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Calcium and related channels in fertilization and early development of *Fucus*

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SUMMARY

Unfertilized eggs of *Fucus serratus* are primed to respond rapidly to the fertilizing sperm. The unfertilized egg plasma membrane is excitable due to the presence of voltage-regulated Ca^{2+} and K^+ channels. Sperm–egg interaction elicits a fertilization potential as the first observable fertilization event. It is speculated that sperm-gated Na^+ channels are responsible for the initial depolarization phase, leading to opening of Ca^{2+} channels, allowing Ca^{2+} influx and further depolarizing the membrane to the threshold for outward K^+ channels. K^+ efflux repolarizes the membrane and the zygote plasmalemma quickly becomes dominated by a large K^+ conductance. The involvement of Ca^{2+} in axis formation and fixation is not clear. Ca^{2+} carries a proportion of the inward current at the future rhizoid pole and asymmetric ^{45}Ca influx has been detected in polarizing zygotes. However, there is no requirement for external Ca^{2+} in axis fixation. In contrast, Ca^{2+} influx is required for expression of polarity and rhizoid growth. New developments in patch clamping can now enable localized areas of the plasma membrane in polarized cells to be studied. So far, both inward and outward single channel currents have been observed in the growing rhizoid tip, most probably carrying Cl^- and K^+ respectively. These channels can be related to the currents identified by previous studies using the extracellular vibrating probe.

1. INTRODUCTION

Eggs and zygotes of members of the brown algal order Fucales show several fundamental developmental features which are common to many plant systems. These include the activation of a haploid female gamete by sperm leading to the production of a zygote with typical plant features including the production of a cell wall, generation of turgor, formation and fixation of a polar axis and polarized tip growth of the rhizoid cell. Ca^{2+} has been implicated in all of these processes and here we discuss the essential evidence for the role of Ca^{2+} in early development. The plasma membrane plays a central role in all of these processes although the involvement of Ca^{2+} and Ca^{2+}-related channels in this control has only recently begun to be critically investigated.

Unfertilized eggs and zygotes have fundamentally different signalling-response requirements. The unfertilized egg is primed to respond rapidly to a single specific stimulus (the fertilizing sperm), beginning with the generation of the fertilization potential and leading ultimately to the resumption of the arrested cell cycle and the production of a walled zygote, the primary aim of which, under normal circumstances, is to fix a polar axis in response to the direction of the prevailing incident light. In contrast to the almost immediate cascade of events following fertilization, the formation and fixation of the polar axis requires the presence of a prolonged stimulus and occurs over several hours (Whitaker & Lowrance 1936). This dramatic change in strategy occurs within a few minutes of activation of the egg. Despite these fundamental differences, the primary stimuli (sperm and light) both act at the plasmalemma (Jaffe 1958; Brawley 1991; Taylor & Brownlee 1992*a*).

2. EGG ACTIVATION

(*a*) *Plasma membrane channels in unfertilized eggs*

The fertilization potential is the first indication of fertilization in *Fucus* eggs and shows similarities to several vertebrate and invertebrate systems with respect to magnitude, pattern and duration (e.g. sea urchin (Chambers & Armendi 1979) and *Xenopus* (Kline & Nuccitelli 1987)). A role for the fertilization potential in the prevention of polyspermy has been postulated in *Fucus* (Brawley 1991) on the basis of experiments where substitution of external Na^+ for N-methyl glucamine reduced the magnitude and duration of the fertilization potential and increased the incidence of polyspermy. We have investigated the ion channels and currents likely to underlie the fertilization potential in *Fucus serratus* using a single electrode voltage and current clamp (switch clamp) technique (Finkel & Redmann 1984) which allows voltage or current clamping with a single intracellular electrode and thus minimizes impalement damage and non-specific leaks associated with two electrode voltage clamping (Weisenseel & Jaffe 1974; Taylor & Brownlee 1992*a*).

The unfertilized egg is excitable. In current clamp, injection of depolarizing current pulses elicits an action potential spike (figure 1*a*). The duration of this

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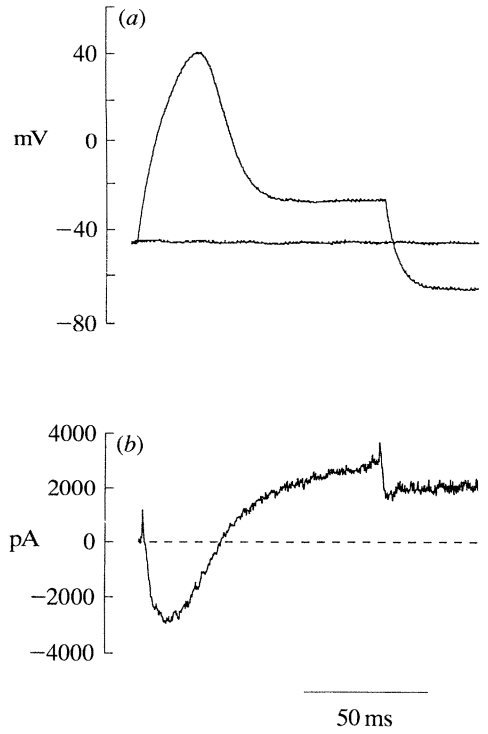

Figure 1. (*a*) Voltage response of an unfertilized *Fucus serratus* egg to injection of a 1 nA, 100 ms depolarizing current in current clamp. (*b*) Inward and outward currents evoked in response to depolarizing voltage clamp with a single electrode. The voltage was held at -50 mV and clamped to +10 mV for 100 ms.

spike is around 50 ms and is followed by a repolarization phase and a prolonged post-stimulus hyperpolarization. The action potential is dependent on the presence of external Ca^{2+} or Ba^{2+} and can be blocked by La^{3+}. The K^+ channel blocker tetraethyl ammonium (TEA) blocked the recovery phase of the action potential.

The currents underlying the action potential can be studied in voltage clamp. The inward current responsible for the depolarizing phase of the spike is activated by depolarization (figure 1*b*). This current is dependent on external Ca^{2+} and can be carried by Ba^{2+} but not Mg^{2+}. Sr^{2+} and La^{3+} both blocked this current, although the Ca^{2+} channel blockers verapamil and nifedipine, which have been shown to be effective in blocking Ca^{2+} channels in other plant systems (for recent reviews see Tester 1990; Johannes *et al.* 1991; Schroeder & Thuleau 1991), had no effect at concentrations up to 10^{-4} M. N-methyl glucamine substitution showed that the inward current was not dependent on the presence of external Na^+. Inward current could be elicited by clamping the membrane potential to values more positive than −35 mV from a resting potential of around −60 mV. This current had rapid activation kinetics (mean time constant 3.53 ms when clamped from −50 to −10 mV) and did not inactivate during stimulation but de-activated rapidly on return of the membrane potential to normal resting values. Under normal circumstances, depolarization of the plasma membrane would result in an influx of Ca^{2+} into the egg through these channels.

Further depolarization to values more positive than −5 mV elicited an outward current with slower kinetics (mean activation time constant 24.1 ms when clamped from −50 to +20 mV). This current was blocked by TEA and La^{3+} and its reversal potential (−80 mV) was close to the equilibrium potential of K^+ (−73 mV) for unfertilized eggs, assuming a cytoplasmic $[K^+]$ of 180 mM from the ionic contents data of Allen *et al.* (1972). This current also did not inactivate and deactivated only very slowly on cessation of the voltage clamp. A third type of current was evident on hyperpolarization of the membrane. This had characteristics typical of the inward K^+ rectifier found in several plant and animal cells (see, for example, Standen & Stanfield 1978; Hagiwara *et al.* 1976; Schroeder *et al.* 1987; Kourie & Goldsmith 1992).

(*b*) *Egg activation*

A model summarizing a testable hypothesis for the mechanism of *Fucus* egg activation is shown in figure 2. This model proposes the involvement of four major classes of channel in the plasma membrane of the unfertilized egg based on the data summarized above. The relative dependences of the membrane potential of an unfertilized egg on external ions suggests that it is predominantly controlled by K^+ and to a lesser extent, Cl^- conductances (figure 2*a*) (Taylor & Brownlee 1992*a*). At the resting membrane potential, K^+ conductance is probably the result of K^+ channels with a low but finite open probability. Their likely role is in regulation of membrane potential and intracellular K^+ homoeostasis. Voltage-dependent Ca^{2+} channels are also present in a predominantly closed state. Although voltage-regulated Na^+ channels were not detected in voltage clamp, a role for Na^+ in the generation of the fertilization potential can be inferred from the work of Brawley (1991). This showed that reducing external Na^+ resulted in a reduction of the magnitude of the fertilization potential in *Fucus cerranoides*, *Fucus serratus* and *Pelvetia fastigiata* and also increased the rate of polyspermy. The simplest explanation consistent with both our own data and that of Brawley (1991) is that the fertilizing sperm activates Na^+ channels by some as yet unknown mechanism. In invertebrates such as the sea urchin and ascidian, an initial sperm-binding step and action potential phase of the fertilization potential indicates the gating of cation channels (Dale *et al.* 1978; Chambers & de Armendi 1979; David *et al.* 1988). In the marine worm, *Urechis caupo*, sperm-activated Na^+ channels are gated locally at the site of sperm entry (Gould-Somero 1981).

The nature of the interaction between the sperm and egg in *Fucus* is not known. There is evidence for sperm receptors on the egg plasma membrane (Stafford *et al.* 1992) but it is not known whether these are involved simply in sperm-egg recognition or additionally represent the first step in a signal response chain leading to Na^+ channel opening either involving G-proteins or direct receptor-channel interaction (figure 2*b*). The possibility that Na^+ channels are actually

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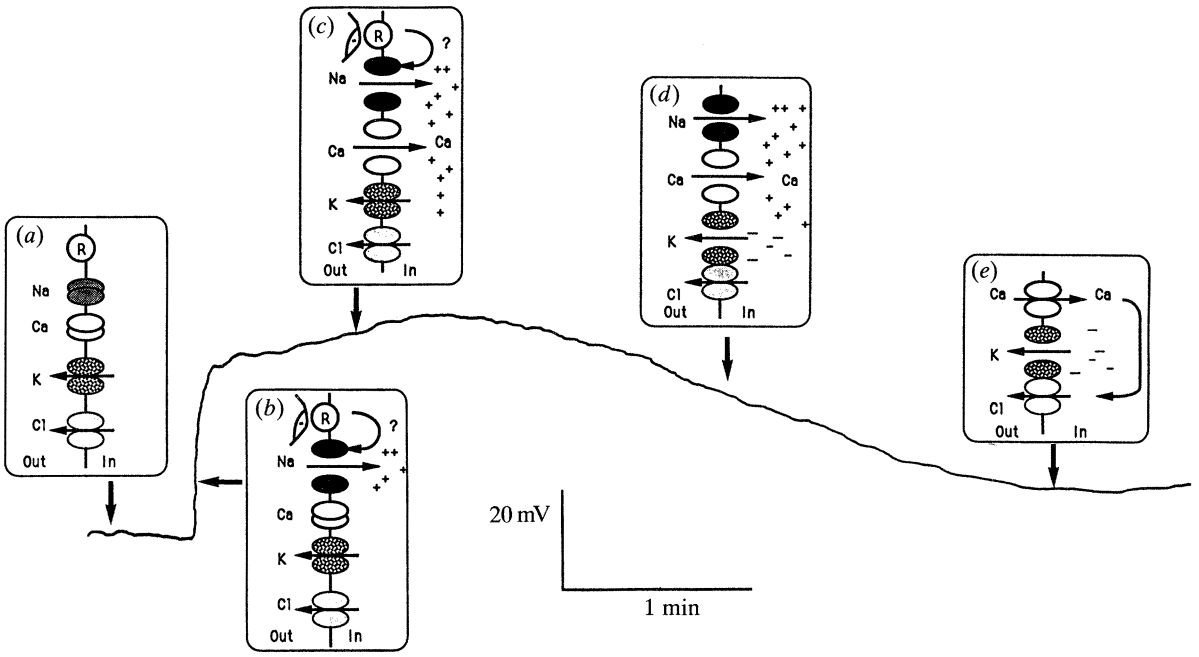

Figure 2. Typical *Fucus serratus* fertilization potential and representation of the ionic events underlying it. (*a*) Unfertilized egg, indicating control of membrane potential by relatively low K^+ and Cl^- conductances. (*b*) Sperm–egg interaction and opening of Na^+ channels during the depolarization phase. (*c*) Voltage-regulated Ca^{2+} channels open to allow Ca^{2+} influx and further depolarize the membrane. (*d*) Voltage threshold for outward rectifying K^+ channels is reached, initiating repolarization. (*e*) Zygote with conductance dominated by open-state K^+ channels.

incorporated into the egg membrane following fusion with the sperm by a mechanism similar to that proposed by Jaffe (1990) for Ca^{2+} channel incorporation into the deuterosome egg plasma membrane by the sperm should also be considered.

Whatever the mechanism leading to onset of the depolarizing fertilization potential, an almost immediate response would be the opening of voltage-gated Ca^{2+} channels (figure 2*c*). This would serve two purposes in allowing Ca^{2+} influx and further depolarizing the plasmalemma until the threshold for the outward rectifying potassium channels was reached (figure 2*d*). K^+ efflux through these channels would serve to restore membrane potential to normal resting levels. The rapid activation and absence of inactivation of the inward current observed in voltage clamp, together with the slow activation, more positive threshold and slow de-activation of the outward K^+ current are consistent with their having a role both in the prevention of polyspermy through the generation of the fertilization potential and in the activation of the egg by allowing Ca^{2+} influx.

Cytoplasmic Ca^{2+}, monitored with a variety of fluorescent indicators (fura-2, indo-1 and fura-2 dextran) has been observed to increase significantly with the onset of the *Fucus* fertilization potential (Roberts *et al.* 1992) and is likely to be involved in triggering egg activation (Jaffe 1983). This is also associated with increased ^{45}Ca influx (Roberts, *et al.* 1992). In the marine worm, *Urechis caupo*, the increase in cytoplasmic Ca^{2+} necessary for egg activation is brought about by increased Ca^{2+} influx through voltage-regulated channels in the plasma membrane (Gould-Somero *et al.* 1979). Treatment of *U. caupo* eggs with high K^+ sea water causes depolarization of the membrane and results in egg activation (Gould-Somero *et al.* 1979). In *Fucus*, exocytosis of cell wall precursors is one of the first structural changes occurring on activation and is easily visualized with the calcofluor white assay (Callow *et al.* 1985). Surprisingly, we have been unable to stimulate exocytosis by depolarizing the *Fucus* membrane to 0 mV for prolonged periods with high K^+ seawater. In addition, we have so far not observed any prolonged increase in cytoplasmic Ca^{2+} in response to depolarization. A mechanism for egg activation based solely on voltage modulation of plasma membrane channels is evidently not sufficient to explain all of these observations. It may be necessary to postulate either a role for intracellular Ca^{2+} release or inhibition of calcium sequestration mechanisms or efflux pump in addition to increased Ca^{2+} influx during activation. There are no data concerning the nature of such mechanisms in *Fucus*. Alternatively, additional factors, such as protein phosphorylation, may be required to hold Ca^{2+} channels open in the course of the prolonged depolarization during the fertilization potential. Although Ca^{2+} channels did not inactivate during the 100 ms voltage-clamp protocol used in our experiments, it is possible that inactivation may occur over longer periods. So far there is no evidence to support a role for intracellular release of Ca^{2+} from stores in the activation of *Fucus* eggs. In deuterosome invertebrate as well as vertebrate eggs, release of Ca^{2+} from intracellular stores causes the dramatic elevation of cytoplasmic Ca^{2+} on fertilization. This probably occurs as a wave of Ca^{2+}-induced Ca^{2+} release (Jaffe 1991). Preliminary experiments in our laboratory

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have shown that microinjection of Ca^{2+} directly into *Fucus* eggs does not lead to a propagated release of intracellular Ca^{2+} (A. R. Roberts & C. Brownlee, unpublished data), suggesting the absence of a role for Ca^{2+}-induced Ca^{2+} release, though the involvement of other intracellular Ca^{2+} release mechanisms still needs to be examined.

3. AXIS FORMATION AND FIXATION

(*a*) *Early changes in the zygote membrane*

The orientation of the polar axis, established in the first few hours following fertilization dictates the plane of the first division and subsequent developmental pattern of the multicellular plant. Polarization and early development in *Fucus* are not closely correlated with changes in gene expression. Proteins required for axis formation and fixation are synthesized from maternal RNA and accumulate before formation and fixation occur (Hetherington *et al.* 1990; Kropf *et al.* 1989; Kropf 1992). Polarization is essentially epigenetic (Jaffe 1958; Harold 1991; Kropf 1992), requiring, as a first step interaction between an asymmetric stimulus (e.g. unidirectional light) and plasma membrane-bound photoreceptor molecules (Jaffe 1958; Brownlee 1990*a*). Possible transduction mechanisms leading to asymmetry following the perception of unilateral light have been discussed elsewhere (Brownlee 1990*a*). Within a few minutes of fertilization the *Fucus serratus* membrane hyperpolarizes (to -80 mV), excitability is lost (Brawley 1991; Taylor & Brownlee 1992*a*) and a large, K^+ conductance dominates (figure 2*e*) (Taylor & Brownlee 1992*a*). Whether this is due to modification of pre-exisiting K^+ channels in the egg plasma membrane or incorporation of a new class of channels which are predominantly in the open state at the zygote resting potential is not known. K^+ and Cl^- concentration in the zygote increases along with an increase in turgor (Allen *et al.* 1972).

(*b*) *Ca^{2+} involvement in polarization*

The earliest measurable processes which may precede axis fixation are localized jelly secretion at the presumptive site of rhizoid germination (Schroter 1978) and the detection, using the extracellular vibrating probe (Jaffe & Nuccitelli 1974) of positive current entry at the same site (Nuccitelli 1978). Localized secretion is detectable within 4.5 h of unilateral illumination following fertilization in *Pelvetia* and is indicative of localized exocytosis. Asymmetric current entry at the presumptive rhizoid is detectable within 5–6 h following fertilization (Nuccitelli 1978). While the timing of the onset of these processes cannot be ascertained precisely, it is possible that localized secretion may precede the current. The fact that cytochalasin treatment inhibits the inward current at the site of rhizoid outgrowth in *Pelvetia* zygotes (Brawley & Robinson 1985) tends to support the role for an actin filament network in incorporation and maintenance of channels in this region. The inward current is carried by K^+ influx and Cl^- efflux (Nuccitelli & Jaffe 1976*a*) and in the pre-germination stages is small (less than 0.5 $\mu A\ cm^{-2}$). Ca^{2+} carries a small (around 2%) proportion of this current (Nuccitelli & Jaffe 1976a; Robinson & Jaffe 1975).

The role of this current and the potential resulting gradients, particularly of Ca^{2+}, in directing axis formation, fixation and subsequent germination is still under debate. Whether channel incorporation into the growing apex is a result of localized secretion (Kropf 1992) or whether the activity of pre-existing channels is modified locally in response to a localized external stimulus (e.g. light) has not been resolved. ^{45}Ca has been shown to enter the future rhizoid end of *Pelvetia* zygotes and to leave the thallus end (Robinson & Jaffe 1975). This asymmetry was most apparent before germination. The presence or involvement of a cytoplasmic Ca^{2+} gradient or locally elevated Ca^{2+} in axis formation and fixation has not yet been convincingly demonstrated (Brownlee 1989). Robinson & Cone (1980) demonstrated that fucoid embryos would germinate towards a source of ionophore A23187, though the significance of this may be less apparent with the knowledge that zygotes will polarize in alignment with a wide range of external gradients (Brownlee 1990*a*; Harold 1991; Kropf 1992). *Pelvetia* zygotes can form and fix an axis in the virtual absence of external Ca^{2+} with just 10 mM KCl and sucrose in the external medium (Hurst & Kropf 1991), and in the presence of Ca^{2+} channel blockers (Kropf & Quatrano 1987), suggesting no requirement for localized Ca^{2+} entry in this process. The result of Speksnijder *et al.* (1989), showing inhibition of development of *Pelvetia* zygotes following microinjection of appropriate concentrations of BAPTA buffers can be interpreted as inhibition of their ability to express axis fixation via inhibition of rhizoid growth. The picture is further complicated by the observation of Kuhtreiber & Jaffe (1990) that *Pelvetia* zygotes will not germinate at external Ca^{2+} concentrations below 1 mM, though once germinated, they will grow at near normal rates with external Ca^{2+} as low as 10 nM. Visualization of Ca^{2+} gradients in the early stages of polarization should now be possible with improvements in high resolution spatial imaging of cytoplasmic Ca^{2+} using fluorescent indicators with scanning laser confocal microscopy or digital deconvolution techniques (Monck *et al.* 1992).

Locally elevated Ca^{2+} can conceivably direct polarization and polarized growth by multiple Ca^{2+}-dependent mechanisms. These include direct control of exocytosis and membrane fusion (e.g. Neher & Marty 1982), direct enzyme modulation or action via calmodulin, and control of actin microfilament polymerization during axis fixation (Kropf *et al.* 1989). Axis fixation has recently been shown to require an axis stabilizing complex comprising two components: the extracellular matrix and the actin filament network (Quatrano *et al.* 1991). Calmodulin has been shown to localize at growth points in both yeast (Sun *et al.* 1992) and pollen tubes (Hausser *et al.* 1984). Ca^{2+} can also play a role in localized regulation of channel activity, resulting in the generation of positive feedback loops (Brownlee 1990*b*), possibly involving direct channel modulation or modulation via protein

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kinase. Though attractive, direct evidence to support this hypothesis is still lacking (see § 4*a*).

4. RHIZOID GERMINATION AND POLARIZED GROWTH

(*a*) *Ca^{2+} and apical growth*

Polar axis fixation is ultimately expressed as the germination of the rhizoid. Rhizoid germination and growth are dependent on the presence of external Ca^{2+} (Kropf & Quatrano 1987) and microinjection of dibromo-BAPTA into growing rhizoids at concentrations likely to eliminate localized Ca^{2+} elevations in the micromolar range inhibits rhizoid growth (Brownlee, Miller & Jaffe, unpublished). Ca^{2+} gradients have now been detected in apically growing cells using a variety of techniques. These include *Fucus* rhizoids (using microelectrodes (Brownlee & Wood 1986) and the fluorescent dye, fura-2 (Brownlee & Pulsford 1988)), and growing pollen tubes (using ester-loaded fura-2/AM (Obermeyer & Weisenseel 1991), indo-1 (Rathore *et al.* 1991) and high molecular weight dextran-linked fura-2 (Miller *et al.* 1992)). In pollen tubes, the presence of a Ca^{2+} gradient was closely correlated with growth. That these elevations are the result of increased Ca^{2+} influx into the growing tip is supported by the direct measurement of Ca^{2+} influx in this region using the extracellular vibrating Ca^{2+} electrode developed by Kuhtreiber & Jaffe (1990). In *Pelvetia fastigiata*, Ca^{2+} influx was observed at the growing rhizoid tip but not into the thallus cell. Similarly, Ca^{2+} influx was directly observed in growing pollen tube tips but not into the pollen grain itself. Similar observations have also been made with the growing apex of the *Funaria* protonema (Saunders 1992). The role of elevated Ca^{2+} in directing exocytosis and actin polymerization in tip growth has been discussed extensively (Brownlee 1990*a*; Steer & Steer 1989; Kropf 1992).

(*b*) *Plasmalemma channels in* Fucus *rhizoids*

Following germination, the steady inward current into the growing rhizoid tip varies from around 0.5 μA cm^2 or less to around 3–6 μA cm^2 (Nuccitelli & Jaffe 1974). Superimposed on this current are much larger pulses (greater than 10 μA cm^{-2}) of up to several minutes' duration. These large pulses appear to be dependent on external Ca^{2+} and to be carried by Cl^- efflux (Nuccitelli & Jaffe 1976*a*). They are probably involved in turgor regulation (Nuccitelli & Jaffe 1976*b*) and may represent opening of Ca^{2+}-regulated Cl^- channels. It has been postulated that these Cl^- pulses depolarize the membrane and are balanced by K^+-efflux (Nuccitelli & Jaffe 1976*a*). A role for the inward current in setting up an internal electric field which could act as a localizing mechanism was postulated by Jaffe (1968, 1981). Although this mechanism may indeed play a role in localization, its limitations are now apparent. Harold (1990) has critically reviewed the role of self organization, electric fields, ion gradients (including calcium) and actin localization in polarization. The conclusion drawn was that at present, no one hypothesis can account for all of the experimental observations. The currents observed entering the growing rhizoid may simply be manifestations of the complex ion fluxes occurring in that region. A clearer picture of the role of ion channels in polarization and polarized growth will only become apparent with more detailed knowledge of the channel types, their distribution and regulation. We have begun to study the activity of single channels in the *Fucus* rhizoid plasma membrane using patch clamp. To be able to study localization and distribution of channels, it is necessary to remove localized regions of the wall for access to the plasma membrane with a patch pipette. We have developed a method using a finely focussed UV laser which can ablate selected regions of the cell wall (Taylor & Brownlee 1992*b*). This method requires no enzyme treatment or protoplast preparation which would cause disruption of polarity (Kropf *et al.* 1988). The first step in this technique involves plasmolysis of the cytoplasm to withdraw the plasma membrane away from the wall, using polyethylene glycol and mannitol in seawater. The cell wall is stained with calcofluor. Plasmolysed zygotes are aligned with the focused laser so that a selected region of the wall is ablated on pulsing the laser. The calcofluor fluorescence allows precise alignment and ablation of a selected region of the wall (figure 3). Careful reflation of the cytoplasm by manipulating the osmotic potential of the external medium allows a small protrusion of cytoplasm from the selected region (figure 3*a*-*c*). High resistance (greater than 5 GΩ) glass-membrane seals can be obtained with a high success rate (greater than 50%, Taylor & Brownlee 1992*b*).

Initial results with this technique suggest the presence of at least two major channel types in the apical membrane. In the cell attached mode, with pipette voltage at 0 mV, channels carrying current both into and out of the cell have been identified in different patches (figure 3*d*,*e*). The main candidates for ions carrying these currents at the assumed transmembrane potential of −80 mV are K^+ for the outward current (figure 3*d*) and Cl^- or Ca^{2+} for the inward current. The Cl^- equilibrium potential (E_{Cl}), assuming a total cytoplasmic [Cl^-] of 320 mM (Allen *et al.* 1972) is −10 mV. However, a significant proportion of this Cl^- is likely to be compartmentalized, giving a more negative E_{Cl}. The driving force for Cl^- would be outward, however, as long as cytoplasmic [Cl^-] was greater than 20 mM. Figure 3*d* shows inward channel events with at least three amplitude levels. Whether these represent sub-conductance states of the same channel or different kinds of channel is not clear at present. These channels can carry very large currents (100 pA in figure 3*e*) and it is tempting to speculate that they are involved in large ion fluxes associated with turgor regulation and ion homoeostasis (Nuccitelli & Jaffe 1976*a*,*b*). A comparison can be made of the potential average current through these channels with the inward current pulses described by Nuccitelli & Jaffe (1976*a*). Simple analysis of the data of which figure 3*e* is representative suggests that the channels

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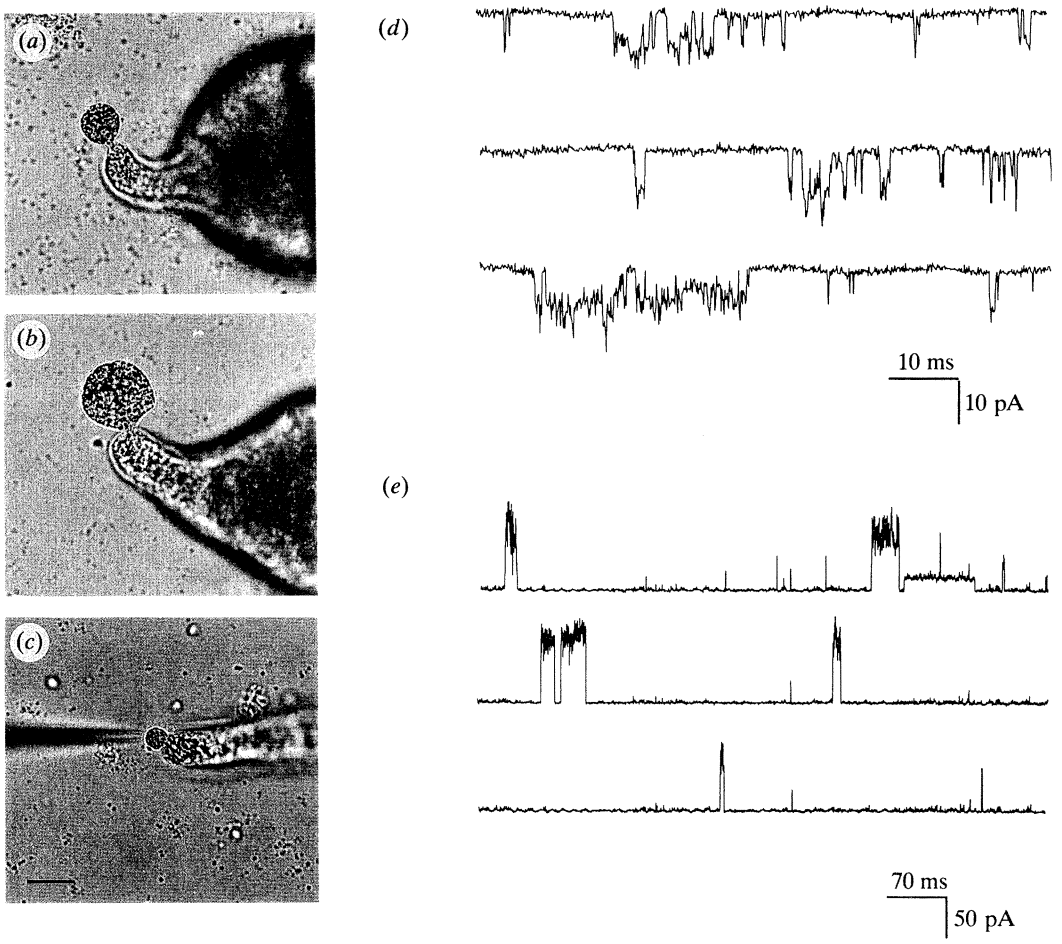

Figure 3. Localized patch clamping of the polarized *Fucus* rhizoid plasma membrane. *Fucus* rhizoids were subjected to UV laser ablation of the cell wall (see § 4*b* for details). Careful control of cell turgor permitted extrusion of plasmalemma-bound cytoplasm to varying degrees (*a*,*b*). High resistance (5–10 GΩ) seals were obtained on cytoplasmic extrusion with a patch clamp electrode (*c*). The technique is being further developed to improve the control of extrusion. Single-channel recordings were obtained from patch clamped membranes such as in (*c*). Currents flowing into the pipette are represented by downward deflections. Outward (from the cell, (*d*)) and inward (into the cell, (*e*)) currents were recorded in different patches. At an assumed transmembrane potential of -80 mV the outward currents are likely to be carried by K^+, and Ca^{2+} or Cl^- (see text for discussion) may account for the very large inward currents (100 pA). The trace of inward currents in (*e*) shows at least three conductance levels. At present it is not possible to ascertain whether these are different channel types or sub-conductance levels of the same channel.

conducting the inward currents are likely to be in an open state for approx 2% of the time. This is equivalent to an average current of 0.16 pA μm^{-2} (assuming a membrane patch of area 12 μm^2). The average current pulses recorded by the vibrating probe (around 10 $\mu A\ cm^{-2}$) are equivalent to 0.1 pA μm^{-2}. This suggests that the large inward currents recorded here could potentially account for the large current pulses into the tip of the growing rhizoid (Nuccitelli & Jaffe 1976*a*). Further work is required to underpin the physiological roles of these single channel currents and to identify other channel types, particularly the much speculated apical Ca^{2+} channels. We are now attempting to characterize the major channel types, their control and distribution in the plasmalemma of *Fucus* rhizoids and at earlier stages in the polarization process.

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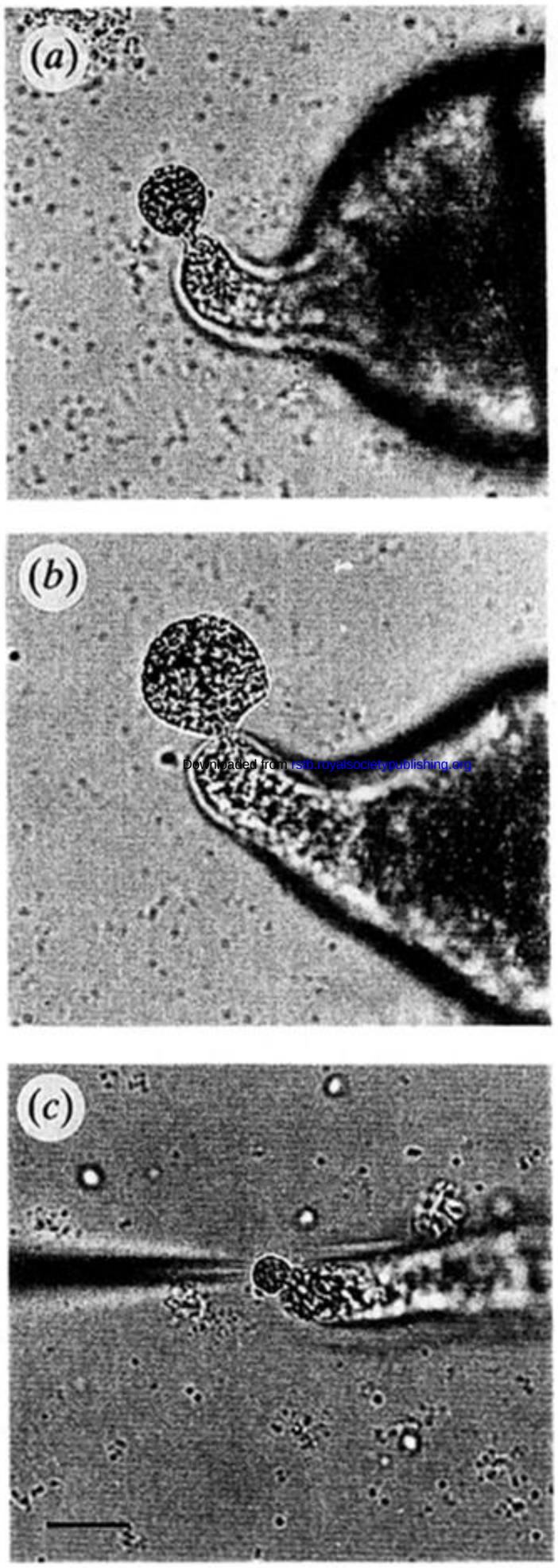

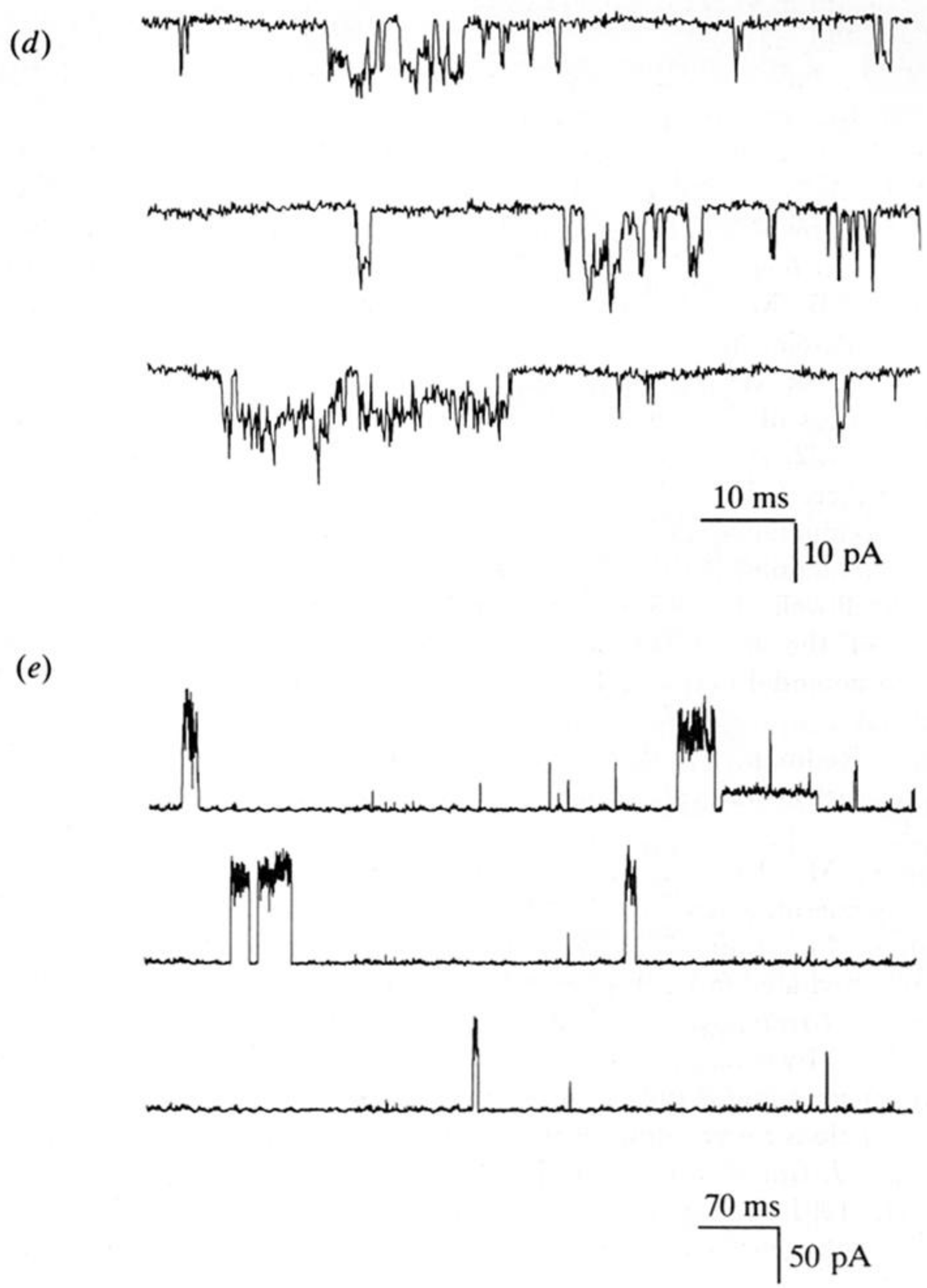

gure 3. Localized patch clamping of the polarized *Fucus* rhizoid plasma membrane. *Fucus* rhizoids were subjected uv laser ablation of the cell wall (see § 4*b* for details). Careful control of cell turgor permitted extrusion of asmalemma-bound cytoplasm to varying degrees (*a*,*b*). High resistance (5–10 GΩ) seals were obtained on toplasmic extrusion with a patch clamp electrode (*c*). The technique is being further developed to improve the ntrol of extrusion. Single-channel recordings were obtained from patch clamped membranes such as in (*c*). urrents flowing into the pipette are represented by downward deflections. Outward (from the cell, (*d*)) and inward nto the cell, (*e*)) currents were recorded in different patches. At an assumed transmembrane potential of -80 mV e outward currents are likely to be carried by K^+, and Ca^{2+} or Cl^- (see text for discussion) may account for the ry large inward currents (100 pA). The trace of inward currents in (*e*) shows at least three conductance levels. At esent it is not possible to ascertain whether these are different channel types or sub-conductance levels of the same annel.