J. exp. Biol. 169, 87–104 (1992)
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ACTIVATION BY MEMBRANE STRETCH AND DEPOLARIZATION OF AN EPITHELIAL MONOVALENT CATION CHANNEL FROM TELEOST INTESTINE

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Accepted 22 April 1992

Summary

The intestine of euryhaline teleosts is an important osmoregulatory organ which actively absorbs Na^+, Cl^- and water from the lumen. This ion-transporting epithelium experiences a variety of physical stimuli resulting from variations in luminal osmolality and distension and from peristaltic contractions. Using patch-clamp techniques in the inside-out configuration, single stretch-activated channels (SA channels) were identified and characterized. These SA channels had a conductance of about 67 pS in symmetrical solutions containing 140 mmol l^{-1} NaCl and were permeable to both Na^+ and K^+ ($P_{Na}/P_K \approx 0.83$) but not to anions. In excised, inside-out membrane patches, channel activity could be enhanced in the absence of membrane tension by strong depolarization of the membrane potential (V_m) to between 0 mV and +90 mV, with V_o [V_m at which the single-channel open probability (P_o)=0.5] at +25.7 mV. In the presence of membrane tension, the voltage-dependence of channel activity was shifted into the physiological range of V_m. Each kPa (10 cmH_2O) of applied pressure (ΔP) generated the same effect on P_o as a membrane depolarization of 49 mV. Membrane tension also increased the single-channel current and single-channel conductance in a dose-dependent manner. The kinetic data suggest that this channel has two open states and three closed states. Both stretch- and depolarization-induced increases in P_o were attributed to prolongation of the lifetime of the longer open state. Possible physiological roles for this channel include the cellular uptake of Na^+ from the lumen as part of the salt and water absorptive process or a yet undefined involvement in cell volume regulation.

Introduction

It has been well documented that the intestine is an important osmoregulatory organ in teleosts, especially in those euryhaline teleosts encountering variations in the external osmotic environment. Ion flux and electrophysiological studies in our laboratory have shown that the goby intestine is able to absorb Na^+, Cl^- and H_2O actively from the lumen (Loretz, 1983). During adaptation to hypertonic environ-

Key words: stretch-activated channel, fish intestinal epithelium, patch-clamp, *Gillichthys mirabilis*.

ments, the induced drinking behavior increases the availability of water for absorption. At the same time, endogenous hormones enhance the efficiency of water absorption (Loretz *et al.* 1985). In sea water, the fish retains the absorbed water and actively secretes NaCl into the external medium at the gill. Other osmoregulatory organs, such as the kidney and urinary bladder, also contribute to the maintenance of a constant internal osmotic environment through their electrolyte and water transport functions. Although scientists have described hormonal and pharmacological effects on intestinal functions, one potentially important factor has been neglected, namely, the mechanical effect of intestinal distension. Distension of the posterior intestine in seawater-adapted gobies suggests a longer residence time for luminal contents in these fishes (allowing more processing) (Loretz, 1983). Further, distension might also enhance ion transport capacity through a mechanosensitive pathway. Distension may result from several causes, including greater fluid delivery to the gut through drinking and less frequent or reduced posterior intestinal evacuation. As the result of this distension, a hydraulic pressure is imposed on the epithelial lining involved in ion and water transport. Along with intestinal distension, the rhythmic contraction of intestinal smooth muscle may also cause periodic increases in tissue tension.

An increasing number of reports have implicated mechanical forces in regulating the biological functions performed by various tissues (Sachs, 1986; Morris, 1990). Specifically, stretch-dependence of membrane ion transport processes has been shown in systems as diverse as regulatory volume decrease in choroid plexus epithelium (Christensen, 1987), hair cell mechanotransduction (Howard *et al.* 1988), initiation of smooth muscle contraction (Kirber *et al.* 1988), mechanotransduction of the Ca^{2+}-mediated synthesis, and release of prostacyclin and EDRF (endothelium-derived relaxation factor) by various agents in aortic endothelial cells (Lansman *et al.* 1987) and induction of appressorium in rust fungus (Zhou *et al.* 1991). In each of these systems, a change in membrane conductance resulting from mechanosensitive channel activity triggers the subsequent physiological response.

In this report, we describe a stretch-activated channel (SA channel) from posterior intestinal cells; these cells are one location of Na^+, Cl^- and water absorption in the goby (Loretz, 1983). We describe the effects of membrane tension on ion channel activity by using standard patch-clamp and manual pressure-clamp methodologies. We also present several biophysical features of the channel (e.g. single-channel conductance, ionic selectivity and voltage-dependence) relevant to its possible role in epithelial transport.

Materials and methods

Dissociated intestinal cell preparation and solutions

All experiments were performed on dissociated epithelial cells stripped from the posterior intestine of the euryhaline goby, *Gillichthys mirabilis* Cooper. Gobies weighing 20–50 g were obtained from commercial suppliers in California and fully adapted in the laboratory to Instant Ocean artificial sea water (Aquarium Systems,

Table 1. *Composition of solutions*

Constituent ($mmol\,l^{-1}$)	GBR*	G35MES-BS	Na_2SO_4-BS	Na-ES	K-ES
Na^+	161.4	35	140	140	–
K^+	2.5	35	–	–	140
Ca^{2+}	2.5	0	1	1	1
Mg^{2+}	1	–	–	–	–
Cl^-	144.5	35	2	142	142
HCO_3^-	5	–	–	–	–
SO_4^{2-}	–	–	70	–	–
HPO_4^{2-}	0.7	–	–	–	–
Isethionate	20	–	–	–	–
Hepes	–	10	10	10	10
MES^-	–	35	–	–	–
Sucrose	–	170	90	60	60
EGTA	–	0.1	–	–	–
Glucose	5	–	–	–	–

GBR, *Gillichthys* bicarbonate Ringer.
The pH of all solutions was 7.6 and it was adjusted with $1\,mol\,l^{-1}$ HCl, $1\,mol\,l^{-1}$ KOH or $1\,mol\,l^{-1}$ NaOH as appropriate.
* Solution gassed with 99 % O_2, 1 % CO_2.

Mentor, OH) at a salinity of 34 ‰. Fish were maintained under a constant 12 h:12 h L:D photoperiod at 12°C. Experiments were conducted at room temperature (18–20°C). Fish were killed by rapid decapitation and pithing; no chemical anesthetics were administered which might have influenced channel activity or function. The epithelium was stripped from the opened goby posterior intestine using a glass microscope slide. Single cells were prepared by treating the stripped epithelium with collagenase ($0.4\,mg\,ml^{-1}$, 20 min). Dissociated cells were then collected by gentle centrifugation (500 *g*, 15 min); the pellet was resuspended in fresh *Gillichthys* bicarbonate Ringer (GBR) and plated onto 35 mm polystyrene culture dishes. Dishes with cells were kept cool (10–15 °C) and used within 4–6 h after preparation. Table 1 shows the basic composition of several solutions used in these experiments; in the text, solutions are referred to by the abbreviations in the table. Two electrolyte solutions, Na-ES and K-ES, were used to fill the patch pipette. The cytoplasmic side of excised membrane patches was bathed by either of these pipette solutions or, alternatively, by one of three other solutions (GBR, Na_2SO_4-BS and G35MES-BS) formulated to probe ionic selectivity of specified channels. With K-ES in the pipette, the formulation of G35MES-BS was such as to yield unique calculated reversal potentials for each of the major ionic species, specifically: K^+, +35 mV; Na^+, a large undefined negative potential; Cl^-, −35 mV; Ca^{2+}, a large undefined positive potential. Osmotic pressures of all solutions were routinely monitored and never differed from $320\,mosmol\,l^{-1}$ by more than $5\,mosmol\,l^{-1}$; where necessary, osmotic pressure was adjusted by addition of mannitol or sucrose.

Patch-clamp apparatus

The patch-clamp system used in our laboratory has been fully described (Loretz and Fourtner, 1988; Chang and Loretz, 1991). As before, seal formation was nearly spontaneous following pipette placement on the cell; occasionally, transient application of slight suction aided seal formation, after which low levels of spontaneous channel activity could often be observed. The only modification to our published system was the installation of a 1 ml syringe and a manometer to the suction tubing of the pipette holder for the controlled application and measurement of hydrostatic pressure.

Because the application of suction often moved the pipette off the cell, all membrane patches examined in this study were of the excised inside-out configuration. For these patches, the membrane potential (V_m) was reported relative to the pipette solution. Inward (+) current refers to the flow of cations from the extracellular side to the cytoplasmic side of the membrane patch, i.e. from the pipette solution into the bath solution. Excision of the patch from the cell sometimes resulted in vesicle formation in the pipette tip; brief exposure of the pipette to air generally restored observable channel activity. Only those channel currents that could be activated reversibly by suction were recorded and analyzed. In some membrane patches, the initial application of suction activated large numbers of channels (more than 5 or 6) which proved too difficult for analysis of single-channel properties. We typically excluded these membrane patches from further study. Data collection from patches generally began following the appearance of channel activity induced by brief test applications of suction. We recorded spontaneous channel activity, when present, at various membrane holding potentials before any other experimental manipulations. In all, over 13 membrane patches containing SA channels were studied.

Suction application

To study the stretch-dependence of channel activity, we applied suction to the membrane patch simply by retracting the plunger of a 1 ml plastic syringe; we calibrated the syringe system by manometry. Each 0.1 ml of suction generated a pressure gradient (ΔP) of -0.8 kPa (-8 cmH_2O). A negative pressure gradient is defined as a pressure gradient from the cytoplasmic side to the extracellular side of the membrane patch. To study the dependence of single-channel current and conductance on suction, pipette suction was sequentially held at -0.4, -0.8, -1.6 and -2.4 kPa, and at each level of pipette suction the membrane potential was stepped from -80 mV to $+80$ mV in 10 mV increments. From the above manipulations, single-channel conductance at each pipette suction can be calculated from a plot of I_c *versus* V_m as the slope of the plot in the physiological range of V_m.

Data analysis and statistics

For analysis, recorded data were filtered (eight-pole low-pass Bessel filter) to

yield an effective bandwidth with corner frequency (f_c) of 1.5 kHz (−3 dB). Unless otherwise specified, the filtered data were digitized at 10 kHz and stored on computer hard disk. The digitized data were further analyzed by the IPROC-2 computer program (Axon Instruments, Inc., Burlingame, CA), which is an automated event detection program designed for the analysis of single ion channels. Using this program, the single-channel current (I_c) was calculated as the mean current during validated channel openings. Single-channel conductance (g_c) was calculated as the slope of plots relating I_c to V_m; regression analysis was applied to these I_c *versus* V_m plots over a range of V_m from 0 to −90 mV that included the physiological range of membrane potentials. Since it was difficult to discriminate very small channel currents from background current noise, the exact membrane potential at which no current flowed though the channel could not be graphically determined. Therefore, we determined the reversal potential (V_r; the V_m at which there was no current flow through the channel) by linear regression of those data points bracketing the intercept on the abscissa. For membrane patches containing only a single functional channel, the single-channel open probability (P_o) was calculated from the total current amplitude histogram with areas under the two peaks representing the closed and open states taken to be proportional to the time spent in those conductance states. For membrane patches with multiple SA channels (as seen in Fig. 1A), we first determined the number of channels by applying suction to the pipette or strongly depolarizing the membrane patch, either of which elicits high channel activity. Next, under the assumption that channels in the membrane patch function independently of one other, we calculated the P_o from the binomial distribution of the time spent in the current levels corresponding to different numbers of simultaneously open channels.

Curve fittings were accomplished using the Easyplot computer program (Spiral Software, Brookline, MA). In assessing the number of components in the exponentially distributed channel kinetics, goodness of fit was determined using the residual squared error. Statistical significance of differences was assessed using Dunnett's test (Crunch Software, Berkeley, CA). The data are reported as the mean±S.E.M.

Results

Fig. 1 illustrates the sensitivity to suction (negative pressure) of this SA channel. Application of positive pressure to the patch pipette was also effective in eliciting channel activity. Because of the difficulty in maintaining gigaohm seals under this experimental treatment, we limited our investigations to the application of suction. From the study of excised patches, we found channel activity could be enhanced by hydrostatic pressure (ΔP) generated by suction applied to the patch pipette. A minimum ΔP of −0.8 kPa was required to increase the channel activity significantly. Stretch activation was observed within the physiological range of membrane potential, which lies between −40 mV and −70 mV.

In Fig. 2A, with Na-ES in both pipette and bath, the V_r was nearly zero

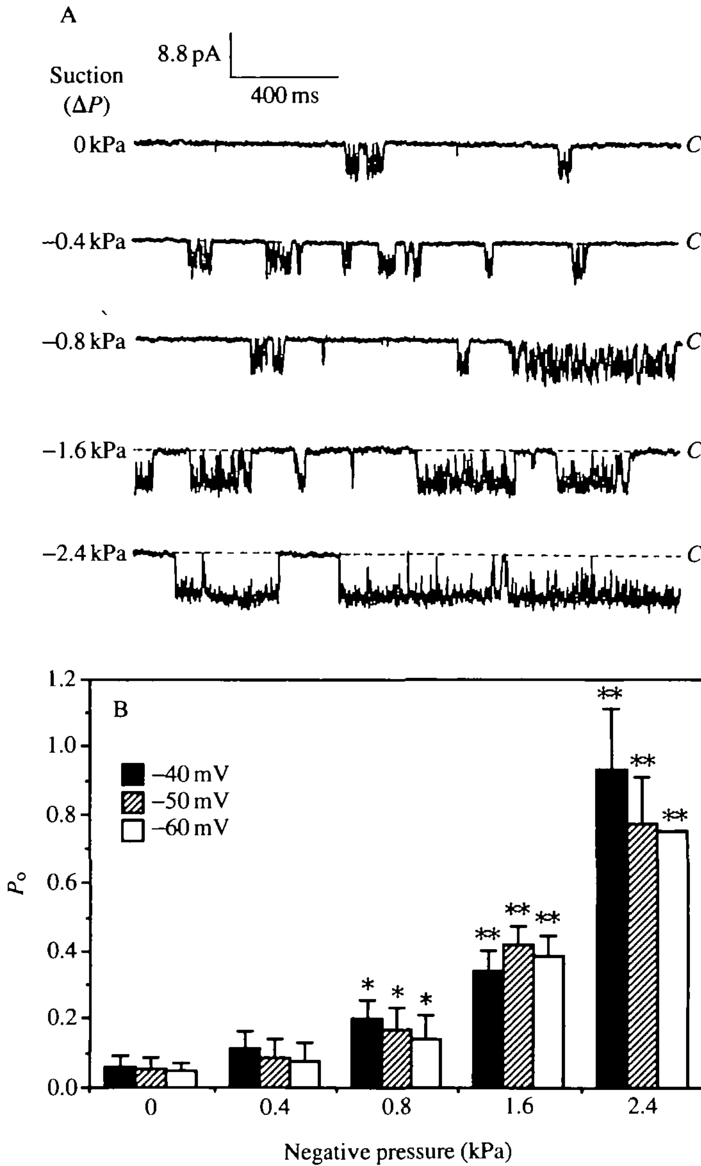


Fig. 1. Single-channel activity increased with applied suction through the pipette. (A) Single-channel current traces were recorded from a membrane patch bathed in GBR with K-ES in the pipette. V_m was clamped at −60 mV. *C* represents the closed state of the channel during which no current flowed. The current records were filtered at 500 Hz. (B) P_o increased with applied suction in the physiological range of membrane potential. The data were pooled from 13 inside-out membrane patches without regard to bath or pipette solutions. Dunnett's test was used to compare the effects of pressure on P_o at each level of V_m. * $P<0.05$; ** $P<0.01$. Bars represent S.E.M.

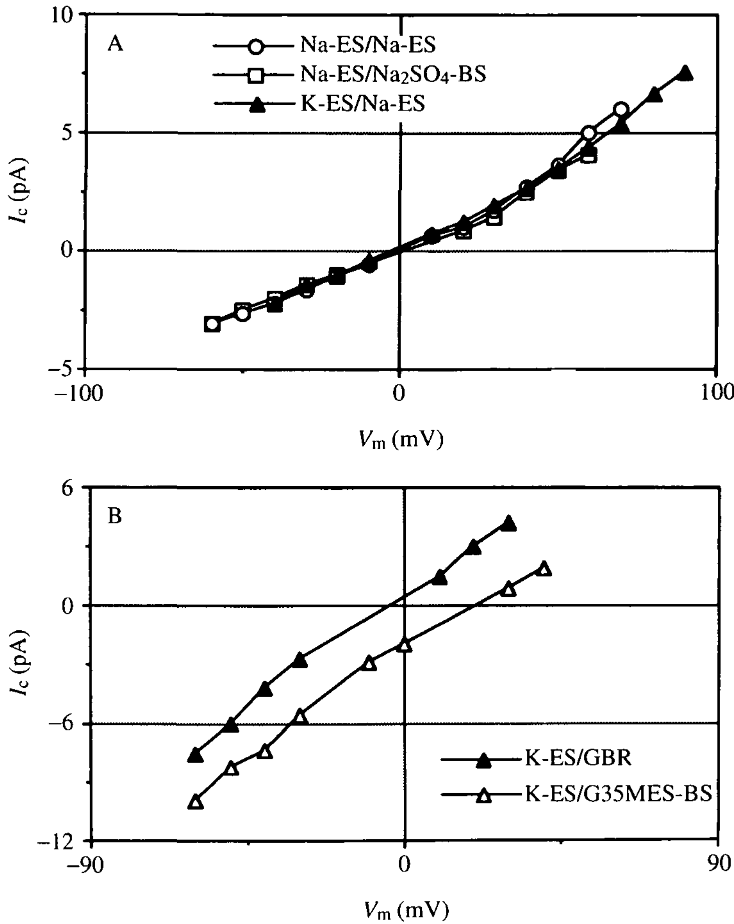


Fig. 2. The I_c *vs* V_m relationship of channel current in the ion substitution experiments. The inset legend indicates the solutions used in pipettes and bath (pipette/bath). From the V_r of the channel current with K-ES in the pipette and Na-ES in the bath, P_{Na}/P_K was calculated as 0.83 using the biionic equation.

(1.7±1.9 mV, N=2). The replacement of the Na-ES in the bath with Na_2SO_4-BS to establish an anion gradient did not shift V_r (2.8±0.7, N=2), suggesting that the channel is no more permeable to Cl^- than to the larger SO_4^{2-}. With K-ES in the pipette and Na-ES in the bath, the V_r of channel current was again very close to zero (4.7±2.9 mV, N=3), suggesting that the channel is permeable to both Na^+ and K^+. The lack of selectivity between Na^+ and K^+ was confirmed by the I_c *vs* V_m relationships presented in Fig. 2B. With K-ES in the pipette, the V_r was 1.4±2.9 mV (N=4) when GBR was in the bath and about +20 mV (N=1) when G35MES-BS was in the bath. This reduction of monovalent cation concentration in the bath from 164 to 70 mmol l^{-1} shifted V_r towards a positive potential, consistent with monovalent cation selectivity of the channel. From the V_r of the

channel current, when K-ES was used in the pipette and Na-ES was used in the bath, P_{Na}/P_K was calculated to be about 0.83 by using the biionic equation derived from the Goldman constant-field equation.

SA channels in patches bathed in GBR, with K-ES in the pipette, displayed a slope conductance of 82±9 pS (N=4). When Na-ES was used as the bath solution, the slope conductance was 67±8 pS (N=2) with Na-ES in the pipette and 78±19 pS (N=3) with K-ES in the pipette. For one channel when K-ES was used in both pipette and bath, the conductance was measured to be 84 pS.

Fig. 3 shows the voltage-dependence of SA channel activity in the absence of membrane stretch. P_o increased when the membrane was strongly depolarized. Over the physiological range of membrane potential, however, channel activity was low in the absence of suction and independent of membrane potential. The relationship between P_o and V_m was sigmoidal and followed a modified Boltzmann distribution (Hille, 1984) described as follows:

$$P_o = 1/\{1 + \exp[nF(V_o - V_m)/RT]\} + P^o,$$

where P_o is the single-channel open probability, n is the apparent gating charge, V_m is the membrane potential, V_o is the membrane potential at which P_o is equal to 0.5, F is the Faraday constant, R is the gas constant, T is the absolute temperature (equal to 293 K at room temperature) and P^o represents the low-level baseline channel activity that is not voltage-sensitive. From pooled data of 13 membrane patches, in the absence of pipette suction, the best-fitting values for these variables were n=1.49, V_o=25.7 mV and P^o=0.04. The voltage-dependence of the channel activity was shifted to more negative membrane potentials with the applied ΔP (Fig. 4A). In the presence of pipette suction, less membrane depolarization was needed to induce channel activity. As shown in Fig. 4B for one particular patch, V_o was shifted from +38 mV to −80 mV by the applied ΔP of −2.4 kPa, without significant change in P^o. The apparent gating charge decreased substantially from 1.52 to 1.14, reflecting reduced sensitivity to voltage change (i.e. the slope of the P_o *vs* V_m plot over the range of voltage gating), when ΔP was stepped from −0.8 to −1.6 kPa.

In addition to the increase in P_o, membrane stretch increased both channel current (I_c) and channel conductance (g_c). In some membrane patches, I_c and g_c were increased by as much as 90 % and 100 %, respectively, when ΔP was stepped from 0 to −2.4 kPa (Fig. 5). The V_r for channel current did not shift significantly with applied hydrostatic pressure, indicating no change in the ion-selectivity ratio with membrane tension.

Fig. 6 shows the channel open-time and closed-time distributions in the presence or absence of membrane tension and/or membrane depolarization. In the absence of membrane tension and membrane depolarization, when P_o is small, the channel open-time distributions can be best fitted by two exponential components (Fig. 6A), suggesting two channel open states with time constants τ_{o1} (short open-time constant) and τ_{o2} (long open-time constant). Under this same experimental condition favoring low P_o, the SA channels spent most of their time

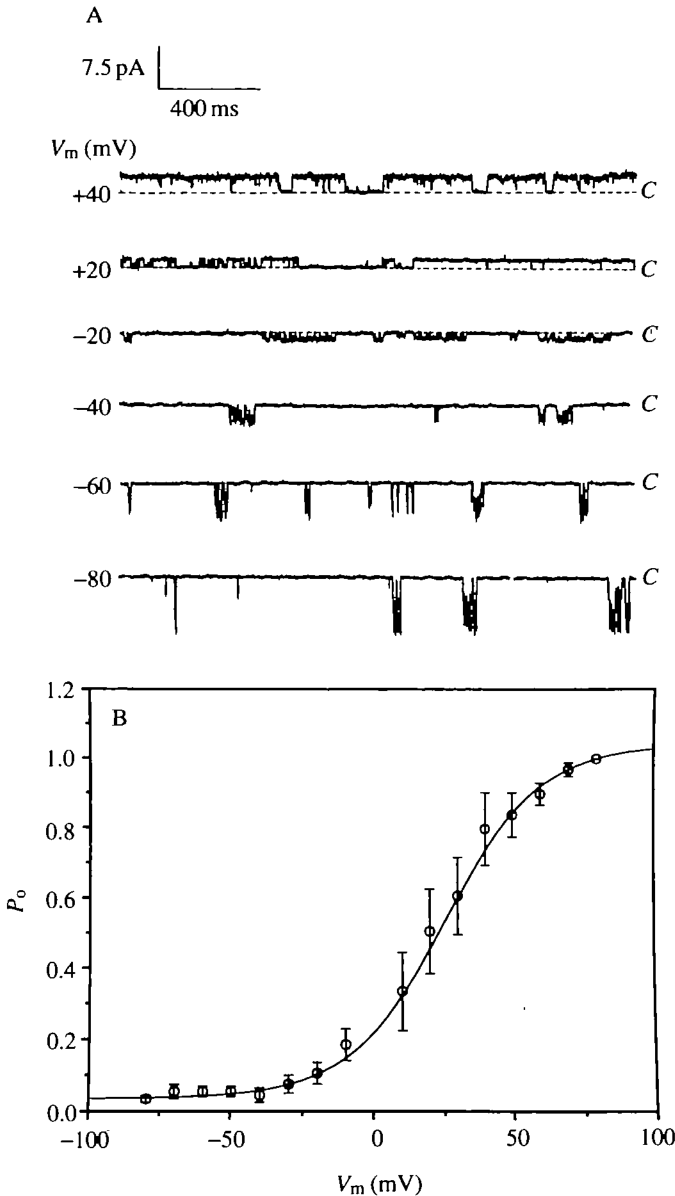
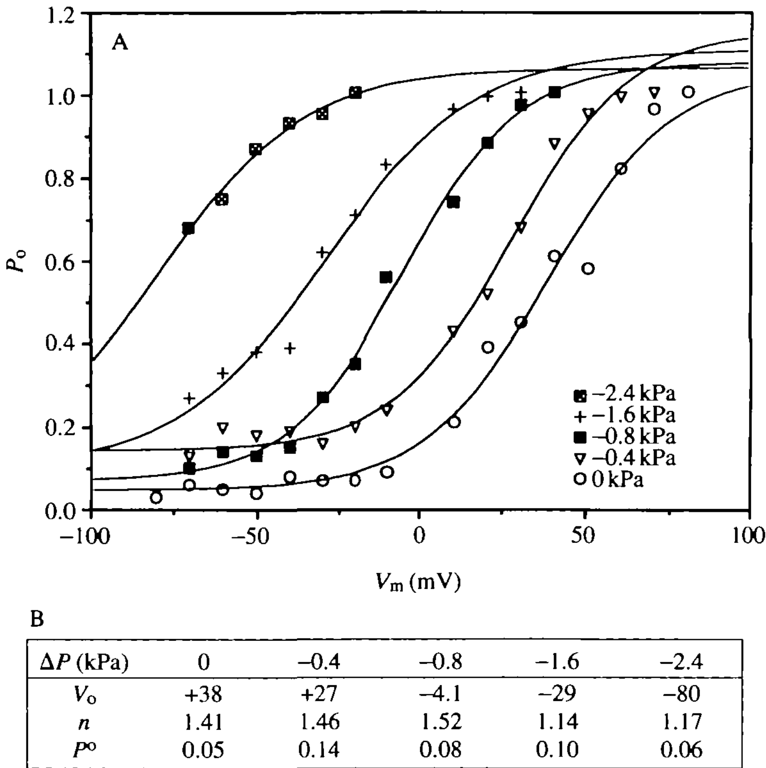


Fig. 3. The P_o *vs* V_m relationship in the absence of pipette suction. (A) The current records were recorded from an inside-out patch clamped at different voltages with a pipette containing K-ES and bathed in GBR. Upward deflections represent inward (+) current and downward deflections represent outward current. *C* represents the closed state of the channel in which no current flowed. The current records were filtered at 500 Hz. (B) The P_o *vs* V_m relationship in the absence of applied suction. P_o represents the mean±S.E.M. of single-channel open probabilities calculated for 13 inside-out patches voltage-clamped at the indicated membrane potentials. Data points were best fitted by the modified Boltzmann equation, $P_o=1/\{1+\exp[nF(V_o-V_m)/RT]\}+P^o$. The values of best fit were $n=1.49$, $V_o=25.7$ mV and $P^o=0.04$. See text for discussion.



B

ΔP (kPa)	0	−0.4	−0.8	−1.6	−2.4
V_o	+38	+27	−4.1	−29	−80
n	1.41	1.46	1.52	1.14	1.17
P^o	0.05	0.14	0.08	0.10	0.06

Fig. 4. (A) The P_o *vs* V_m relationships for different amounts of applied suction. The data were collected from an inside-out membrane patch bathed in GBR with a pipette containing K-ES. Increasing steps of suction were applied sequentially to the patch with each test period separated by a 20–30 s interval of zero suction. The data for I_c *vs* V_m plots were collected immediately after each level of suction had been applied. The inset legend indicates the amount of suction (ΔP, kPa) applied to the pipette. The data were fitted as in Fig. 3. (B) A summary of the fitted variables.

in the long closed state. Owing to the short recording time (20–30 s) and long duration of that closed state (400–800 ms per event), we can only detect those events with very low frequency, making statistical analysis difficult. Excluding those long channel-closed events, we could fit the remaining closed-time event distribution with two exponential components (Fig. 6E). These results indicate that this channel has one very long-duration closed state and two shorter-duration closed states with time constants, τ_{c1} (short closed-time constant) and τ_{c2} (medium closed-time constant).

Only a single time constant was influenced by membrane tension or voltage. The long open-time constant, τ_{o2}, increased with both applied pressure (Fig. 6B,C) and membrane depolarization (Fig. 6D). Membrane tension and depolarization had no significant effects on the short open-time constant τ_{o1} or on either the short closed time constant τ_{c1} or the medium closed-time constant τ_{c2} (Fig. 6F,G,H).

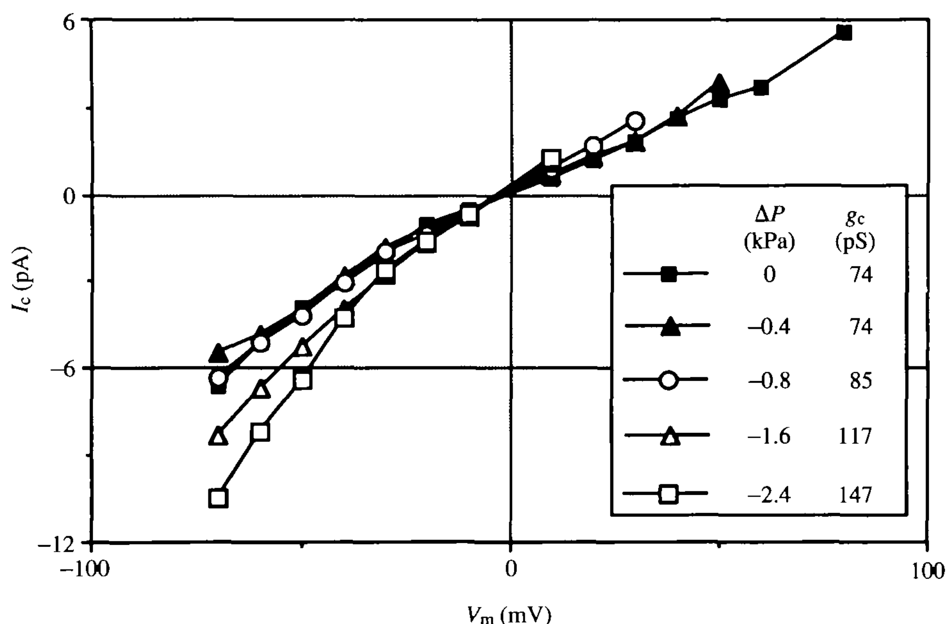


Fig. 5. The relationships between single-channel current and V_m for various amounts of applied suction. The data were collected from an inside-out patch bathed in GBR with a pipette containing K-ES. The inset shows the amount of suction applied and the calculated slope conductance (g_c) at each ΔP. Slope conductances were calculated from the linear portion of the I_c *vs* V_m plots including the physiological range of membrane potentials.

Hydraulic pressure and membrane depolarization clearly and dramatically decreased the duration of long closed events (Figs 1A and 3A). Since we do not have sufficient data for curve fitting, we cannot describe quantitatively the decrease in time constant for that long closed state.

Discussion

Ever since Guharay and Sachs (1984) identified the first stretch-activated ion channel, scientists have started to reappraise the effects of mechanical forces, a fundamental feature of our natural environment, on ion transport functions of biological membranes. The intestine of the euryhaline teleost is responsible for nutrient, salt and water absorption, thereby fueling metabolism and keeping the internal osmotic environment in balance. The intestine experiences a variety of physical stimuli, such as osmotic gradients, hydraulic pressure and even the physical contact of ingested objects. Without a mechanosensor, it cannot establish a successful response to those mechanical signals. In this report, we identify a membrane ion channel that is sensitive to membrane tension; this cation-selective

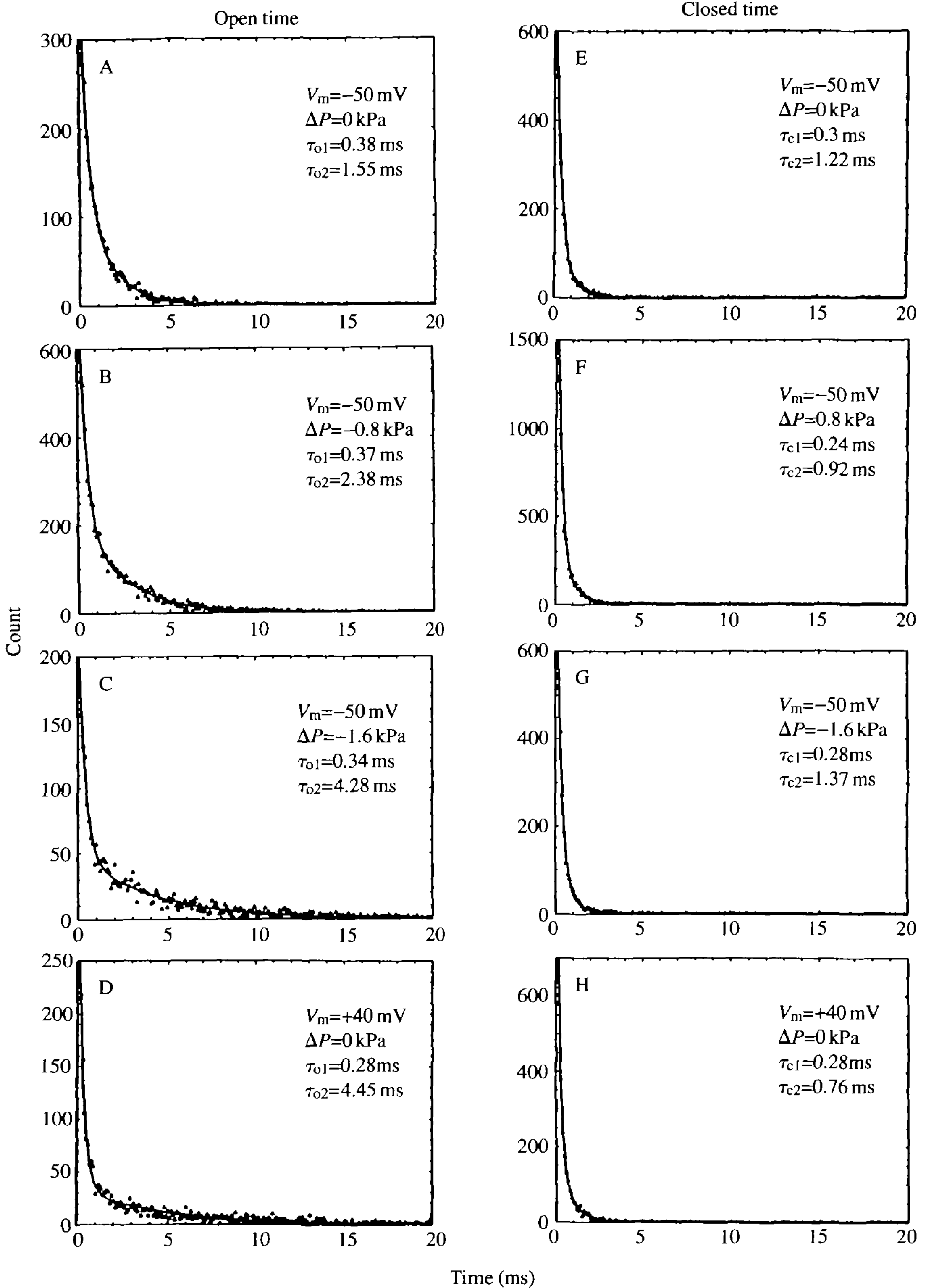


Fig. 6

Fig. 6. Curve fittings of open-time and closed-time histograms. Histograms were best fitted by an equation containing two exponential components. The generalized equation is $y=y_1\times\exp(-\tau_1/x_1)+y_2\times\exp(-\tau_2/x_2)$, where y_1 and y_2 are the y-intercepts of component 1 and 2 and τ_1 and τ_2 are the time constants for components 1 and 2. The insets in each panel indicate the membrane potential, applied ΔP and time constants. τ_{o1}, τ_{o2}, τ_{c1} and τ_{c2} represent the short open-time constant, long open-time constant, short closed-time constant and medium closed-time constant, respectively.

ion channel may constitute one mechanism for the transduction of mechanical stimuli.

Stretch-activated channel activity is dependent on membrane tension

In contrast to several other types of ion channels exhibiting spontaneous activity in our patch-clamp study system, we observed one class of channel that showed reversible activation with the application of negative (hydraulic) pressure (ΔP) in the patch pipette. At physiological V_m, the spontaneous single-channel open probability (P_o) of this channel was low ($\approx$0.02), and P_o could be increased significantly by application of -0.8 kPa ΔP. The maximum channel activity (P_o near 1) could be induced by applied pipette suction without breaking the gigaohm seal. Similar suction-dependence of channel activity has been observed in the urinary bladder epithelium of this same species (Chang and Loretz, 1991) and in other cell types from different species (Lansman *et al.* 1987; Sigurdson and Morris, 1987; Kawahara, 1990; Zhou *et al.* 1991). These results indicate that the SA channel performs its biological function in response to membrane tension.

Guharay and Sachs (1984) have proposed that SA channels are activated by energy transduced from membrane tension resulting from the transmembrane pressure difference. These authors (Guharay and Sachs, 1984) have modelled the SA channel as a flexible cylindrical plug with membrane tension expanding the cylinder. The activation of SA channels may result from the direct physical alteration of the channel protein's structure by the attached submembrane intracellular cytoskeleton, the supporting evidence coming from the ability of cytochalasins to alter channel sensitivity to membrane stretch (Guharay and Sachs, 1984). Alternatively, a conformational change in a gating particle may be the physical basis for stretch sensitivity rather than a global molecular expansion of the cylinder. Consequently, distension of the cell membrane may change the local environment of the channel protein and bring about gating *via* an interaction between the protein and the lipid bilayer in a more limited spatial domain.

This SA channel from intestinal epithelium is generally similar in its properties to that from the urinary bladder epithelium described earlier by Chang and Loretz (1991). Both channels display a relatively large g_c, which increases concomitantly with stretch activation, and both channels are only slightly more permeable to K^+ than to Na^+ (P_{Na}/P_K=0.83 for the intestinal SA channel *vs* 0.75 for the urinary bladder SA channel). Two differences are notable, however, between the channels. First, the SA channel from the intestine has a lower threshold ΔP for

stretch activation compared with the SA channel from urinary bladder (approximately −0.8 kPa *vs* −1.6 kPa). And second, the SA channel from intestine did not exhibit inactivation following suction application, as did the urinary bladder channel. Although differences in channel protein structure cannot be ruled out as the basis for these differences, neither can differential influences of the membrane on the channel. Differences in submembranous cytoskeleton or in local plasma membrane interactions with the channel proteins may also affect their activity in these two tissues. Further study may elucidate the basis for these differences and their physiological significance.

Stretch-activated channel activity is voltage-dependent

In the absence of suction, the SA channel reported herein showed voltage-dependent activation in the absence of suction only when the membrane was highly depolarized (V_m between 0 mV and +80 mV). During the application of suction, the dependence of the channel on voltage was shifted in the negative direction towards the physiological range of membrane potential (from 0 mV to −90 mV), suggesting the possibility of dual regulation by voltage and membrane tension. Sensitivity to both voltage and stretch has also been reported in the urinary bladder epithelium of this same species (Chang and Loretz, 1991), an osteoblast-like cell line (Duncan *et al.* 1989), toad stomach smooth muscle (Kirber *et al.* 1988) and protoplast membrane patches of the fungus *Uromyces* (Zhou *et al.* 1991).

Membrane stretch, in addition to shifting voltage-dependence into the physiological range of membrane potential, also decreased the sensitivity of P_o to membrane voltage, as indicated by n, the apparent gating charge. This finding may be significant in two respects. First, the stretch-dependence and voltage-dependence of gating may not be fully independent of one another; they may, for instance, both affect the same gating particle or domain. And second, this independence might suggest a mechanism more complex than a simple elastic cylinder serving as the center for the focusing of membrane stretch by cytoplasmic elements. We have described the curves in Fig. 4 relating P_o to V_m using a modified Boltzmann equation. Over the entire range of pressure tested, applied suction reduced V_o, the voltage at which P_o=0.5. We quantified the relationship between V_o and ΔP in Fig. 7 and calculated that −1 kPa ΔP is equivalent to a membrane depolarization of 49 mV in terms of its effect on P_o. The sensitivity of P_o to voltage, expressed in the variable n in the equation, decreased in a stepwise manner by about one-third when ΔP exceeded −0.8 kPa. This decrease in n may reflect an allosterically derived reduction in the effective gating charge or a conformational change in the channel protein or surrounding lipid bilayer, possibly affecting the transmembrane electrical field. With respect to the former, membrane stretch might induce a conformational change in that part of the molecule functioning as the voltage gate so as to redistribute charged moieties, resulting in a diminished gating charge. Alternatively, stretching of the cell membrane, or the consequent deformation of the channel protein, with the

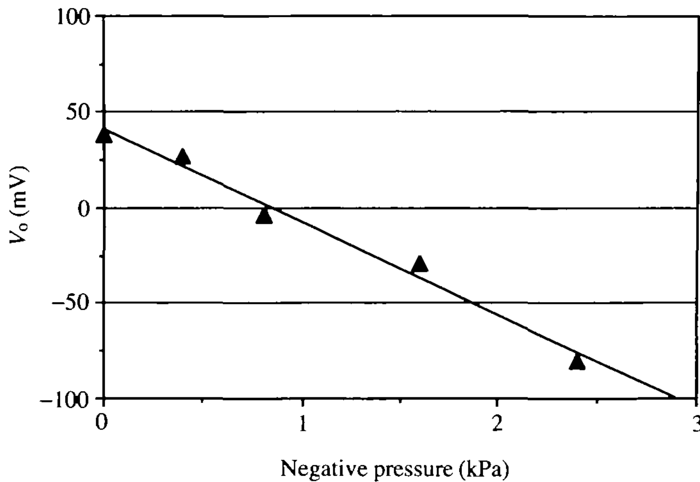


Fig. 7. The relationship between V_0 and applied suction (ΔP). The V_0 values are from Fig. 4B. The V_0 *vs* ΔP relationship can be described by a first-order linear equation with a slope of $-49\,\mathrm{mV\,kPa^{-1}}$.

imposition of hydrostatic pressure might affect the mobility of a gating particle independently of any decrease in gating charge. Although our data will not permit us to distinguish among changes in these several components contributing to n, they do suggest the equivalence between the energies of protein conformation and electrical field in the gating process.

Single-channel conductance is dependent on membrane stretch

Membrane stretch increased the single-channel current and single-channel conductance without changing ionic selectivity. These results indicate that membrane stretch in response to ΔP changed the dimension of the conducting pore of the channel without affecting the selectivity filter and, further, support the notion that the conformation of the channel protein can be altered by the mechanical forces imposed on the cell membrane. Similar results were also reported for the urinary bladder epithelium of this same species (Chang and Loretz, 1991). Any proposal regarding the nature of stretch-induced conformational changes to the protein would be speculative in the absence of structural data. Others have reported stretch-induced increases in P_0 without changes in single-channel conductance (Guharay and Sachs, 1984; Sackin, 1987); together with ours, such findings support independent mechanosensitive loci for gating and conductance.

This stretch-activated channel has at least two open states and three closed states

The kinetic studies show that this SA channel shows three closed states and two open states, as evidenced by the number of exponential components required to fit adequately the closed- and open-time distributions, respectively. The time

constants τ_{o1}, τ_{c1} and τ_{c2} were unaffected by ΔP and/or membrane depolarization. Only τ_{o2}, the longer open-time constant, was sensitive to changes in voltage and stretch (suction); both suction and membrane depolarization increased τ_{o2}. These findings suggest that the energy transduced from either membrane tension or membrane depolarization affects the same conformational transition of the channel protein and, therefore, explains the equivalency of ΔP and membrane depolarization in increasing P_o. Because of the lack of kinetic data, discussion on the voltage- and stretch-sensitivity of the longest closed state must be reserved. Nevertheless, we mention the possibility that, based on its slow transition rate, this state may derive from a covalent modification such as phosphorylation/dephosphorylation.

This stretch-activated channel functions as a Na^+ channel in situ

From the ion substitution experiments, this SA channel shows its characteristic selectivity to the monovalent cations Na^+ and K^+, with P_{Na}/P_K=0.83, and its exclusion of the anion Cl^-. Our observations are in general agreement with the general finding for SA channels from other tissues of a relative non-selectivity among cations, with K^+ being more permeable than Na^+ in those tissues where selectivity has been tested (Guharay and Sachs, 1984; Sackin, 1987; Kirber *et al.* 1988; Chang and Loretz, 1991; Zhou *et al.* 1991). However, to assess the *in situ* function of this SA channel as a pathway for K^+ or Na^+ movement, we need to consider the electrochemical driving forces for K^+ and Na^+ maintained across the cell membrane. The resting membrane potential of posterior intestinal epithelial cells of goby is about −70 mV, a value close to the equilibrium potential for K^+, E_K, when the tissue is bathed in a solution containing 5 mmol l^{-1} K^+ and assuming intracellular K^+ activity to be about 80 mmol l^{-1} (Loretz *et al.* 1985). Although K^+ is more permeant than Na^+ through this SA channel, the Na^+ electrochemical gradient will drive Na^+ influx through this channel at the resting membrane potential. Therefore, we propose that this SA channel functions predominantly as a Na^+ channel *in situ*. Stimulation of SA channel activity may, consequently, produce a slight membrane depolarization, limited by K^+ current flow as V_m moves away from E_K. Membrane depolarization resulting from SA channel activity will also activate voltage-dependent $K^+(Ca^{2+})$ channels, which are highly selective for K^+ over Na^+ (P_{Na}/P_K=0.04), thereby serving to maintain V_m near E_K (Loretz and Fourtner, 1991).

Physiological significance of the stretch-activated channel in teleost intestine

From studies of scanning and transmission electron micrographs, we found more than 90 % of this cell preparation to be epithelial cells. The remainder were blood cells of clearly smaller size. In our experiments, we can easily, based on size, avoid patching those non-epithelial cells. Although we can ascribe the distribution of this channel specifically to the epithelial cell of the posterior intestine, we cannot specify with certainty the location within the cell. It could be in either the apical membrane or the basolateral membrane. If it is in the apical membrane, it

can facilitate Na^+, Cl^- and water reabsorption from the lumen by increasing the Na^+ conductance of the apical membrane, which is an intrinsic part of the ion absorptive function of posterior intestine in goby (Loretz, 1983). Alternatively, this SA channel may be part of a cell volume regulatory mechanism (Sackin, 1987; Bear, 1990), and its cellular location may not be limited to the apical membrane. We have not yet successfully made recordings in the whole-cell configuration, although these measurements could demonstrate the function of these channels in the intact cell under physiologically meaningful levels of membrane stretch induced by osmotic challenge.

Scientists have described the significance of rhythmical intestinal contractions in the mixing of ingested food with digestive enzymes and in the movement of the digesta along the gastrointestinal tract. However, there has been little emphasis on how the absorptive tissues respond to the mechanical changes during peristaltic contraction. We also know very little about the effects of intestinal distention, resulting from the rapid intake of either solid or liquid materials, on the absorptive ability of intestinal epithelium. Perhaps the presence of digesta in the lumen and the associated peristaltic contractions facilitate salt absortion *via* these mechano-transducing ion channels in a local manner temporally appropriate to the delivery of substrate. Here, we report a membrane ion channel that is sensitive to cell membrane tension. This channel may be part of a local regulatory mechanism responding to a change in physical state at the cellular or tissue levels.

This study was supported by NSF Grants DCB-8718633 and DCB-9105874 to C.A.L. We thank Dr C. R. Fourtner for valuable discussion.

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