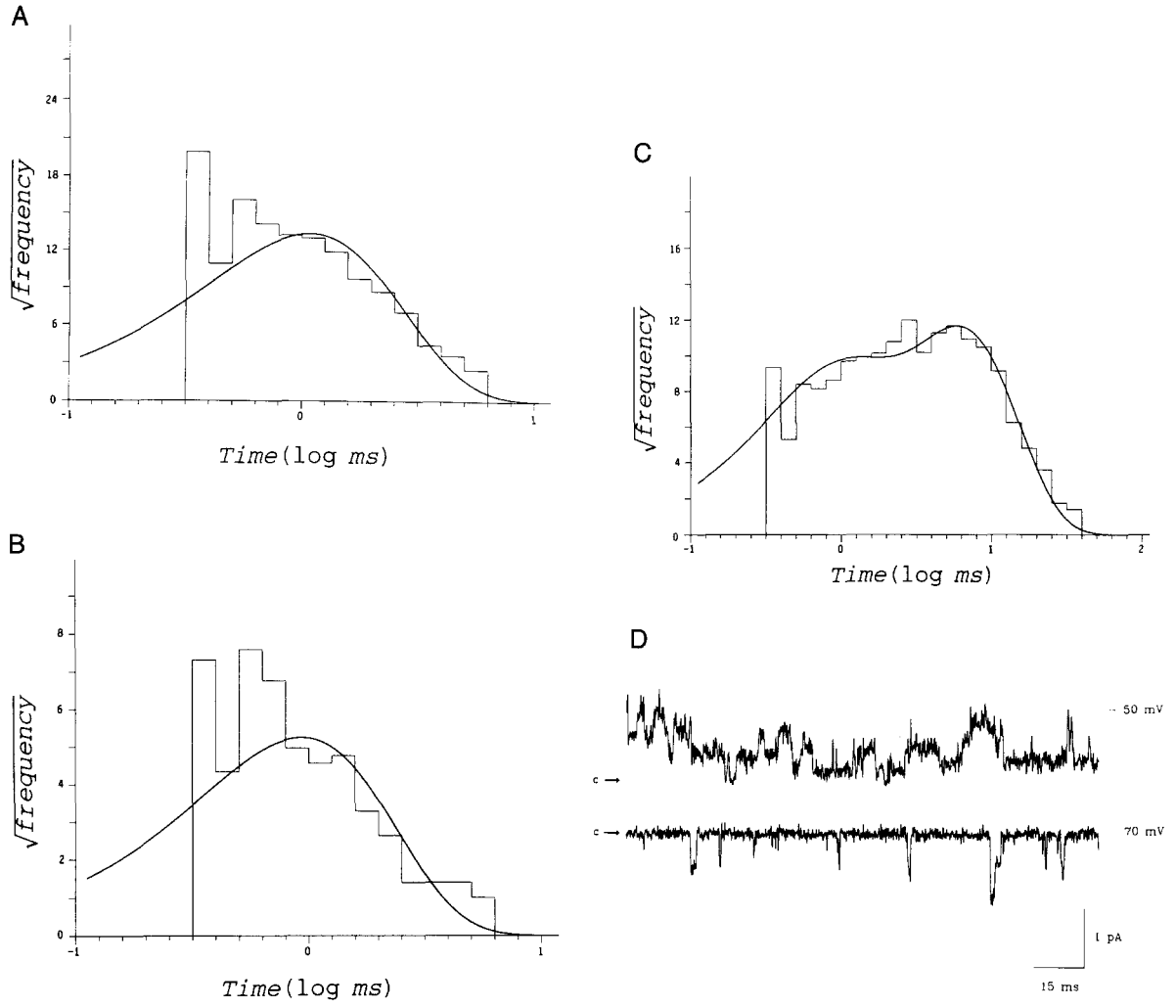

Fig. 9. (*A*) Open-time distribution for the 15-pS conductance state of the F-channel. Mean open time: 1.1 msec. (*B*) Open-time distribution for the 41-pS conductance state of the F-channel. Mean open time: 0.9 msec. (*C*) Distribution for all closed times of the F-channel. Time constants: 0.4 and 4.5 msec; same data as in Fig. 8. Bandwidth = 2,000 Hz. (*D*) Voltage dependence of the probability of being open of the F-channel. Top trace: channel activity at a pipette potential of −50 mV. Bottom trace: channel activity at 70 mV. The arrow indicates the closed state of the channel.

fraction of the patches developed irreversible spontaneous channel activity in the absence of cGMP. This spontaneous activation was sometimes associated with the excision procedure or manipulations of the patch, but on other occasions it occurred without any mediating manipulation. This irreversible spontaneous activity could be either one of two easily distinguishable types. We compared the spontaneous channels with the light-dependent channel to establish the nature and significance of the spontaneously-active channels observed in excised patches. The two types of spontaneously active channels were clearly different than the light-dependent channel, although each of them has important features in common with the light-dependent channel.

The similar features between the two spontaneous channel types and the light-dependent channel, in addition to the fact that excised patches containing spontaneously active channels presented only light-dependent channels prior to excision, support the possibility that both the F- and S-type channels are artificially induced anomalous expressions of the light/cGMP-dependent channel.

F-Channel

The F-channel activity consists of two size events, both quite similar to the two substrates of the light-dependent channel. The mean open time of both event types is approximately the same as that of the

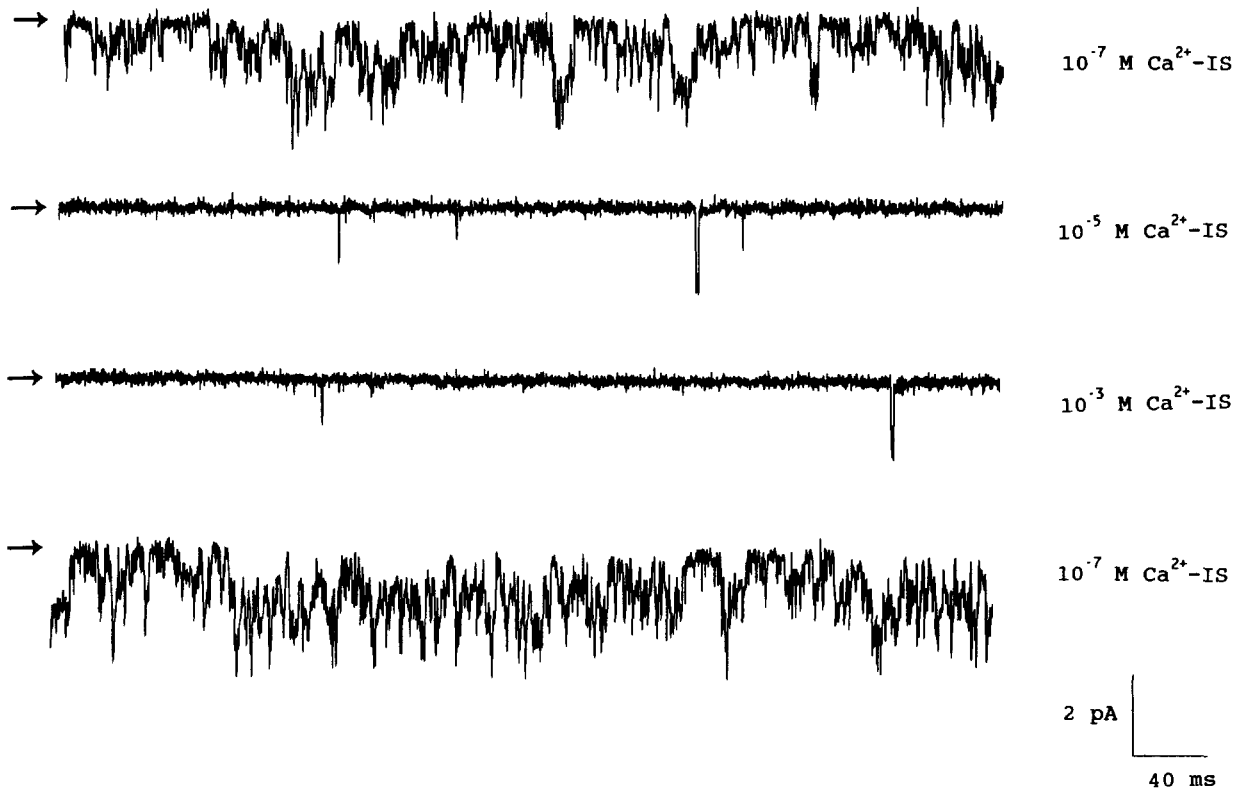

Fig. 10. Suppression of F-channel activity by Ca^{2+}. High activity of the channel in control-IS (top trace) decreased in the presence of 10^{-5} M Ca^{2+}-IS (second trace) or 10^{-3} M Ca^{2+}-IS (third trace), but recovered when the patch was returned to control-IS (bottom trace). Bandwith = 1,500 Hz. The arrow indicates the closed state of the channel.

light-dependent channel. In addition, the F-channel has a voltage dependence closely resembling that of the light-dependent channel (*see* Johnson et al., 1991). The major difference between both channel types is that the F-channel is active in the absence of second messenger, whereas the light-dependent channel requires second messenger—likely cGMP (Bacigalupo et al., 1990; Bacigalupo et al., 1991)—to open. These observations suggest that the F-channel and the light-dependent channel are different forms of the same channel and that the F-channel results from some alteration of the light-dependent channel, possibly at a cGMP-binding site. This binding site could be part of a channel made of a single type of polypeptide chain—as the vertebrate rod photoreceptor light-dependent channel (Kaupp et al., 1989)—or it could be located in a separate subunit of the protein that may be lost from the channel protein in the excised patch. In either case, it is likely that excision of the patch into ASW makes the channel available to attack by proteases. These proteases may become activated by exposure to the high Ca^{2+} levels of ASW. Ca^{2+}-activated proteases have been found in invertebrate photoreceptors (Oldenburg & Hubbel, 1990). We have observed that when patches were excised into IS and then transferred to ASW, F-channel activity was not induced. This situation could be explained if the Ca^{2+}-dependent protease detached from the patch after excision. This would also explain why a spontaneous activation of the channel was not induced after a patch containing cGMP-dependent channels was exposed to millimolar Ca^{2+} concentration following excision into IS (Bacigalupo et al., 1991).

It is interesting that while the probability of the F-channel to be open is often high compared with the normal channel under bright illumination or under rather elevated concentrations of cGMP, the two channels have roughly the same mean open time. The reason for this is that the frequency of opening of the F-channel is much higher. The F-channel activity occurs in the absence of cGMP. We suggested above that the F-channel is a form of the light/cGMP-dependent channel that has lost its cGMP sensitivity, based on the similar features of both channels. Thus, a reasonable possibility is that the mean open time of the light-dependent channel is not determined by the dissociation constant of cGMP from

the channel, but by some other intrinsic feature of the channel protein.

Another interesting aspect of the F-channel form of the light-dependent channel is its Ca^{2+}-dependence, because of the possible implications it may have for understanding the role of this ion in light-adaptation in *Limulus* photoreceptors. Calcium ions have been shown to mediate light-adaptation in *Limulus* photoreceptors (Lisman & Brown, 1971), and to be an important factor in determining the normal kinetics of the light response (Payne & Fein, 1986). The $[Ca_i^{2+}]$ at which the F-channel activity was affected falls within the range in which $[Ca_i^{2+}]$ varies during bright illumination in the ventral photoreceptor (Levy & Fein, 1985; O'Day & Gray-Keller, 1989). The F-channel was active at 0.1 μM free Ca_i^{2+} and lower, but it was inactive when free Ca^{2+} levels were raised above 1 μM. This observation of the calcium sensitivity of the F-type channel activity may have important implications for adaptation. Our observations suggest that the light-dependent channel has at least one Ca^{2+} regulatory site, so that as the Ca^{2+} levels increase during bright lights, channels would close, decreasing the effect of light in opening these channels. If fewer channels are available to be opened by cGMP under elevated Ca^{2+} conditions, the sensitivity of the cell to light would be reduced. Preliminary evidence with the cGMP-sensitive channel activity indicates that there may be a calcium regulatory site on the channel (Bacigalupo et al., 1991). We have observed that the cGMP-dependent activity subsided much faster when the cGMP-IS bathing the patch was replaced by 10 μM Ca^{2+}-IS rather than 0.1 μM CA^{2+}-IS (Bacigalupo et al., 1990). Further studies on the effect of Ca^{2+} on the cGMP-dependent channel in excised patches are necessary for establishing the effect of Ca^{2+} on the light-dependent channel and its possible physiological role. In addition to reducing the gain by blocking the light-dependent channel, calcium affects the other components of the transduction cascade that contribute to gain reduction (Bolsover and Brown, 1985; Payne & Fein, 1986), although the nature of such components still remains to be determined. cGMP-dependent channels of vertebrate rod and cone photoreceptors have been shown to be highly sensitive to divalent cations (Haynes, Kay & Yau, 1986; Zimmermann & Baylor, 1986; Haynes & Yau, 1990) and recent evidence suggests the existence of two Ca^{2+}-regulatory sites on those channels (Tanaka & Furman, 1991).

It has previously been reported that pressure injection of inositol trisphosphate and Ca^{2+} into *Limulus* ventral photoreceptors activated the light-dependent conductance in the dark (Payne, Corson & Fein, 1986). This observation raised the possibility of a direct action of Ca^{2+} in opening the light-dependent channel. However, it has been shown that the light dependent-channel is opened by cGMP and not by Ca^{2+} in isolated patches from *Limulus* ventral photoreceptors (Bacigalupo et al., 1991). Furthermore, our observation presented here that the F-channel activity is blocked by Ca^{2+}, is inconsistent with the possibility that this directly causes the opening of the light-dependent channel, but rather suggests that the excitatory effect of Ca^{2+} is exerted at some other step in the transduction cascade. Recent evidence indicates that Ca^{2+} regulates cyclic-GMP metabolism and that the excitation via the PI-Ca^{2+} pathway is through acting on Ca^{2+} metabolism. Recent evidence indicates that Ca^{2+} regulates cyclic-GMP metabolism and that excitation induced via Ca^{2+} pathway may be explained by an effect of Ca^{2+} on cGMP metabolism (Johnson, 1990; O'Day, Johnson & Baumgard, 1991; Johnson & O'Day, 1992).

The S-Channel

The S-channel has more differences with the light-dependent channel than the F-channel. Both channels have similar reversal potentials and one similar conductance state of approximately 40 pS, but the S-channel appears to lack the 14-pS state. The S-channel is insensitive to cGMP and to voltage, and its kinetics is quite different from that of the light-dependent channel. One of the two S-channel mean open times is an order of magnitude larger than the mean open times of both conductance states of the light/cGMP-dependent channel and the F-channel. And unlike the F-channel, the S-channel is insensitive to Ca^{2+}. Thus, if the F- and the S-channels are altered forms of the normal light-dependent channel, our results would suggest that while the F-channel has simply lost the cGMP sensitivity, the S-channel form in addition has lost its sensitivity to Ca^{2+} and to voltage. This would suggest further that these three regulatory features of the channel may reside on separate functional domains of the protein that can be independently modified. It remains unclear what induces the light-dependent channel to adopt the S-channel form, but as in the case of the F-channel, a protease attack is a likely possibility. We have not observed transitions from the F-channel form into the S-channel form that could indicate that the S-channel is a more extreme product of a protease attack on the light-dependent channel than the F-channel. This would require that the proteases remain with the patch after excision. This is consis-

tent with our explanation of why we do not observe patches containing cGMP-dependent channels turning into spontaneously active patches after exposing them to ASW (see above).

To our knowledge, this is the first case of a spontaneously active channel that appears to be an altered form of a physiologically relevant channel. It appears likely that through further understanding of the nature of the alteration of the channel that leads to its spontaneous activation, it will become possible to gain insight on the protein domains that are involved in the functional aspects of the light/cGMP-dependent channel.

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