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Expression of an Outward-Rectifying Potassium Channel from Maize mRNA and Complementary RNA in Xenopus Oocytes

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Injection of Xenopus oocytes with poly(A)$^+$ mRNA isolated from different plants (maize, cucumber, and squash) results in the appearance of a voltage- and time-dependent, potassium-selective, outward current that is similar to the outward-rectifying potassium current recorded in many higher plant cells. Maize shoots were found to be especially enriched in mRNA encoding such activity. A cDNA library of maize shoot mRNA was constructed in the vector λZAPII and was used to synthesize RNA complementary to the cDNA (cRNA). Injection of the cRNA gave rise to an outward-rectifying potassium current with properties similar to the currents obtained by poly(A)$^+$ mRNA injection. These results demonstrate that higher plant mRNA can be properly translated into a product that produces a voltage-regulated potassium channel in the plasma membrane of Xenopus oocytes. Thus, Xenopus oocytes can be used as a heterologous expression system for the functional identification and isolation of plant ion channel genes as well as for the study of structure-function relationship of plant ion channels.

INTRODUCTION

Potassium channels are a class of plasma membrane proteins that selectively facilitate the flow of potassium ions down their electrochemical gradient. Potassium channels have been found in a wide variety of tissues and cells of many plant species (for reviews, see Hedrich and Schroeder, 1989; Tester, 1990). Results from detailed biophysical, pharmacological, and cell biological investigations have shown that potassium channels may provide major pathways for potassium transport across the plasma membrane and contribute significantly to such tissue- and cell-specific functions as control of turgor pressure and stomatal action (Schroeder et al., 1984, 1987; Schroeder, 1988; Fairley-Grenot and Assmann, 1991), leaf movements (Moran et al., 1988, 1990), ion uptake and transport (reviewed by Kochian and Lucas, 1988), and electrochemical homeostasis in the cell (Colombo et al., 1991; Schroeder and Fang, 1991).

To date, two major classes of potassium channel currents have been characterized in higher plant cells (Schroeder et al., 1987). One type facilitates potassium influx upon membrane hyperpolarization (inward-rectifying potassium channels). The other type facilitates potassium efflux upon membrane depolarization (outward-rectifying potassium channels). Both channel types are voltage- and time-dependent and show no significant inactivation (Schroeder, 1988). Therefore, it was hypothesized that these channels allow long-term potassium fluxes as a physiological consequence to a prolonged shift in membrane potential from the potassium equilibrium potential (Schroeder et al., 1984). This role of plant potassium channels as long-term potassium transporters is different and unique from the role of animal potassium channels, which is mainly to reset the membrane potential after the induction of short-term potential changes (Hodgkin and Huxley, 1952).

The regulation of potassium channels involves intrinsic and environmental signals and multiple regulatory mechanisms. Light (Lew et al., 1990), cytosolic Ca^{2+} concentration (Schroeder and Hagiwara, 1989; Ketchum and Poole, 1991), abscisic acid (Blatt, 1990), and GTP binding proteins (Fairley-Grenot and Assmann, 1991) all affect potassium channel activation. In addition, proton ATPases (Assmann et al., 1985; Slayman, 1987), anion channels (Keller et al., 1989; Schroeder and Hagiwara, 1989), and Ca^{2+} permeable channels (Schroeder and Hagiwara, 1990) have been suggested to participate in the control of potassium channel activation by regulation of the membrane potential.

Although the physiological importance of potassium channels has been well documented, little is known about the molecular structure of these proteins in plants, mainly because of the low abundance of these proteins and the lack of characterized potassium channel mutants in higher plants. Conventional molecular genetic approaches by either cDNA library screening or polymerase chain reaction with heterologous DNA sequences derived from animal potassium channel

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genes have been so far difficult in plants. Recently, a breakthrough was achieved when two cDNA clones that complemented yeast potassium transporter deficient mutants were isolated from Arabidopsis (Anderson et al., 1992; Sentenac et al., 1992). Sequence analysis of these clones suggests that they encode potassium channel-like proteins. An electrophysiological assay is now needed to prove that the encoded proteins have potassium channel activity.

To aid the analysis of putative potassium channel genes and to develop an alternative strategy for cloning such genes, we examined Xenopus oocytes as a potential heterologous expression system. Xenopus oocytes efficiently translate exogenous mRNA from both animals and plants (Melton, 1987; Wallace et al., 1988; Aoyagi et al., 1990) and are suitable for voltage and patch clamp analysis (Gundersen et al., 1984). Xenopus oocytes have been used extensively to investigate neurotransmitter receptors and ion channels from animal cells (Dascal, 1987). For example, the Drosophila *Shaker* gene has been expressed in oocytes to study the functional properties of this class of potassium channels (e.g., Iverson et al., 1988; Timpe et al., 1988), including the molecular mechanisms of channel inactivation (Hoshi et al., 1990; Zagotta et al., 1990) and ion selectivity (reviewed by Miller, 1991).

In this study, we present direct evidence of the expression of a plant ion channel in Xenopus oocytes. We show that plant mRNA, when injected into Xenopus oocytes, is expressed to produce potassium channel activity in the plasma membrane of oocytes. In addition, complementary RNA (cRNA) from a plant cDNA expression library produces potassium channel activity in oocytes. This system provides a method for isolating plant ion channel and transporter genes using a functional assay and for studying the detailed structure and function of these proteins.

RESULTS

Monitoring Endogenous Currents and Expression Efficiency in Xenopus Oocytes

To explore the possibility of using Xenopus oocytes as an expression system for plant ion channels, we first examined the endogenous currents present in uninjected or water-injected oocytes by applying a sequence of voltage pulses to the plasma membrane of oocytes. As shown in Figure 1A, the membrane potential was held at −60 mV and then stepped in 20-mV increments to a series of pulse voltages ranging from −180 to +80 mV (Figure 1A, inset). The resulting inward and outward currents were measured (Figure 1A). (Inward current corresponds to the movement of positive charge into the cytoplasm from the external solution and appears as a downward deflection of the signal to negative values.) Time-dependent inward currents developed when oocytes were hyperpolarized to membrane potentials more negative than −160 mV (Figure 1A). Depolarization of the membrane potential to values more positive than −30 mV resulted in small and slowly inactivating (decaying) outward currents in oocytes produced by most frogs

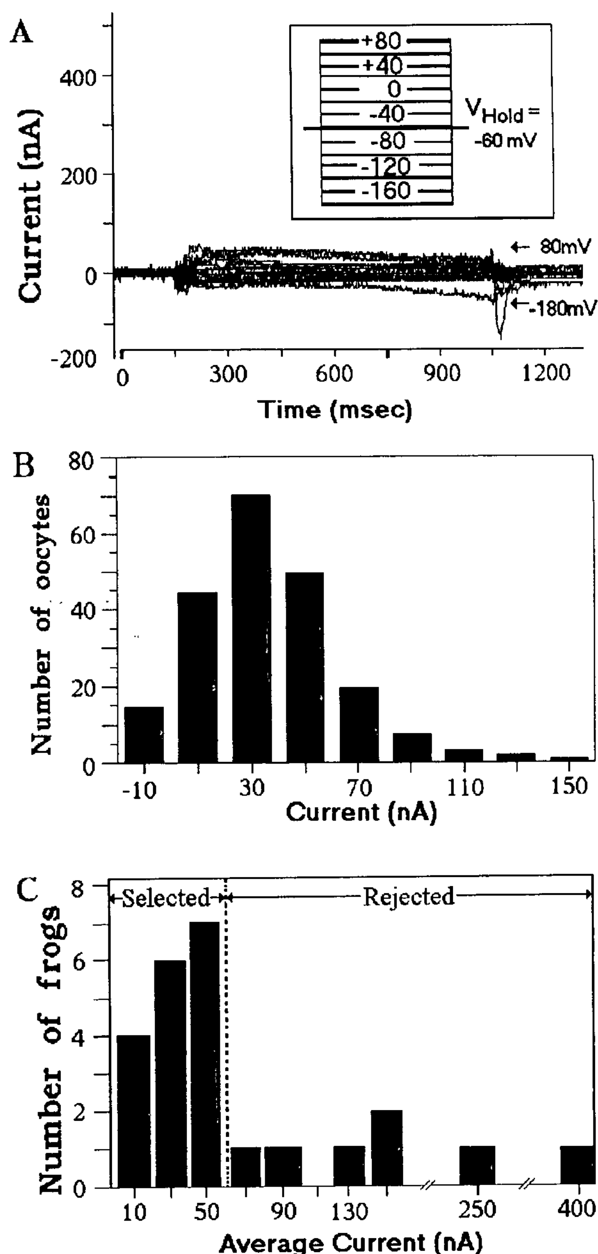

Figure 1. Endogenous Currents in Uninjected or Water-Injected Oocytes.

(A) Voltage-clamp recording of an oocyte injected with water. The membrane potential of the oocyte was held at −60 mV and then shifted for 900 msec to a series of pulse potentials ranging from −180 to +80 mV, as indicated in the inset. The graph shows superimposed current traces recorded during these potential shifts.

(B) Distribution of endogenous outward currents in oocytes from batches selected for low endogenous currents.

(C) Distribution of average currents measured in oocytes from 24 frogs. Oocytes from frogs with less than 60 nA of average endogenous currents were used for subsequent expression studies; oocytes from frogs with higher endogenous currents were rejected.

In **(B)** and **(C)**, outward currents were measured after voltage pulse stimulation had proceeded for 200 msec at a membrane potential of +40 mV.

(Figure 1A; Miledi, 1982). From these frogs, the variation in endogenous outward currents from one oocyte to the next was small (Figure 1B; Lu et al., 1990). In a few cases, a batch of oocytes from a frog showed high and variable endogenous currents (Figure 1C; Parker and Ivorra, 1990), but oocytes from these frogs were not used for subsequent expression studies. These results indicate that one can select a batch of oocytes with low and predictable endogenous currents after testing several oocytes from that batch.

To monitor the efficiency and fidelity of foreign mRNA expression in each batch of oocytes, we used a cDNA clone (XSha2) that encodes a Xenopus outward-rectifying potassium channel protein. The XSha2 gene is expressed in the nervous system but not in the oocytes of frogs (Ribera, 1990). RNA transcripts of the cDNA (cRNA) were synthesized in vitro and then injected into oocytes. After 1 to 3 days of incubation to allow translation of the RNA in vivo, currents elicited by both depolarization and hyperpolarization pulses were measured, as shown in Figure 2. An outward-rectifying potassium current could be easily detected in almost every injected oocyte, whereas no change in magnitude of inward current could be detected. These results match those previously reported for XSha2 cDNA (Ribera, 1990). The average amplitude of potassium current expressed from XSha2 cRNA varied among oocytes from different frogs by severalfold (data not shown), indicating that expression efficiency was somewhat variable among different batches of oocytes.

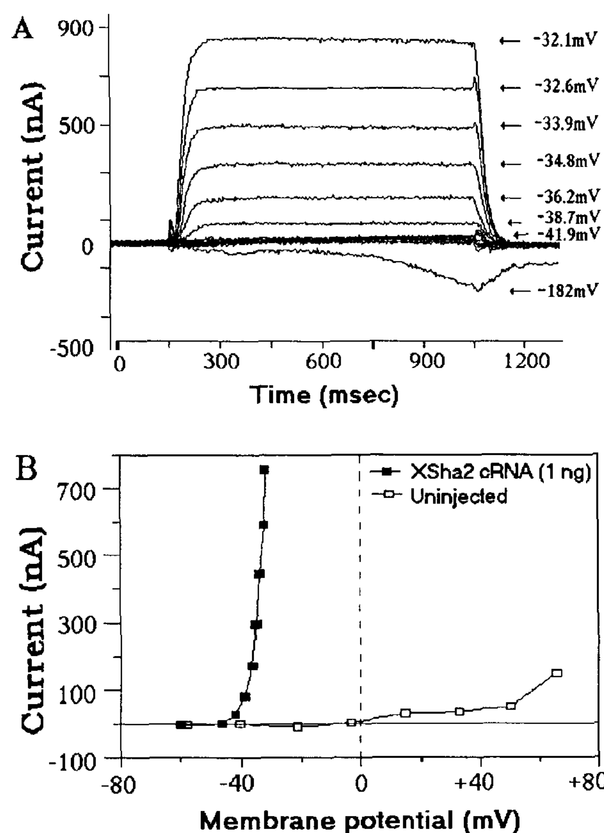

Figure 2. Currents Measured in a Xenopus Oocyte Expressing the Xenopus Potassium Channel Gene XSha2.

(A) Voltage-clamp recording of an oocyte 3 days after injection of 1 ng of XSha2 cRNA. Currents across the plasma membrane during each depolarization and hyperpolarization pulse were measured and superimposed. The membrane potential was held at −60 mV. The values of each depolarization pulse are indicated to the right of each outward current trace. Hyperpolarization pulses were performed in −20-mV increments from the holding potential, and the value of the most negative pulse is indicated next to the correspondent current trace.
(B) Current-to-voltage relationship. Outward currents in cRNA-injected (■) and uninjected (□) oocytes were plotted as a function of membrane potential during the voltage pulses. Currents were measured at the end of 200 msec of stimulation after subtraction of leakage.

These results confirm that Xenopus oocytes can be used to express potassium channel mRNA and measure potassium current carried by these channels. In each subsequent experiment, oocytes from the same donor were used. Endogenous currents (Figure 1) and expression efficiency (Figure 2) of the oocytes were monitored in all experiments to ensure the reliability of the subsequent expression experiments.

Expression of an Outward Current from Plant Poly(A)+ mRNA in Oocytes

To assay whether mRNAs from plants might encode ion channels that could be assayed in the Xenopus oocyte expression system, poly(A)+ mRNA from maize shoot and maize root were injected into Xenopus oocytes. Figures 3A and 3B show typical voltage-clamp recordings of ionic currents across the plasma membrane of Xenopus oocytes 5 days after injection of maize shoot and root mRNA, respectively. Depolarization of the membrane from a holding potential of −60 mV to values more positive than −40 mV produced outward currents that were voltage- and time-dependent and were five to 15 times larger than the control (uninjected or water-injected) oocytes (Figures 3C and 3D). No increase in the magnitude of inward currents was detected in the oocytes expressing the outward current (Figures 3A and 3B); thus, the new channel activity in the mRNA-injected oocytes was exclusively outward-rectifying and had no measurable inward-rectifying component. Other sources of poly(A)+ mRNA from cucumber and squash were also tested and showed expression of a similar outward current (data not shown). The largest and most reproducible voltage-dependent outward currents were recorded when maize shoot mRNA was injected into the oocytes. Thus, ion channel activity could be detected in oocytes injected with plant poly(A)+ mRNA.

The outward currents expressed from maize mRNA in oocytes showed no obvious inactivation during 900 msec of stimulation but showed variable time courses of initial activation. The time required for maximum activation ranged from 50 to 200 msec in maize shoot mRNA-injected oocytes and 100 msec to 1 sec in oocytes injected with maize root mRNA (Figures 3A and 3B, and data not shown).

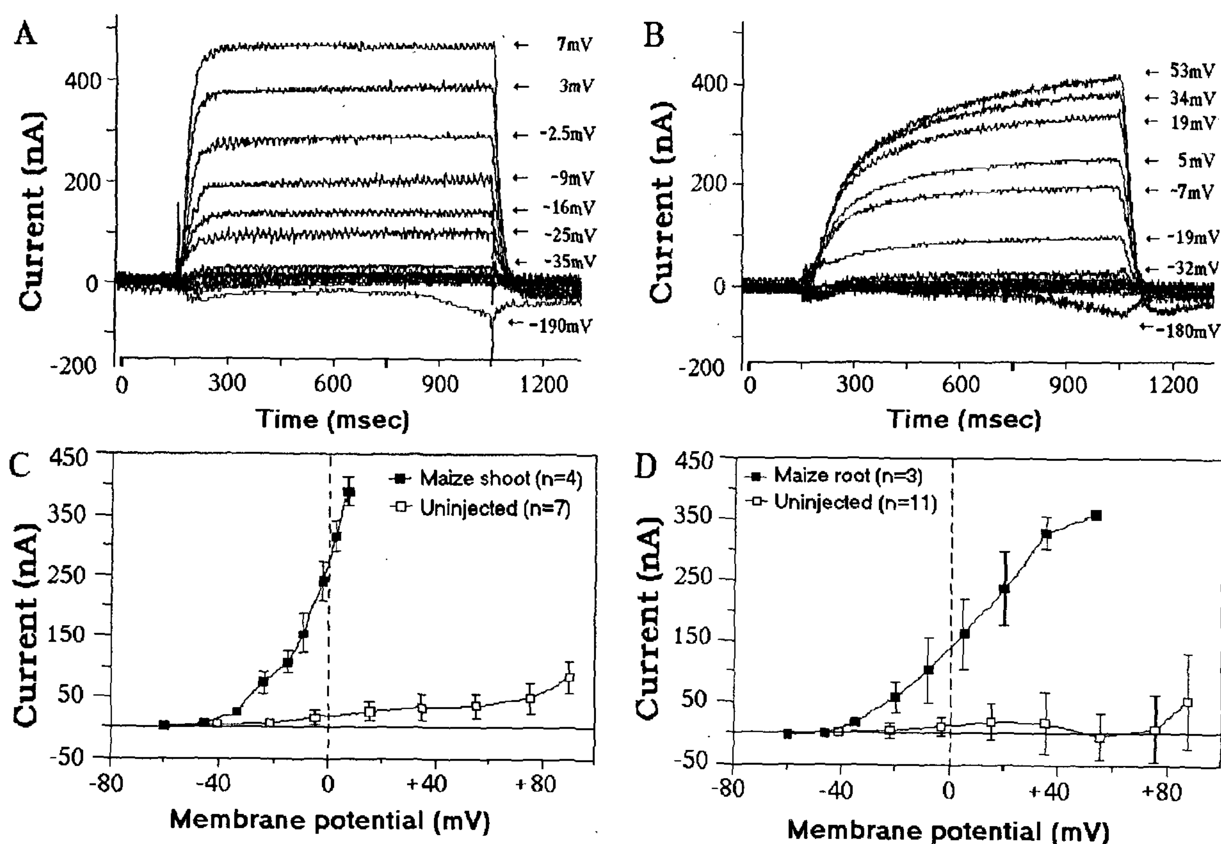

Figure 3. Expression of Outward Currents in Xenopus Oocytes Injected with Poly(A)$^+$ mRNA Isolated from Maize Shoots and Roots.

(A) and **(B)** Voltage-clamp recording of oocytes injected with maize mRNA. Currents were measured 5 days after injection using the voltage protocol described in the legend of Figure 2. Oocytes were injected with approximately 50 ng of mRNA from maize shoot **(A)** and maize root **(B)**.
(C) and **(D)** Current-to-voltage relationship. Outward currents in maize shoot **(C)** or root **(D)** mRNA-injected (■) and uninjected (□) oocytes were plotted as a function of membrane potential. Vertical lines indicate the standard deviation in measurements taken from different oocytes. Data from one batch of oocytes were used in each plot. Similar results were obtained using at least two additional batches of oocytes from different frogs.

Outward Currents Are Carried by Potassium Ions

To determine which ions carried the outward currents across the plasma membrane, the reversal potential was measured by relaxation ("tail") current analysis (Hille, 1984). Relaxation current experiments were performed by depolarizing the plasma membrane from a holding potential of −60 mV to a pulse potential of 0 mV for 750 msec, leading to a large outward current in maize mRNA-injected oocytes. From the pulse potential of 0 mV, the membrane potential was stepped back to more negative potentials (V_{tail}) at which the decay of the outward currents (deactivation) could be monitored, as illustrated in Figure 4. The direction of the decaying "tail" currents reversed at −78 ± 22 mV (mean ± SD, $n = 4$). The predicted reversal potential calculated from the Nernst equation for a purely potassium permeable current is in the range of −91 to −105 mV (Barish, 1983; Dascal, 1987). But considering a low permeability of Na^+ ions to some plant potassium channels (Schroeder et al., 1984, 1987; Moran et al., 1990), the expected reversal potential can shift to a potential of −49 mV under the imposed recording conditions, calculated from the Goldmann equation (Hille, 1984). Other possible ions that can carry a current under the same recording conditions (e.g., Na^+, Ca^{2+}, and Cl^-) have a reversal potential more positive than −26 mV (Barish, 1983; Dascal, 1987). These findings indicate that the outward currents expressed from maize mRNA in Xenopus oocytes are carried mostly by potassium ions and may have ion selectivity properties similar to those of outward-rectifying potassium channels in higher plant cells. Additional evidence is presented below.

Expression of an Outward Potassium Current from cRNA in Oocytes

One useful application of the Xenopus oocyte expression system would be to isolate a cDNA clone responsible for the functional expression of outward-rectifying plant potassium

channel activity. To determine the feasibility of this approach, we first prepared a directional cDNA library. The first-strand cDNA was synthesized using poly(A)$^+$ mRNA from maize shoot and a NotI-oligo(dT) linker primer. The second-strand cDNA was synthesized; then EcoRI adaptors were ligated to the product. NotI-digested cDNA was ligated to EcoRI, NotI double-digested λZAPII vectors and then packaged into phages. The resultant cDNA library had a complexity of 5 × 10^6, with 85% of the phages being recombinant.

Pools of 50,000 recombinant phages (independent cloning events) from the maize shoot cDNA library were amplified and used to prepare DNA templates for RNA synthesis. RNA was prepared using T7 RNA polymerase and NotI-digested phage DNA. Following microinjection of in vitro–synthesized RNA (cRNA), Xenopus oocytes were tested for the appearance of voltage-dependent outward currents. In 3 to 5 days after injection, an outward current could be detected that was three to 10 times larger than the endogenous current and was very similar to the currents expressed from maize shoot poly(A)$^+$ mRNA in oocytes. A typical example is shown in Figure 5A. Figure 5B shows the average current–voltage relationship elicited upon depolarization of injected oocytes from a holding potential of −60 mV. The outward current in cRNA-injected oocytes was activated at potentials of −35 mV and was voltage- and time-dependent.

The current in cRNA-injected oocytes was also K^+-selective based on its reversal potential of approximately −55 mV determined by relaxation current measurements, as shown in Figure 6. Further evidence that depolarization-activated outward currents were carried by potassium channels resulted from pharmacological studies with potassium channel blockers. As shown in Table 1, both Ba^{2+} and tetraethylammonium, commonly used blockers of potassium channels, reduced the

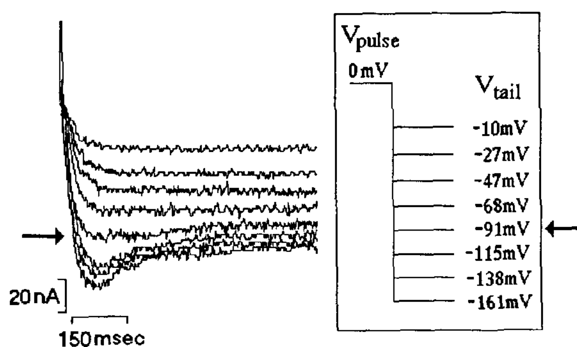

Figure 4. Outward Currents Expressed from Maize Shoot mRNA Are Carried by Potassium Ions.

Relaxation current analysis was performed as described in Results. Current relaxation graph at left shows superimposed traces of deactivation (tail) currents in a maize shoot mRNA-injected oocyte following a step repolarization from a membrane potential of 0 mV to V_{tail} potentials according to the voltage protocol shown at right. The approximate potential at which the current reversed direction is indicated by the arrows.

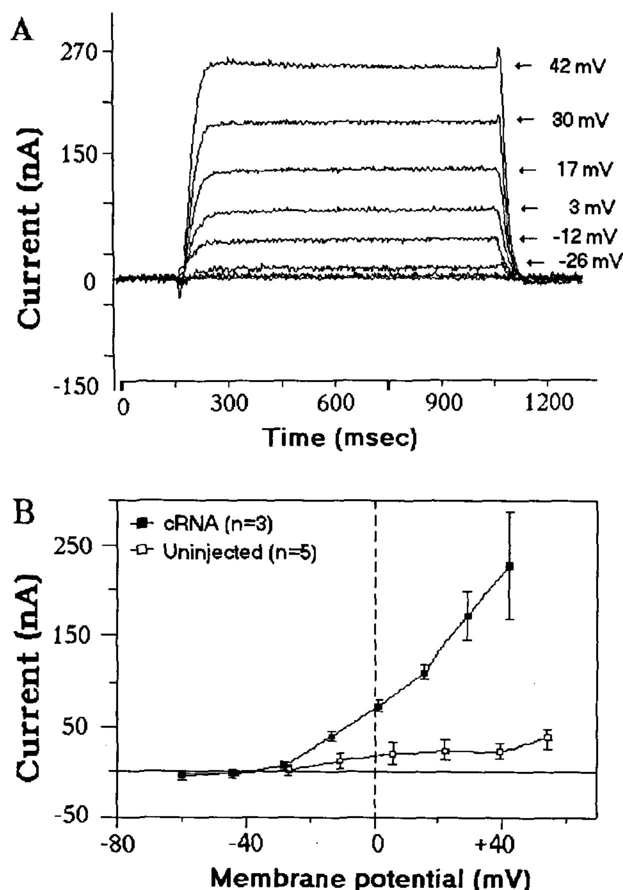

Figure 5. Expression of Outward Currents in Xenopus Oocytes Injected with Maize Shoot cRNA.

(A) Voltage-clamp recording of an oocyte injected with cRNA. Oocytes were injected with approximately 20 ng of cRNA. Five days later, outward currents were measured across the plasma membrane during membrane potential shifts from a holding potential of −60 mV to the potentials indicated next to each trace.

(B) Current-to-voltage relationship. Mean of outward currents of cRNA-injected oocytes (■) or uninjected oocytes (□) were plotted as a function of membrane potential. Similar results were obtained in three separate batches of oocytes from different frogs.

magnitude of outward currents expressed from cRNA at concentrations normally used to block outward-rectifying potassium channels in maize (Ketchum and Poole, 1990). Ba^{2+} inhibition of outward-rectifying potassium channels was fully reversible upon washing out of extracellular Ba^{2+}.

DISCUSSION

Xenopus oocytes are widely used as a model system to study neurotransmitter receptors and ion channels in animal systems because of the unique features oocytes possess

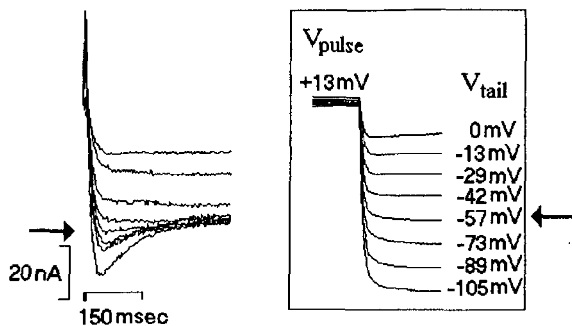

Figure 6. Determination of the Reversal Potential of the Outward Current Expressed in a cRNA-Injected Oocyte.

Relaxation current analysis was performed as described in Results. Graph at left shows superimposed traces of tail currents in a cRNA-injected oocyte following a step repolarization from a membrane potential of +13 mV to V_{tail} potentials shown at right. The approximate potential at which the current reversed direction is indicated by the arrows.

(Gundersen et al., 1984; Dascal, 1987). For example, Xenopus oocytes are very large (up to 1.3 mm in diameter) and thus suitable for microinjection, voltage-clamping, and other electrophysiological manipulations. Oocytes readily translate injected mRNA, producing functional exogenous proteins. Recently, Xenopus oocytes have been exploited to isolate several ion channel and receptor genes from animal cells, such as a delayed rectifier K^+ channel gene (drk1) from rat brain (Frech et al., 1989) and a slow voltage-gated K^+ channel gene from rat kidney (Takumi et al., 1988). Xenopus oocytes may also provide a powerful expression system to isolate and analyze ion channel, receptor, and transporter genes from plants. To explore this possibility, we injected oocytes with plant mRNA and observed the expression of functional ion channels. Outward-rectifying potassium currents were observed when either maize shoot mRNA or cRNA was injected into oocytes, and these currents were significantly larger than the endogenous currents of uninjected oocytes. The expression level was comparable to that found for animal poly(A)$^+$ mRNA-injected oocytes (Takumi et al., 1988; Frech et al., 1989). These results indicate that Xenopus oocytes express functional plant ion channels and may be used for characterizing clones of putative ion channel genes (Anderson et al., 1992; Sentenac et al., 1992) and studying ion channel regulation and structure–function relationships. In addition, this system provides an alternative method for cloning ion channel genes that may yield clones not identified by other procedures (e.g., Takumi et al., 1988).

The properties of the outward currents expressed from maize mRNA in oocytes are similar to those of outward-rectifying potassium channels recorded in higher plant cells (Schroeder et al., 1987; Moran et al., 1988, 1990; Ketchum and Poole, 1990). For example, both the expressed oocyte currents as well as the native outward-rectifying potassium currents in higher plant cells are K^+-selective and voltage- and time-dependent with activation potentials in the range of −40 to −30 mV. Both outward currents in maize suspension cells (Ketchum and Poole, 1990) and in Xenopus oocytes are inhibited by Ba^{2+} and tetraethylammonium at similar concentrations. At strongly depolarized potentials, potassium current saturation became apparent (e.g., Figures 3B and 3D), which correlates to saturation of single-channel current amplitudes (Gradmann et al., 1988), although yet unidentified "bipolar" gating has alternatively been suggested as a mechanism for saturation (Blatt, 1988). In addition, the outward currents in oocytes and higher plants show no significant inactivation (Schroeder, 1988). These similarities indicate that plant potassium channels can be faithfully synthesized, properly processed, and integrated into the plasma membrane of Xenopus oocytes.

Outward-rectifying potassium channels in various higher plant cells show variation in their activation time course (e.g., Schroeder, 1989; Ketchum and Poole, 1990). Similarly, we saw variation in the activation time course of plant potassium channels expressed in Xenopus oocytes (Figures 3A and 3B). The Xenopus oocyte expression system should provide a potent approach for determining whether these variations in potassium channel activation times can be attributed to different potassium channel gene isoforms or post-translational modification. Similarly, variation in activation times of potassium channels has been observed in animal systems (for review, see Jan and Jan, 1990).

Several sources of poly(A)$^+$ mRNA from cucumber, squash, maize shoot, and maize root were tested for expression of potassium channels by injection into Xenopus oocytes. In each case, potassium channel activity was detected (Figure 3 and data not shown). These results support the hypothesis that outward-rectifying potassium channels are ubiquitous in higher plants (Hedrich and Schroeder, 1989). The amplitude of the expressed currents varied depending on the source of the mRNA. The different amplitudes could arise from differences in the abundance of potassium channel mRNA or in the

Table 1. Inhibition by Potassium Channel Blockers of Outward Currents Expressed in cRNA-Injected Oocytes

Blocker	Concentration (mM)	Inhibition (%) ± SD
Ba^{2+}	1	23.2 ± 4.5 (n = 2)
	10	41.8 ± 5.5 (n = 5)
	20	63.0 (n = 1)
	50	87.5 (n = 1)
TEA	1	18.4 ± 4.2 (n = 2)
	10	28.0 ± 0.4 (n = 3)
	50	39.3 ± 6.0 (n = 2)

Data were collected from oocytes that showed the expression of an outward current from cRNA. Blockers were applied externally to the bath solution. Peak outward currents elicited by depolarization pulses were measured for statistical analysis. TEA, tetraethylammonium.

translational efficiency of the particular plant mRNA in Xenopus oocytes.

In addition to outward-rectifying potassium channels, which are activated upon membrane depolarization, another major class of potassium channel, inward-rectifying potassium channel, has also been identified in many plant cells (Schroeder et al., 1987; Bush et al., 1988; Colombo and Cerana, 1991). Inward-rectifying potassium channels are activated upon hyperpolarization; these channels have been suggested to provide a pathway for potassium uptake into higher plant cells (Schroeder et al., 1984, 1987; Schroeder and Fang, 1991). It is interesting to note that putative Arabidopsis potassium channel genes have homology to outward-rectifying potassium channel genes from animal systems (Anderson et al., 1992; Sentenac et al., 1992). On the other hand, these homologs complement potassium uptake that has been ascribed to inward-rectifying potassium channels (Schroeder et al., 1987), leaving open the question of the function of these ion channel homologs. In our heterologous expression system, we were unable to detect any expression of inward potassium current in the oocytes expressing outward potassium currents from plant mRNA. This observation supports the hypothesis that inward- and outward-rectifying potassium channels are two discrete channel types (Schroeder et al., 1987) and suggests that they may be encoded by two different genes.

In conclusion, Xenopus oocytes are a useful expression system for studying plant genes involved in ion transport. Maize shoot tissue is a good source of mRNA for expression of outward-rectifying potassium channel activity in oocytes. The maize mRNA can be replaced by cRNA synthesized in vitro from a maize cDNA expression library. It is hoped that by fractionating this library, we will be able to identify a cDNA clone that encodes a functional outward-rectifying potassium channel from maize.

METHODS

Plant Materials and Poly(A)$^+$ mRNA Isolation

Maize seeds were obtained from R. J. Schmidt, University of California at San Diego (La Jolla, CA). Cucumber and squash seeds were from Ferry-Morse Seed Company (Fulton, KY). Seeds were germinated on vermiculite in the dark at 30°C and were irrigated with distilled water every other day. Four to 5 days after planting, the etiolated seedlings were harvested, washed in distilled water, frozen in liquid nitrogen, and then stored at −80°C until needed. Shoots and roots from maize seedlings were separated before freezing.

Poly(A)$^+$ mRNAs were isolated as described by Wilkinson and Crawford (1991). A yield of 2 to 5 mg of poly(A)$^+$ mRNA per g (fresh weight) of tissue was obtained.

cDNA Library Construction

For cDNA synthesis, about 5 μg of poly(A)$^+$ mRNA from maize shoot was used. First- and second-strand cDNAs were synthesized using a cDNA synthesis kit (Pharmacia LKB Biotechnology) except that 5 μg of NotI-oligo(dT) primer (CTGAAGCGGCCGCTTTTTTTTTTTT-TTTTT) was used. An EcoRI adaptor (Stratagene) was ligated to the cDNA. The resultant products were phosphorylated by T4 polynucleotide kinase (Pharmacia LKB Biotechnology). Following NotI digestion, the cDNA was further purified on a Sephacryl S-300 spun column (Pharmacia LKB Biotechnology). The resultant cDNA had different cohesive ends (EcoRI at 5′ end and NotI at 3′ end) and were unidirectionally ligated to EcoRI, NotI double-digested arms of λZAPII as described by the manufacturer (Pharmacia LKB Biotechnology). Phage DNA was packaged using GigapackII Gold packaging extract (Stratagene).

Complementary RNA Synthesis

For the preparation of XSha2 complementary RNA (cRNA), an XSha2 cDNA clone (inserted into the downstream region of the T7 promoter of the pSp73 plasmid; Ribera, 1990) was linearized by PstI digestion and then incubated with 20 to 50 units of T7 RNA polymerase (Stratagene) for 60 min at 37°C in the following buffer: 20 mM NaH_2PO_4/Na_2HPO_4, pH 7.7; 10 mM DTT; 8 mM $MgCl_2$; 4 mM spermidine; 0.4 mM each of ribosomal ATP, GTP, CTP, and UTP (Promega); 0.4 mM cap analog (pGpppG; Pharmacia LKB Biotechnology); 1 unit/μL RNAsin (Promega). The reaction products were phenol extracted, ethanol precipitated, and resuspended in deionized H_2O at a final concentration of 0.5 to 1 mg/mL.

For the preparation of cRNA from a maize shoot cDNA library, a pool of 50,000 phages from the library was amplified, and phage DNA was extracted following the standard protocols of Maniatis et al. (1982). Phage DNAs were linearized by digestion with NotI and then used as templates for in vitro transcription as described above.

Xenopus Oocyte Preparation and Microinjection

Adult female *Xenopus laevis* frogs were anesthetized and placed on ice. A small incision was made on the side of the abdomen to remove several ovarian lobes. The lobes were torn apart and immersed in sterile Barth's solution (88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 10 mM Hepes-NaOH, 0.33 mM $Ca[NO_3]_2$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, pH 7.4) supplemented with gentamycin (1 mL/L; Sigma). Oocytes were defolliculated by incubation in Barth's solution containing 5 mg/mL collagenase (type A; Boehringer) at room temperature for 1 to 2 hr and in potassium phosphate solution (100 mM K_2HPO_4, pH 6.5 [adjusted with HCl], 1 g/L BSA, fragment V) at room temperature for another hour on a circulatory shaker (60 rpm). Oocytes were washed five times with Barth's solution containing BSA (1 g/L) at the end of each incubation. Stage 5 to 6 oocytes (Dumont, 1972) were selected and stored at 18°C in the dark in Barth's solution containing gentamycin (1 mL/L) for 1 day before microinjection. Transcripts (1 to 50 ng per oocyte) were injected in a final volume of 50 nL. Oocytes were incubated in Barth's solution supplemented with 1 mL/L gentamycin for 1 to 6 days at 18°C in the dark before recording.

Voltage Clamp

Microelectrode voltage clamp experiments were performed with a two-electrode amplifier (Dagan, Minneapolis, MN) in a continuously perfused bath at room temperature. The two microelectrodes were filled

with 3 M KCl. The data were low-pass filtered with 8-pole Bessel characteristics at a cut-off frequency of 60 Hz. The time resolution of voltage-clamped oocytes was determined by simultaneous recording of oocyte membrane potentials during all pulses. Voltage protocols and data acquisition were performed by a pCLAMP program (Axon Instruments, Inc., Burlingame, CA). Leakage currents were subtracted using a digital P/6 linear leakage subtraction method (Bezanilla and Armstrong, 1977) in the range of −60 mV. Control experiments without leakage subtraction were performed to ensure that a linear background conductance was subtracted. Membrane currents were measured in Ringer solution that contained 115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 1 mM $NaHCO_3$, 10 mM Hepes, 1 mM $MgCl_2$, pH 7.4.

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