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A Calcium Homeostasis Mechanism Induced by Heterologous Expression of Total RNA from Chicory Leaves in *Xenopus* Oocytes

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Abstract. *Xenopus* oocytes were injected with total RNA from chicory leaf tissues and then examined by the voltage-clamp technique.

A double-step voltage protocol was used, with an initial hyperpolarization step from the holding potential of −35 to −140 mV followed by a second depolarization step to +60 mV. Two different outward currents were observed, one noninactivating (I_{ni}), and one inactivating (I_i). Only the noninactivating outward current (I_{ni}) could be induced by depolarization from −35 to +60 mV. The mean amplitude of I_{ni} was 2915 ± 848 nA (n = 11). This current, carried by chloride ions, declined nearly to the baseline in 153 ± 64 sec (n = 13), and was highly dependent on intracellular calcium. After the rundown of I_{ni}, the same oocyte was depolarized from −140 to +60 mV. This protocol induced an inactivating outward current (I_i) with a mean amplitude of 4461 ± 1605 nA (n = 13). I_i was also carried by chloride ions and dependent on extracellular calcium. I_i was strongly inhibited by 100 μM extracellular La^{3+}.

These two types of chloride currents were also observed after IP_3 injection in control oocytes. I_{ni} and I_i were not observed in noninjected oocytes or water-injected oocytes.

We suggest that the expression of total chicory leaf tissue RNA in *Xenopus* oocytes reveals a calcium homeostasis mechanism responsible for calcium mobilization from internal stores and subsequent calcium entry.

Key words: Plant — *Cichorium* — *Xenopus* oocytes — Calcium — Chloride conductances — Voltage-clamp

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Introduction

Many physiological plant signal transduction processes involve calcium ions as a key element. Variations in cytosolic free calcium concentrations in response to a variety of external stimuli have been observed in several cell types (for reviews, *see* Bush, 1995; Webb et al., 1996). Numerous studies have reported that calcium may be involved in higher plant somatic embryogenesis (Timmers, De Vries & Schel, 1989; Jansen et al., 1990; Overvoorde & Grimes, 1994). In vitro somatic embryogenesis has been defined as a process producing an embryo from a somatic cell. Somatic embryogenesis provides a valuable system for studying early embryo development events in plants. In carrots, an increase in cytosolic calcium concentration has been described during somatic embryo development from the late globular to the torpedo-shaped stage (Timmers et al., 1996). In the chicory hybrid ''474'', embryogenic cells were characterized, in leaf tissues after five days of culture, by accumulation of calcium in the vacuole (Verdus et al., 1993) and by a callose deposit in the cell wall (Guedira, Dubois & Vasseur, 1990). This callose deposit may be triggered by calcium as shown in *Catharenthus roseus* (Kauss, Waldmann & Quader, 1990).

No electrophysiological data about calcium homeostasis in *Cichorium* have been published so far. However some calcium transport systems have been described in other plant cell types. Two major pathways for calcium increase in the cytosol have been shown, i.e., entry from the extracellular compartment (apoplast) through the plasma membrane and/or release from internal stores, mainly through the tonoplast (vacuolar membrane). Studies have provided evidence for the existence of mechanosensitive calcium channels (Cosgrove & Hedrich, 1991) and voltage-gated calcium channels in

the plasma membrane (White, 1993, 1994; Thuleau et al., 1994*a,b;* Piñeros & Tester, 1995, 1997). Several different calcium transport systems have been described in the tonoplast: a ligand-gated calcium channel, activated by IP_3 (Inositol 1,4,5-trisphosphate) (Schumaker & Sze, 1987; Ranjeva, Carrasco & Boudet, 1988; Alexander et al., 1990; Brosnan & Sanders, 1990; Johannes, Brosnan & Sanders, 1992; Canut et al., 1993; Allen, Muir & Sanders, 1995; Muir et al., 1997), an IP_3-sensitive Ca^{2+} store other than the vacuole has been reported (Muir & Sanders, 1997), a voltage-gated calcium channel (Johannes, Brosnan & Sanders, 1992; Ping, Yabe & Muto, 1992; Gelli & Blumwald, 1993; Allen & Sanders, 1994; Ward & Schroeder, 1994; Ward, Pei & Schroeder, 1995) and a cyclic-ADP-Ribose (cADPR)-gated pathway (Allen et al., 1995; Muir et al., 1997).

Xenopus oocytes have provided a powerful heterologous expression system for animal as well as plant genetic material. Several studies have already been published showing the expression in *Xenopus* oocytes of plant transport proteins, including a KAT_1 potassium channel (Schachtman et al., 1992; Cao et al., 1992), a chloride channel (Lurin et al., 1996), a nitrate transporter (Tsay et al., 1993), and an H^+/hexose cotransporter (Boorer et al., 1992).

In the present study, we suggest for the first time that the expression of total RNA from chicory leaf tissues in *Xenopus* oocytes reveals a calcium homeostasis mechanism responsible for calcium mobilization from internal stores and subsequent calcium entry from the external medium.

Materials and Methods

Plant Material and Culture Conditions

The chicory hybrid clone ''474'' (*Cichorium intybus* L. × *Cichorium endivia* L.) was propagated in vitro from styles by somatic embryogenesis as previously described by Dubois et al., 1988. Plantlets were grown on Heller medium containing 15 mM sucrose, in a growth chamber with a 12 hr light/12 hr dark regime. The temperature was 22°C/24°C with a cool-white fluorescent light (50 μM · m^{-2} · sec^{-1}). Leaves were delicately cut off four to six week-old plantlets and put into liquid nitrogen for RNA extraction.

Extraction and Purification of Total RNA

The total RNA extraction method was derived from Chirgwin et al., 1979. Leaves stored in liquid nitrogen at –70°C were ground to a powder using a mortar and pestle, then solubilized in GIT buffer (isothiocyanate guanidium 4 M; β-mercapto-ethanol 0.1 M; sodium acetate 25 mM, pH 6). After centrifugation (15,000 × *g,* 10 min, 4°C), the supernatant was settled in a cesium chloride solution (5.7 M) with potassium acetate (25 mM), pH 6. After ultracentrifugation (125,000 × *g,* 21 hr, 20°C), the RNA pellet was solubilized in a sodium acetate solution (0.3 M) and precipitated by adding ethanol (v/v 0.3:0.7) for 18 hr at –20°C. The pellet was drained after centrifugation (10,000 × *g,* 5 min, 4°C) and, finally, suspended in sterile water. All solutions, except the final sterile water, were treated with DMPC (dimethyl pyrocarbonate v/v 1:100).

Oocyte Preparation

Ovaries were dissected from tricaine methane sulphonate-anaesthetized female *Xenopus laevis* (CRBM Montpellier, France). Ovarian follicles were removed and oocytes were isolated in normal Ringer (ND96), without calcium, containing 2 mg/ml collagenase A (Boehringer, France). Stage V and VI oocytes (Dumont classification, 1972) were selected for electrophysiological measurements. Oocytes were injected with 60 nl of a 5 mg/ml solution of total RNA from *Cichorium* leaves. Noninjected oocytes were used as negative control; oocytes injected with sterile water had a behavior similar to that of noninjected oocytes (as previously described by Fournier et al., 1989; Tomaselli et al., 1990; Schroeder et al., 1994; Tosco et al., 1998). Oocytes could be maintained for 2–6 days at 19°C in a ND96 medium containing (in mM): NaCl, 96; KCl, 2; $CaCl_2$, 1.8; $MgCl_2$, 2; HEPES, 5; pH 7.45 with NaOH; and supplemented with 50 μg/ml gentamicin.

Electrophysiological Measurements

Electrophysiological measurements were performed from day three to day five after injection, using the standard two-microelectrode voltage-clamp technique with the TEV-200 amplifier (Dagan Instruments, Minneapolis, MN). Oocytes were placed in a recording chamber (300 μl) and impaled with 3 M KCl-filled electrodes (0.5–1.5 MΩ resistance). In all experiments, oocytes were depolarized every 40 sec from –35 or –140 mV to different test potentials for 1 sec or 1.2 sec. Stimulation of the preparation, data acquisition and analysis were performed using pCLAMP software (ver. 5.5, Axon Instruments, Burlingame, CA).

Solutions

Solutions were applied externally by addition to superfusate (gravity-driven superfusion).

We used: (i) a chloride-free medium (in mM): NaOH, 96; KOH, 2; $MgOH_2$, 2; $CaOH_2$, 1.8; HEPES, 5; pH 7.45 titrated with methane sulfonate; (ii) a calcium-free medium (in mM): NaCl, 96; KCl, 2; $MgCl_2$, 2; HEPES, 5; EGTA, 1; pH 7.45 with NaOH.

Internal perfusion

Oocytes were impaled with a third additional micropipette (Nichiryo Digital Micropipette). We injected 40 nl of a 25 mM solution of K_4. BAPTA or 1 mM IP_3, dissolved in HEPES KOH (pH 7.2), to give, respectively, a final calculated concentration in the oocyte of 1 mM or 50 μM.

Analysis

Results were expressed as mean ± SE, *n* indicating the number of oocytes tested and *N* the number of frog donors.

Results

Membrane Currents in *Xenopus* Oocytes Injected with Total RNA from *Cichorium* Leaves

The double-step voltage protocol designed to reveal membrane currents in oocytes consisted of an initial hy-

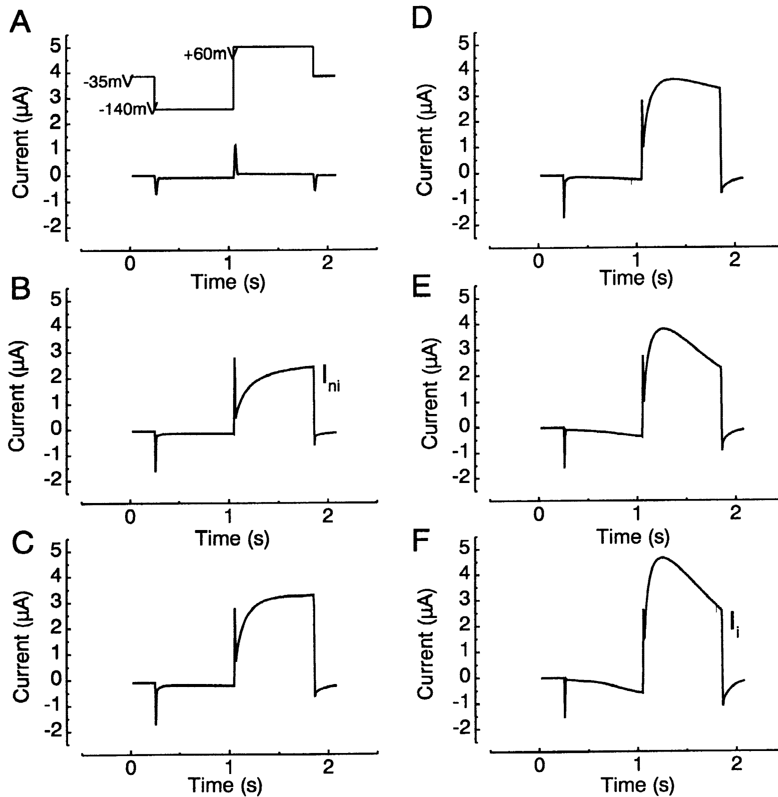

Fig. 1. Change of outward current kinetics recorded on oocytes injected with total RNA from *Cichorium* leaf tissues. (*A*) Typical outward current evoked from −140 to +60 mV in a noninjected oocyte (n = 32, N = 9). (*B–F*) Typical traces of the outward current induced by depolarizing pulses from −140 to +60 mV lasting 1 sec. The kinetics of the outward current gradually changed over time. (*B*) At the start of the test and (*C*) after 40 sec, the current did not inactivate. (*D*) 80 sec, (*E*) 120 sec and (*F*) 160 sec later, the outward current inactivated and increased in amplitude.

perpolarization step from the holding potential of −35 mV (resting potential of oocytes 3 days after incubation in ND96 medium) to −140 mV, followed by a second depolarization step to +60 mV. This protocol was repeated every 40 sec. In noninjected oocytes, this double-step voltage protocol induced only a small outward current at +60 mV (Fig. 1*A*). In *Xenopus* oocytes injected with total RNA from *Cichorium* leaves, a large outward current was observed under the double-step voltage protocol. The membrane currents elicited by depolarization steps from a holding potential of −140 to +60 mV exhibited complex behavior. During the initial stimulation, a noninactivating outward current was observed (Fig. 1*B*). After 40 sec, the current amplitude was increased without any change in the kinetics (Fig. 1*C*). Eighty seconds later (Fig. 1*D*), the current kinetics changed gradually to an inactivating behavior (Fig. 1*E–F,* 120 and 160 sec after the first stimulation, respectively). As demonstrated later, this outward current displayed (i) a noninactivating component (I_{ni}) with a decay in amplitude over time, known as ‘‘rundown’’, and (ii) an inactivating component (I_i).

Characterization of the Noninactivating Outward Current

To study the noninactivating outward current, depolarization steps from −35 to +60 mV were applied to the oocytes. This protocol was used to isolate the noninactivating outward current as the inactivating outward current was never observed under these conditions. This protocol did not induce any current in noninjected oocytes (n = 32, N = 9; Fig. 2*A*). When the same protocol was used on oocytes injected with total RNA, a noninactivating outward current (I_{ni}) was observed, with rapid biexponential activation kinetics (τ_1 = 68.74 ± 15.49 msec; τ_2 = 467.20 ± 65.29 msec; n = 12, N = 2; Fig. 2*B*). The mean amplitude of I_{ni} at +60 mV was 2915 ± 848 nA (n = 11, N = 2). This current declined nearly to the baseline in 153 ± 64 sec (n = 13, N = 2; Fig. 2*B-C*).

We investigated the ionic nature of I_{ni}. As indicated in Fig. 3*A*, substitution of external chloride ions completely abolished I_{ni} (n = 5, N = 2). As *Xenopus* oocytes contain a large density of endogenous calcium-activated chloride channels (Barish, 1983; Miledi & Parker, 1984; Hartzell, 1996), calcium dependence of I_{ni} was then explored. The removal of external calcium had no effect on I_{ni} (n = 12, N = 3; Fig. 3*B*). The mean amplitude and run-down kinetics of I_{ni} recorded in Normal Ringer or Ca-free external solution were similar (2915 ± 848 nA, 153 ± 64 sec; n = 11, N = 2 for I_{ni} recorded in Normal Ringer and 3772 ± 1507 nA, 106 ± 18.8 sec; n = 12, N = 3 for I_{ni} recorded in Ca-free external solution). Perfusion of Ca-free external solution for 10 min had no effect on control oocytes depolarized

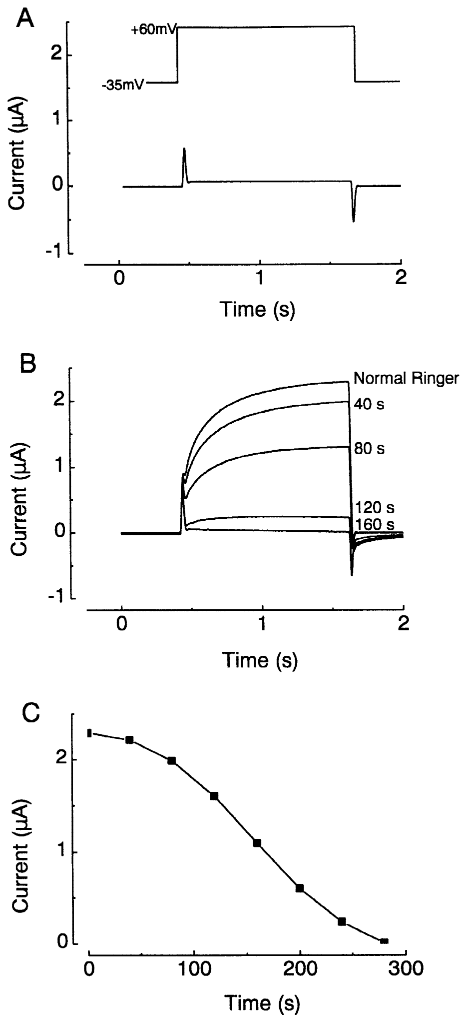

Fig. 2. Electrophysiological properties of the noninactivating component of the outward current. (*A*) Noninjected and (*B* and *C*) injected oocytes were depolarized from −35 to +60 mV for 1.2 sec (the time between each pulse was 40 sec). (*A*) Current trace recorded in a noninjected oocyte ($n = 32$, $N = 9$). (*B*) Typical traces showing the amplitude decrease of I_{ni} ($n = 11$, $N = 2$). (*C*) Time course of the decrease of I_{ni}.

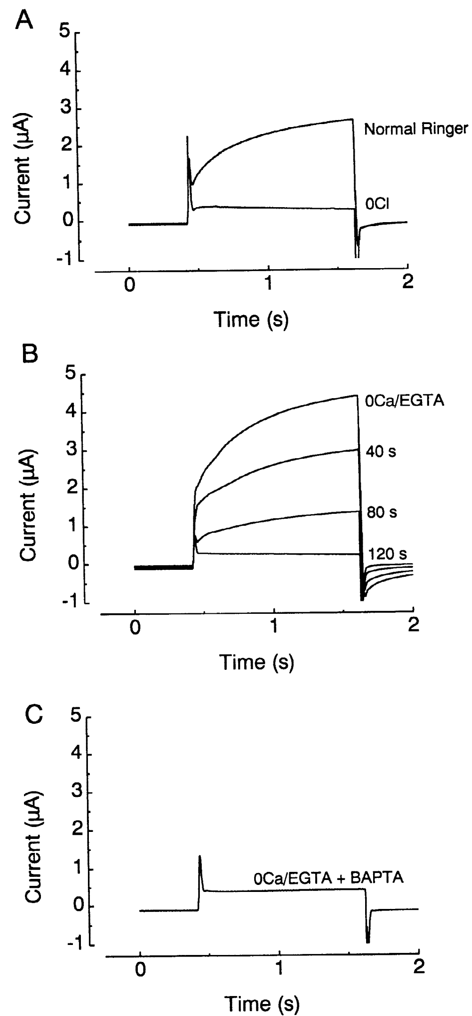

Fig. 3. Pharmacology of the noninactivating outward current. I_{ni} was induced by depolarization from −35 to +60 mV for 1.2 sec. (*A*) Effect of the substitution of chloride ions by methane sulfonate on I_{ni}. Traces before (Normal Ringer) and after (0 Cl) perfusion of 0 Cl solution ($n = 5$, $N = 2$). (*B*) Effect of a Ca-free solution on I_{ni}. Note that the time course of the decrease of I_{ni} in Normal Ringer and in Ca-free solution was similar ($n = 12$, $N = 3$). (*C*) Intracellular injection of 40 nl of 25 mM K_4. BAPTA completely abolished I_{ni}.

from −35 to +60 mV. To assess the role of intracellular calcium in I_{ni} development, we injected K_4. BAPTA into the oocytes. We then compared the expression of I_{ni} in BAPTA-injected oocytes ($n = 19$, $N = 4$) and in oocytes from the same batches not injected with BAPTA ($n = 28$, $N = 4$). BAPTA injection reduced the number of oocytes expressing I_{ni} by 76% (Fig. 3*C*).

Thus, we suggest that I_{ni} is a chloride current highly dependent on intracellular calcium concentration.

Characterization of the Inactivating Outward Current

As already shown, in oocytes injected with total RNA, depolarization from a holding potential of −35 up to +60 mV induced a large noninactivating outward component displaying a rapid run-down (Fig. 2*B*). After total disappearance of I_{ni}, the same oocyte was depolarized from −140 to +60 mV. This protocol induced an inactivating outward current (I_i) which displayed monoexponential fast activation ($\tau = 47.61 \pm 13.59$ msec; $n = 17$, $N = 3$) and biexponential inactivation ($\tau_1 = 218.95 \pm 38.6$ msec; $\tau_2 = 398.14 \pm 16.47$ msec; $n = 6$, $N = 2$; Fig. 4*B*). The mean amplitude of I_i was 4461 ± 1605 nA at +60 mV ($n = 13$, $N = 2$). These currents were never observed in noninjected oocytes (Fig. 4*A*). Figure 4*C* illustrates a typical current-voltage relationship showing an activation threshold around −25 mV (−22.33 ± 7.06 mV; $n = 8$, $N = 2$).

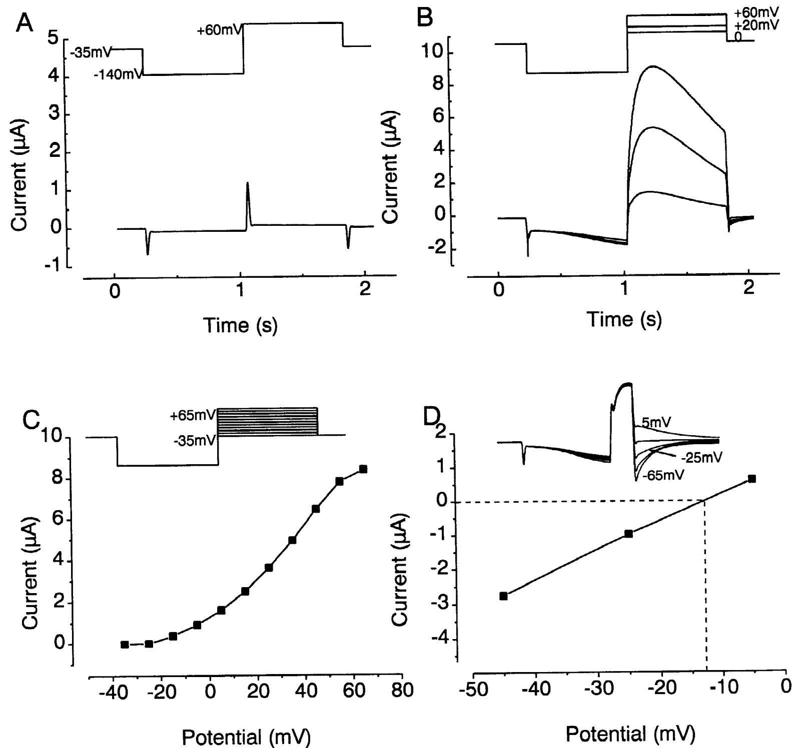

Fig. 4. Characteristics of the inactivating outward current. (*A*) Current trace recorded on a noninjected oocyte depolarized from −35 to −140 mV for 1 sec then to +60 mV for 1 sec ($n = 32$, $N = 9$). (*B*) Typical traces recorded at 0, +20 and +60 mV ($n = 13$, $N = 2$). (*C*) Current-voltage relationship of I_i. (*D*) Tail current-voltage relationship of I_i.

To identify the reversal potential and therefore the ionic nature of the inactivating outward current, we studied tail currents following repolarization to different holding potentials. As shown in Fig. 4*D*, the tail current following a test pulse to +60 mV was clearly inward at −25 mV and outward at +5 mV. From the current-voltage relationship of the tail currents, the reversal potential of the outward current was estimated to be −13.54 ± 8.17 mV ($n = 11, N = 3$). The reversal potential was close to the chloride equilibrium potential in our ionic conditions, suggesting that I_i was predominantly carried by chloride ions. Moreover, the substitution of external chloride ions led to a total disappearance of the inactivating outward current ($n = 7$, $N = 2$; Fig. 5*A*).

In contrast to I_{ni}, which was activated by intracellular calcium, I_i was dependent on extracellular calcium. I_i was completely abolished by perfusing total RNA-injected oocytes with a Ca-free solution ($n = 13, N = 2$; Fig. 5*B*). Extracellular perfusion of Ca-free solution for 10 mn had no effect on control oocytes depolarized from −140 to +60 mV.

These results are consistent with the hypothesis that calcium influx is required for I_i activation. Our interpretation is that depletion of calcium stores activates I_{ni}, which, in turn, stimulates the capacitative calcium entry responsible for I_i activation. To test this hypothesis, we used capacitative calcium entry blockers. The inorganic calcium channel blocker La^{3+} was the most potent capacitative current blocker in *Xenopus* oocytes (Gillo, Sealfon & Minke, 1996; Yao & Tsien, 1997). Addition of 100 μM La^{3+} completely abolished I_i ($n = 12, N = 3$; Fig. 6*A*). Moreover, the amplitude of I_i increased when the driving force for calcium entry was higher (Fig. 6*B*). In this protocol, oocytes were first hyperpolarized to different potentials (between −35 and −135 mV) and then depolarized to +60 mV, which completely activated I_i. Like the capacitative calcium entry in *Xenopus* oocytes previously demonstrated by Hartzell (1996), the inactivating outward current was only activated at negative potentials greater than −50 mV. The current-voltage relationship recorded in injected oocytes shows that the inactivating outward current was activated only when the membrane was hyperpolarized to negative potentials greater than −50 mV ($n = 7$, $N = 3$; Fig. 6*B*).

Recently, two endogenous chloride currents were reported following IP_3 injection in *Xenopus* oocytes (Hartzell, 1996; Centinaio, Bossi & Peres, 1997). Currents recorded in oocytes injected with IP_3 and oocytes, from the same batches, injected with total RNA from *Cichorium* leaf tissues, shared the same characteristics (Fig. 7). Figure 7*A* and *B* illustrate currents recorded in oocytes following IP_3 injection ($n = 27$, $N = 6$). During the early phase following IP_3 injection (Fig. 7*A*), depolarization steps from −35 to +60 mV induced an outward current, I_{STORE}, with the same characteristics as I_{ni} (Fig. 7*C*). The mean amplitude of I_{STORE} was 6730 ± 1968 nA ($n = 17$, $N = 3$), declining nearly to the baseline in 113 ± 48 sec ($n = 17$, $N = 3$). Induction of

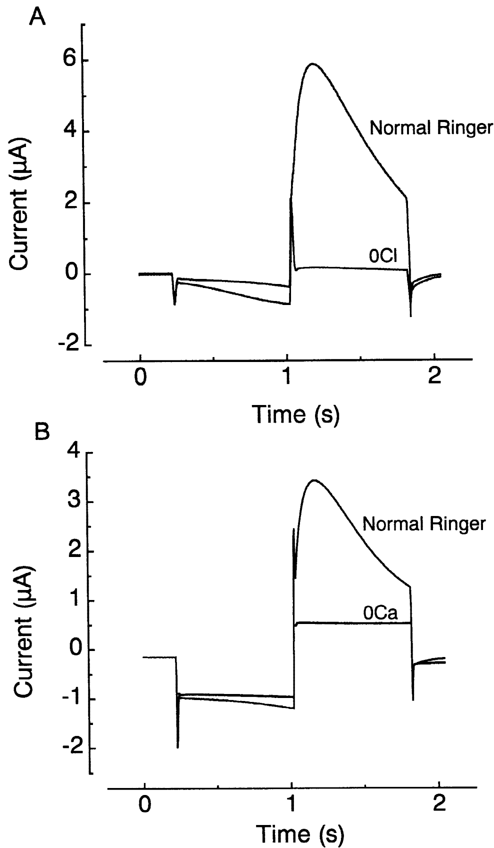

Fig. 5. Dependence of the inactivating outward current on the extracellular chloride and calcium ions. The oocyte was depolarized from −140 to +60 mV for 1 sec. (*A*) Current traces recorded before (Normal Ringer) and after (0 Cl) the perfusion of 0 Cl solution ($n = 7, N = 2$). (*B*) Currents recorded in Normal Ringer and when the oocyte was shifted into a Ca-free solution (0 Ca) ($n = 13, N = 2$).

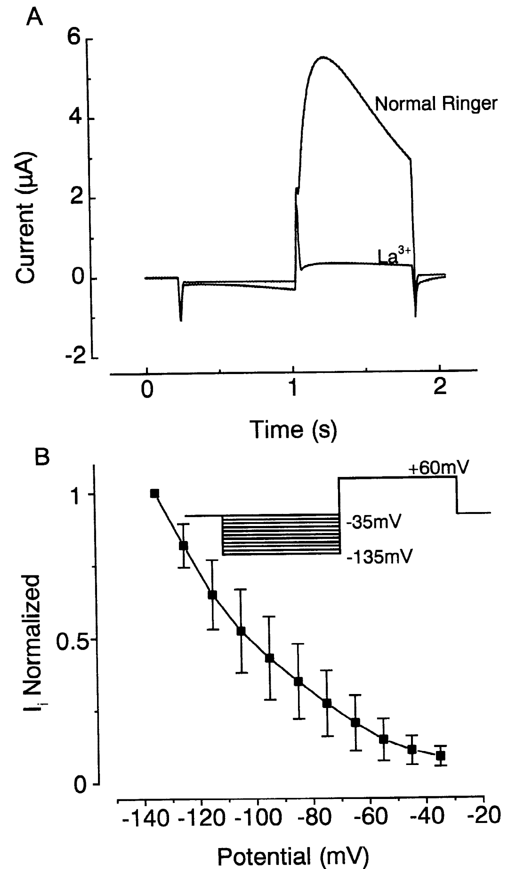

Fig. 6. Effect of extracellular La^{3+} on the inactivating outward current and activation curve of I_i. (*A*) Current traces recorded in Normal Ringer and when 100 μM La^{3+} was added to the bath ($n = 12, N = 3$). (*B*) The I_i activation curve was determined by hyperpolarizing the membrane from a holding potential of −35 mV to different prepotentials between −35 and −135 mV for 1 sec and then depolarizing to a test potential of +60 mV for 1 sec. The current for each oocyte was normalized ($n = 7, N = 3$).

I_{STORE} was independent of extracellular calcium and was abolished by intracellular K_4. BAPTA injection ($n = 5, N = 2$; *data not shown*). This current (I_{STORE}) is a calcium-activated chloride current induced by calcium release from internal stores. After the development of I_{STORE}, a second current was recorded on the same oocyte under a depolarization from −140 to +60 mV ($n = 15, N = 3$; Fig. 7*B*). This current (I_{CCE}) had the same characteristics as I_i (Fig. 7*D*). The mean amplitude of I_{CCE} was 5610 ± 2100.8 nA ($n = 15, N = 3$), it was highly dependent on extracellular calcium and was blocked by extracellular application of 100 μM La^{3+} ($n = 4, N = 2$; *data not shown*). Like I_i, this current was significantly activated only at negative potentials greater than −50 mV ($n = 6, N = 3$; *data not shown*). This current (I_{CCE}) was the consequence of an extracellular calcium influx through store-operated channels after calcium-release from internal stores.

Discussion

For the first time, we have characterized a mechanism responsible for intracellular calcium regulation in cells from chicory leaf tissues, by injecting total RNA into *Xenopus* oocytes. We reported that injection of total RNA led to two different outward calcium-activated chloride currents in *Xenopus* oocytes.

I_{ni} IS A CA-ACTIVATED CHLORIDE CURRENT INDUCED BY CALCIUM-RELEASE FROM INTRACELLULAR STORES

We propose that I_{ni} is a chloride current activated by calcium released from intracellular stores for the following reasons: (i) I_{ni} is highly dependent on extracellular chloride ions (Fig. 3*A*). (ii) I_{ni} is independent of extra-

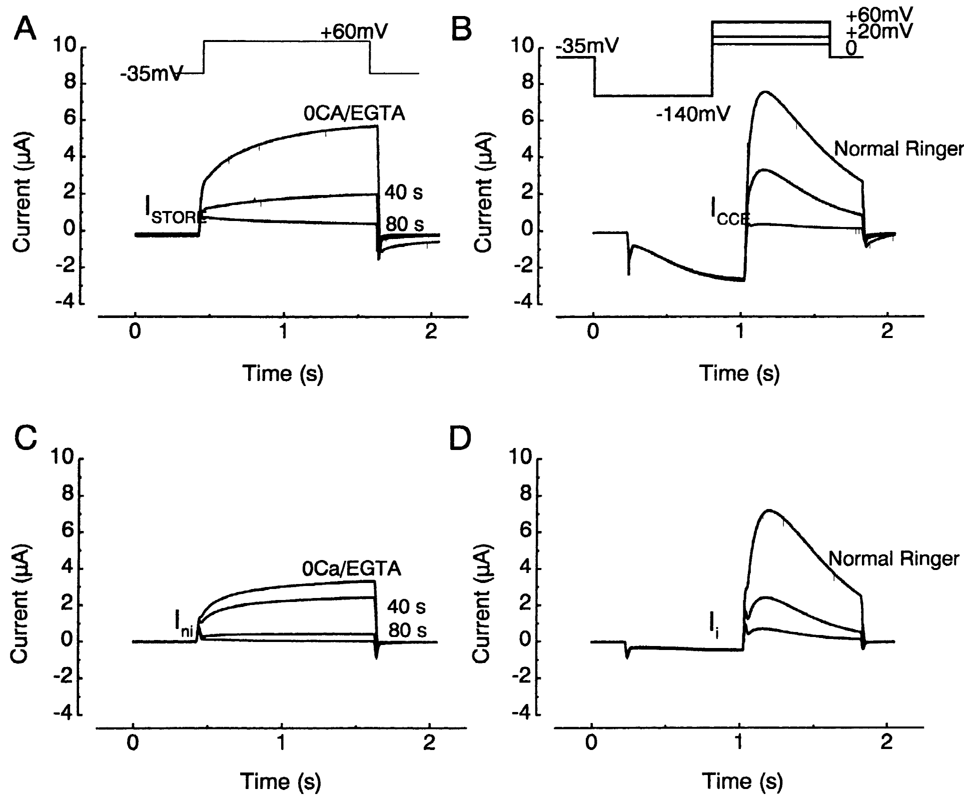

Fig. 7. Comparison of currents recorded on oocytes injected with IP_3 and oocytes injected with total RNA from chicory leaf tissues. (*A* and *B*) Current traces obtained in oocytes following IP_3 injection. (*C* and *D*) Current traces obtained in oocytes injected with total RNA from chicory leaf tissues. (*A*) Typical traces recorded after 40 nl injection of 1 mM IP_3 solution in a Ca-free extracellular solution ($n = 27, N = 6$). (*B*) Typical traces recorded at 0, +20 and +60 after a prepulse at −140 mV from a holding potential of −35 mV in Normal Ringer ($n = 15, N = 3$). (*C*) Typical traces of I_{ni} in a Ca-free extracellular solution ($n = 12, N = 3$). (*D*) Typical traces of I_i in Normal Ringer ($n = 13, N = 2$).

cellular calcium. The current is activated by depolarization even when calcium is removed from the external medium (Fig. 3*B*). (iii) I_{ni} requires intracellular calcium for activation, as it was abolished by intracellular injection of K_4. BAPTA (Fig. 3*C*). (iv) The I_{ni} amplitude at +60 mV declines to the baseline in 153 ± 64 sec (Fig. 2*B* and *C*). These kinetics may reflect the time course of calcium store depletion.

I_i is a Ca-activated Chloride Current Induced by Capacitative Calcium Influx

We propose that I_i is a chloride current stimulated by capacitative calcium influx for several reasons: (i) I_i is highly dependent on extracellular chloride ions (Fig. 5*A*). (ii) Contrary to I_{ni}, I_i is highly dependent on extracellular calcium (Fig. 5*B*). (iii) As demonstrated by Putney (1990), calcium-release from internal stores in *Xenopus* oocytes triggers extracellular calcium influx. In these studies, we have shown that I_i is activated after I_{ni} (Fig. 1*B–F*). Thus, I_i may be the consequence of CCE. (iv) I_i is activated at negative potentials greater than −50 mV, at which capacitative calcium entry was significantly activated in *Xenopus* oocytes (Hartzell, 1996). (v) The inorganic calcium channel blocker La^{3+}, the most potent capacitative calcium entry blocker in *Xenopus* oocytes (Gillo et al., 1996; Yao & Tsien, 1997), completely abolished I_i (Fig. 6*A*).

These results are supported by studies of two different chloride currents in *Xenopus* oocytes activated by calcium release from internal stores and capacitative calcium influx induced after injection of IP_3 (Hartzell, 1996; Centinaio et al., 1997). The characteristics of currents recorded in oocytes injected with total RNA extracted from *Cichorium* leaf tissues are similar to those observed after IP_3 injection (Fig. 7).

In summary, we suggest that injection of total RNA from *Cichorium* leaves induces the activation of two outward chloride currents which are the consequence of (i) calcium-release from internal stores and (ii) capacitative calcium entry.

Calcium Release in Higher Plants

Several transport mechanisms capable of triggering calcium release from internal stores, mainly the vacuole, have been reported in plant cells. Schumaker & Sze (1986), first reported the existence of IP_3-gated calcium channels in the vacuolar membrane vesicles of oat roots. They suggest that IP_3 may operate as a second messenger in the mobilization of intracellular calcium in plant cells. Another calcium-release channel has been reported in beet vacuoles (Johannes et al., 1992). This channel is voltage-sensitive and channel opening is largely promoted by negative potentials (potential in the cytosol relative to the vacuole). Channel activities are neither affected by IP_3 nor by alteration of cytosolic free calcium concentrations. Only Zn^{2+} and the lanthanide (Gd^{3+}) have been shown to be effective inhibitors. The following calcium transport system reported in the vacuole is another voltage-sensitive calcium-release channel which may be involved in the calcium-induced calcium-release mechanism (Ward & Schroeder, 1994; Ward et al., 1995). This channel is activated at positive potentials and is strictly dependent on cytosolic free calcium. The last calcium transport system was reported in individual plant vacuoles. A cyclic-adenosine 5′-diphosphoribose (cADPR), which interacts with a ryanodine receptor in certain animal cells (Clapper et al., 1987), was also shown to elicit calcium release at the vacuolar membrane of beet storage root and analysis by patch-clamping demonstrated that the cADPR-gated pathway is voltage-dependent (Allen et al., 1995; Muir et al., 1997).

Which of these calcium transports could be expressed in our system? Since IP_3-gated calcium channels has been reported in plant cells, we do not totally exclude the hypothesis of the expression of exogenous IP_3-gated calcium channels in the endoplasmic reticulum of *Xenopus* oocytes. Moreover, neo-expression of exogenous voltage-dependent calcium channels or cADPR-gated calcium channels in intracellular membranes is possible. This would imply a conformational coupling where a portion of the intracellular membrane must lie within 10 nm of the plasma membrane to allow a depolarization-induced calcium release as described in skeletal muscle (Gilly, 1981). A similar close juxtaposition of the endoplasmic reticulum to the cell surface has been described in *Xenopus* oocytes (Gardiner & Grey, 1983; Berridge, 1997). Further experiments are needed to elucidate which of these Ca^{2+} channels are responsible for the calcium-release reported in our study.

In summary, this paper reports for the first time, a calcium homeostasis mechanism induced by heterologous expression of total RNA from chicory leaf tissues in *Xenopus* oocytes.

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