The Potassium Channel KAT1 Is Activated by Plant and Animal 14-3-3 Proteins*

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14-3-3 proteins modulate the plant inward rectifier K+ channel KAT1 heterologously expressed in Xenopus oocytes. Injection of recombinant plant 14-3-3 proteins into oocytes shifted the activation curve of KAT1 by +11 mV and increased the τon. KAT1 was also modulated by 14-3-3 proteins of Xenopus oocytes. Titration of the endogenous 14-3-3 proteins by injection of the peptide Raf 621p resulted in a strong decrease in KAT1 current (−70% at −150 mV). The mutation K56E performed on plant protein 14-3-3 in a highly conserved recognition site prevented channel activation. Because the maximal conductance of KAT1 was unaffected by 14-3-3, we can exclude that they act by increasing the number of channels, thus ruling out any effect of these proteins on channel trafficking and/or insertion into the oocyte membrane. 14-3-3 proteins also increased KAT1 current in inside-out patches, suggesting a direct interaction with the channel. Direct interaction was confirmed by overlay experiments with radioactive 14-3-3 on oocyte membranes expressing KAT1.

Voltage-dependent (Kv) potassium channels mediate an inward K+ current (IKin) at the plasma membrane of plant cells. IKin serves several functions: controlling membrane voltage, nutrient uptake, and osmotic regulation. In guard cells onset of this current typically leads to K+ accumulation triggering stomatal opening (1–3). The molecular characterization of guard cell IKin started in 1992 with the cloning of KAT1 (4, 5), the first Kv channel subunit from Arabidopsis thaliana, and still is a subject of intensive research. One unsolved problem in this field is the rationale for the existence of a large number of Kv channel genes with apparently similar characteristics. Five genes coding for inward K+ channel subunits have so far been found to be expressed in guard cells, KAT1, KAT2, AKT1, AKT3/2, and AtKC1 (6). When heterologously expressed, none of these genes alone forms a channel that fully matches the properties of native IKin with respect to pH, Ca2+ dependence, and block by Cs+ (7). This suggests that the native channel might be composed of different subunits and/or that additional regulatory proteins contribute to native IKin current. KAT1 can form homotrimers with each of the four other K+ channel subunits expressed in guard cells (8–10). At least in the case of KAT1 and KAT2, two very similar proteins (displaying 85% identity in their transmembrane region), the heteromeric channel does not have properties distinguishable from the homomeric channels. It is relevant that the C-terminal cytoplasmic domains of the two channels display the lowest level of sequence similarities; these regions are typically regulatory domains in Kv channels and often provide specific interaction sites with regulatory proteins (11).

Known protein partners of plant Kv channels are β subunits (12), protein kinases, and phosphatases (13). Recent contributions have now extended information on the regulatory effect of 14-3-3 proteins. These are a family of highly conserved proteins with molecular mass of 30 kDa, expressed in all eukaryotic organisms. They exist in a number of isoforms and form homo- and heterodimers. 14-3-3 proteins play a central role in the regulation of many cellular processes, such as cell cycle, differentiation, apoptosis, and mitogenic signal transduction (14). The common feature of 14-3-3s is their ability to bind phosphorylated consensus motifs on target proteins (15, 16); this accounts for their diverse regulatory functions. In plants it has been shown that 14-3-3 proteins accomplish peculiar functions, such as regulation of ion transport, through interaction with the plasma membrane H+ -ATPase (17). Recently, increasing evidence suggests that besides the H+ -ATPase 14-3-3s can also regulate K+ channels. In fact, they have been shown to interact with plant K+ outward rectifiers (18–20), SV channels (21), and K+ inward rectifiers (20).

The effect of 14-3-3 proteins on K+ inward rectifiers has so far only been reported for native channels. Because these studies have not yet exploited the question of subunit specificity, we have tested the possibility that 14-3-3 proteins interact with the guard cell subunit KAT1.

Here we show that the homotetrameric KAT1 channel expressed in Xenopus oocytes is modulated by 14-3-3 proteins via a direct interaction with the channel protein and that this interaction changes the voltage-dependent properties and the activation kinetics of the channel. We also show that KAT1 is
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regulated by the interaction with endogenous 14-3-3 proteins present in the oocyte.

EXPERIMENTAL PROCEDURES

Expression of KAT1 in Oocytes—KAT1 cDNA was cloned into pSGEM vector (a modified version of pGEM HE). cRNA was transcribed in vitro using T7 RNA polymerase (Promega) and injected (25 ng/oocyte) into Xenopus laevis oocytes prepared according to standard methods (22). Measurements were performed 2–4 days after injection. The average diameter of the oocytes (stage IV and V) was considered to be 1 mm, and on this basis we have calculated the final concentration of injected 14-3-3 proteins and peptides when mentioned in the text.

Electrophysiology—Two-electrode voltage clamp recordings were made using a two-electrode voltage clamp amplifier (GeneClamp 500; Axon Instruments, Foster City, CA) under control of pCLAMP 8 software (Axon Instruments). Electrodes were filled with 3 M KCl and had a resistance of 0.4–0.8 MΩ in 50 mM KCl. Experiments were performed at room temperature (20–25 °C). LEAK subtraction was performed manually after acquisition.

A linear amplifier (Axon Instruments) with borosilicate pipettes coated with paraffin wax. Data acquisition and analysis were performed according to standard methods (23). In Fig. 4A, the soma of the oocyte was held at a voltage of −40 mV and steps from −60 to −180 mV. Current-voltage relations report the experimentally measured voltages, which can slightly deviate from command voltages.

Voltage coefficient (23). In Fig. 4E the tail current values are plotted as absolute.

Escherichia coli Strains—E. coli strain DH5α was used for plasmid propagation, and strain BL21 (DE3) pLysS was used for protein expression. E. coli strains were grown at 37 °C in Luria Bertani liquid medium or agar plates (15 g/liter). Ampicillin was used at a concentration of 100 µg/ml.

Expression and Purification of Recombinant 14-3-3 Proteins—The maize 14-3-3 isoform GF14-6 was expressed in E. coli as GST fusion protein as already described (24). K56E GF14-6 mutant was produced and expressed as previously described (25).

For production of His-tagged GF14-6, a cDNA fragment was removed from pGEX-2TK, using a 5′-Ncol restriction site and a 3′-EcoRI restriction site, and inserted into Ncol-EcoRI-cut PET-32B vector (Novagen, Madison, WI), previously deprived of the thioredoxin coding region by NdeI digestion. Transformation of E. coli was performed according to standard methods.

Induction of protein expression was performed by adding 1 mM isopropyl-1-thio-β-D-galactopyranoside to liquid cultures. After 3 h of induction, cells were collected by centrifugation and stored at −80 °C. Cells were lysed in a buffer containing 10 mM Tris-HCl, pH 8, 1 mM EDTA, 10 mM dithiothreitol, 20 µg/ml leupeptin, 0.5 mM phenylmethylsulfonyl fluoride, and 0.75% N-lauryl sarcosine and sonicated until a clear, non-viscous solution was obtained (26). Particulate matter was removed by centrifugation (15 min at 12,000 × g), and the soluble fraction was used for protein purification.

His-GF14-6 was purified by affinity chromatography on Ni²⁺-nitrolacetic acid-agarose (Qiagen, Hilden, Germany) equilibrated with 50 mM sodium phosphate, pH 8, 300 mM NaCl, 5 mM β-mercaptoethanol, and 20 mM imidazole; after extensive washing, protein was eluted by addition of increasing concentrations (50, 100, 200, 400 mM) of imidazole to the above described buffer. Fractions containing the purified fusion protein were extensively dialyzed against 5 mM bis-tris propane- Hepes, pH 7, in 10% (v/v) glycerol and stored at −80 °C.

Raf 621p—Raf 621p is 15 amino acids long (LPKINR-SASPEPSLHR), corresponding to amino acids 613–627 of Raf 1 (15). Serine 621 is phosphorylated. The sequence highlighted in bold mimics the mode I recognition site of 14-3-3 proteins (16).

Isolation of Oocyte Membranes—Membrane fractions were obtained according to De Jong et al. with minor modifications (27). 40 control or KAT1-expressing oocytes were homogenized in 800 µl of 20 mM Tris-HCl, pH 7.4, containing 5 mM MgCl₂, 5 mM NaH₂PO₄, 1 mM EDTA, 80 mM sucrose, 1 mM phenylmethylsulfonyl fluoride, 5 µg/ml leupeptin, and 5 µg/ml pepstatin and centrifuged two times for 10 min at 3000 × g at 4 °C to remove yolk proteins. Membranes were pelleted by centrifugation for 30 min at 14,000 × g at 4 °C and resuspended in 20 mM Tris-HCl, pH 7.4, containing 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 5 µg/ml leupeptin and 5 µg/ml pepstatin, and 20% (v/v) glycerol.

SDS-PAGE and Overlay Assay—SDS-PAGE was performed according to Laemmli (28) in a Mini Protean apparatus (Bio-Rad). The system used for expression of GF14-6 proteins produces a glutathione S-transferase-fused 14-3-3 containing a cAMP-dependent protein kinase phosphorylation site and a thrombin site between the two polypeptides. The 32P-labeled GF14-6 was obtained as described (24). Specific activity of the labeled protein was 3 MBq/mg. The overlay assay was carried out according to Ref. 24 with minor modifications. Briefly, 20 µg of membrane fractions from KAT1-expressing Xenopus oocytes were subjected to SDS-PAGE and blotted onto nitrocellulose membrane, using a semidry LKB apparatus (2 h, 0.8 mA cm⁻²). The membrane was blocked with 5% fatty acid-free milk in 25 mM Hepes-OH, 75 mM KCl, 5 mM MgCl₂, 1 mM diithiothreitol, 0.1 mM EDTA, 0.05% Tween 20, pH 7.5 (buffer H) and then incubated overnight at 4 °C in the same buffer containing 2% fatty acid-free milk, 3 µg of 32P-labeled GF14-6 (corresponding to 9 kBq/
Channels Expressed in Xenopus Oocytes

RESULTS AND DISCUSSION

Recombinant 14-3-3 Proteins Increase the Activity of KAT1 Channels Expressed in Xenopus Oocytes—Fig. 1A shows the current traces recorded from KAT1 channels expressed in a Xenopus oocyte. Clamping the oocyte with a voltage step protocol ranging from −60 mV (holding potential) to −150 mV, elicited the slowly activating, inward K+ currents I\textsubscript{KAT1} typical of this channel (5). The measured current values were stable over time; when monitored over a period of >30 min they varied by <1% (not shown).

To examine the effect of 14-3-3 proteins on KAT1 currents the same oocyte was removed from the voltage clamp apparatus and injected with 46 nl of a buffer containing 4 mg/ml of purified recombinant 14-3-3 proteins from maize GF14-6 (final concentration in the oocyte ~1 μM). The oocyte was kept 10 min at room temperature to recover from the injection and to allow 14-3-3 diffusion and challenged with the same voltage protocol used prior to injection. Fig. 1A shows a strong increase in KAT1 current after 14-3-3 injection. The steady state current/voltage (I/V)\textsuperscript{2} relationship of the currents from Fig. 1A are shown in 1B. Fig. 1C shows the mean I/V relationship from 10 oocytes from three different batches. The increase in current induced by 14-3-3 is voltage dependent; at −110 mV the current is increased by 200% (control, −0.36 ± 0.02 μA; 14-3-3, −1.1 ± 0.24 μA), whereas at −140 mV is increased by 100% (control, −5.1 ± 0.7 μA; 14-3-3, −10.3 ± 2.3 μA).

To test the specificity of the 14-3-3 effect on I\textsubscript{KAT1}, we performed several control experiments, summarized in Fig. 2. The effect of injection of 14-3-3 on I\textsubscript{KAT1} at the reference voltage of −120 mV, is compared with that of heat-denatured 14-3-3 proteins, buffer only, and 14-3-3 proteins bearing the K56E mutation. This mutation, lysine 56 replaced by glutamic acid, is known to disrupt the interaction with target proteins, as shown with the plant proton pump (25). In our experiments, K56E 14-3-3 proteins as well as heat-denatured wild-type proteins and resuspension buffer did not enhance I\textsubscript{KAT1}, indicating that the effect observed after 14-3-3 injection is specific and not related to injection artifacts.

Because 14-3-3 proteins have been reported to modulate the endogenous Cl\textsuperscript{−} channels in Xenopus oocytes (29), we tested whether the increase in inward current was entirely due to a stimulation of KAT1 channels. Fig. 3 shows that addition of 5 mM Cs\textsuperscript{+}, a well known blocker of KAT1 channels (5) with no known effect on Cl\textsuperscript{−} channels, completely blocked the inward current after 14-3-3 stimulation. This shows that the stimulated current is KAT1 specific and is not due to a nonspecific effect on endogenous channels of the oocyte.

2 The abbreviation used is: I/V, current/voltage.
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14-3-3 Proteins Activate KAT1 Channels by Shifting the Activation Curve—KAT1 is a voltage-gated channel, and its open probability strictly depends on voltage. The increase in current observed after injection of the 14-3-3 proteins can, in principle, result from a change in conductance or from a change in open probability.

An increase in conductance due to an increase in the number of active channels at the plasma membrane is a means by which 14-3-3 proteins regulate ion channels in plants (19) and animal cells; in particular, for animal channels it has been shown that 14-3-3 proteins modulate protein trafficking (30, 31). On the other hand, it has also been shown that 14-3-3 proteins regulate the gating of animal and plant channels by shifting the activation curve, that is the plot of open probability over voltage (20, 32).

To discriminate between these two possible modes of action, it was necessary to check whether the maximal value of \( I_{\text{KAT1}} \) was affected by the 14-3-3 proteins. Therefore we had to measure the current of the fully activated channel, in the case of KAT1 at about −200 mV. Such negative voltages can be deleterious for the stability of the membrane; furthermore, they can trigger random activation of endogenous currents. Because the speed of \( I_{\text{KAT1}} \) activation increases at negative voltages (33), we made use of a protocol (shown in Fig. 4A) that progressively reduces the length of the voltage steps at negative potentials; in this case we obtained all the essential kinetic information without any unnecessary long and potentially deleterious polarization of the membrane. Fig. 4B shows a control experiment in which the full activation of KAT1 was achieved. The amplitude of the tail current from the last preconditioning voltage (−196 mV) is the same as that from the preconditioning voltage of −186 mV. This indicates that KAT1 channels had reached full open probability at −186 mV and that our protocol that steps at −200 mV serves the purpose of measuring the current at full activation.

Fig. 4C shows exemplary current traces recorded from the same oocyte before and after injection of 14-3-3. 14-3-3 proteins increased \( I_{\text{KAT1}} \) at the test voltage of −150 mV but had no effect on the amount of current measured at the fully activating voltage of −200 mV. This is also shown by the current/voltage relationships of Fig. 4D, where the control and 14-3-3-treated curves converge to the same value at negative potentials. These data indicate that 14-3-3 proteins do not act by increasing the maximal conductance but rather by changing the voltage dependence of gating. To check the effect of 14-3-3 proteins on gating, we analyzed the channel open probability, plotting the initial value of the tail currents (changed in sign) over the preconditioning voltages (Fig. 4E). By fitting a Boltzmann curve to the data we obtained in control conditions a value for the midpoint of the open prob-

![Image](http://www.jbc.org/)

**FIGURE 4.** 14-3-3 proteins shift KAT1 activation curve and do not change maximal conductance. A, two-step voltage protocol applied to reach the fully activated state of the channel (at −200 mV) from different test voltages (−60/−200 mV). B, representative control experiment showing overlap of the tail currents collected at −200 mV from the last two test voltages, −186 and −196 mV. C, current traces recorded from the same oocyte before and after injection of 14-3-3 at the test voltage of −150 mV, tail at −200 mV. D, current/voltage relationship at all test voltages. 14-3-3 proteins do not increase the maximal conductance of the channel.
ability curve \(V_{1/2}\) of \(-155\) mV and for the inverse slope factor coefficient \(s\) of \(-13\) mV. The rather negative \(V_{1/2}\) value was in the range reported previously for KAT1 in oocytes (33, 34). After 14-3-3 protein injection, the \(V_{1/2}\) shifted by 12 mV to \(-143\) mV and the inverse slope factor coefficient was \(-14\) mV. The maximal current value obtained by the Boltzmann fit was 3.57 \(\mu\)A in both cases, again confirming that 14-3-3 did not affect the maximal conductance but only the open probability.

Averaging normalized open probability curves (Fig. 4F) \((n = 15\) oocytes from seven independent experiments\) shows that 14-3-3 shifted the \(V_{1/2}\) by 11 mV, from \(-146\) to \(-135\) mV while \(s\) was unchanged \((-13\) mV\). The increase in current observed in response to 14-3-3 protein injection can hence be explained by a positive shift of the activation curve of the channel.

The findings that 14-3-3 proteins act directly on a channel protein by altering its voltage dependence is in agreement with that reported for the native inward rectifier from radicle protoplasts (20). Also in this case, an elevation of 14-3-3 protein concentration on the cytosolic side of the membrane resulted in a positive shift in the activation curve of the channel by \(\sim 9\) mV.

To further test whether a positive shift of 11 mV in the channel open probability is sufficient to explain the reported increase in KAT1 current in response to elevation of 14-3-3 proteins, we used the aforementioned Boltzmann parameters \(V_{1/2}\) and \(s\) to reconstruct the measured current/voltage relation. As shown in Equation 2, the cell current \(I\) is

\[
I = i \times N \times P_o
\]

(Eq. 2)

where \(i\) is the single channel current amplitude of KAT1, \(N\) the number of channels in the membrane, and \(P_o\) the open probability. The curves in Fig. 1C were calculated using a unitary KAT1 channel conductance of 10 pS (36) with a reversal voltage at \(-18\) mV and the respective values \(V_{1/2}\) and \(s\) from the Boltzmann fit reported in Fig. 4F. The curves were jointly fitted to the mean current/voltage relations obtained before and after injection of 14-3-3 proteins into oocytes (Fig. 1C); only the parameter \(N\) was used as a fitting variable. A very good agreement between data and calculation was obtained with an \(N\) of \(1.3 \times 10^6\) channels. This means that the entire increase in current observed in response to 14-3-3 injection can be explained on the basis of a shift in the voltage dependence; an increase in the number of channels as a consequence of a 14-3-3 effect on channel trafficking was not needed to reproduce the data.

We then checked whether the shift in the activation curve was accompanied by a change in channel kinetics. As expected, following 14-3-3 treatment \(I_{KAT1}\) activation was accelerated. Fig. 5A shows an example current traces recorded at \(-130\) mV before and after injection of 14-3-3. Traces have been normalized to show, qualitatively, current acceleration due to 14-3-3. Fig. 5B shows the time constant of activation \(\tau_{on}\) plotted against voltage. The \(\tau_{on}\) were estimated by fitting a single exponential function to current activation kinetics. At \(-130\) mV mean \(\tau_{on}\) \((n = 6)\) was \(486 \pm 39\) and \(363 \pm 18\) ms for control and 14-3-3, respectively. No apparent effect was observed on the \(\tau_{off}\) obtained from the deactivation kinetics. The acceleration of activation kinetics together with the positive shift of the activation curve confirms that 14-3-3 proteins act by modifying the kinetic properties of \(I_{KAT1}\).

14-3-3 Proteins Act on KAT1 in a Membrane-delimited Way—To test whether KAT1 is modulated by 14-3-3 proteins independently of regulatory cellular pathways, we recorded \(I_{KAT1}\) from inside-out macropatches. In this way we could access the cytosolic side of the channel and at the same time remove the channel from the cytosolic environment. In excised patches \(I_{KAT1}\) undergoes a fast run down process, which shifts the voltage dependence of the channel (37). Run down of the KAT1 channel could be recovered by the addition of 3 mM ATP to the bath solution as described by Hoshi (37, 38). In this condition we were able to record KAT1 macrocurrents that were stable for the length of the experiment (several minutes) and allowed testing of the effect of 14-3-3 proteins (1 \(\mu M\) final concentration). Fig. 6A shows that addition of 14-3-3 increased \(I_{KAT1}\), as

![FIGURE 5. 14-3-3 proteins accelerate the activation kinetics of KAT1 channels. A, comparison of control and 14-3-3-activated KAT1 current traces recorded upon hyperpolarization to \(-130\) mV from the holding potential of \(-40\) mV. Current values normalized to the same ordinate. B, effect of 14-3-3 on the voltage dependence of activation time constants \(\tau_{on}\) obtained by fitting experimental traces by single exponential curves. Error bars are S.E. (\(n = 6\)).](image1)

![FIGURE 6. Effect of 14-3-3 on KAT1 macrocurrents recorded in excised patches. A, KAT1 currents elicited by hyperpolarizing voltage steps from a holding voltage of \(+40\) mV to the range \(+20\) to \(-160\) mV, 20-mV steps. Addition of 1 \(\mu M\) 14-3-3 to control solution in the bath increased the amplitude of KAT1 currents. B, current/voltage relationship of control (○) and 14-3-3 (□) time-dependent currents of the experiment reported in panel A. C, mean values obtained from three experiments (including the one reported in panel A) ± S.E.](image2)
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FIGURE 7. Injection of peptide Raf 621p reduces KAT1 current in oocytes. A, KAT1 currents elicited by standard clamp protocol, before and after the injection of 50 nl of 1 μM Raf 621p (estimated final concentration in oocyte ~50 nM). B, current/voltage relationship of the experiment shown in panel A, (△) control, (●) + Raf 621p. C, mean current/voltage relationship, n = six oocytes from three independent experiments. Error bars are S.E.

also shown by the steady state I/V relationship in Fig. 6B. A similar stimulation was observed in three of four patches; the mean I/V relationship of the positive responding patches is reported in Fig. 6C.

The occurrence of a rundown process in excised patches already indicates that KAT1 is regulated by cytoplasmic factors. Tang and Hoshi (37) reported that a protein kinase A-mediated phosphorylation is responsible for the shift in the voltage-dependent gating observed on patch excision. In contrast, nonspecific alkaline phosphatase accelerated the rundown time course. Among others, we can probably add 14-3-3 proteins as factors that seem to modulate the channel in a membrane-delimited pathway, given that the channel is in its active phosphorylated form.

Endogenous 14-3-3 Modulate KAT1 Channels—Working with KAT1 channels expressed in oocytes we observed that the position of the activation curve along the voltage axis can vary greatly between experiments. The measured values of V_{1/2} varied in the range −150 to −120 mV. This variability is suggestive of an endogenous regulation of the channel, and therefore we tested the possibility that endogenous 14-3-3 proteins could modulate KAT1.

To examine this possibility we compared currents from KAT1-expressing oocytes before (control) and after the injection of peptide Raf 621p (Fig. 7A). This phosphorylated peptide mimics the mode I recognition site of 14-3-3 proteins (16). When provided in large excess, it presumably titrates the endogenous 14-3-3 (see the use of this peptide in Refs. 15, 20, 39). The injection of this peptide strongly decreased the level of I_{KAT1} in oocytes (70% ± 6 at −150 mV, n = 4); in control experiments (data not shown) heat-inactivated Raf 621p decreased the current at −150 mV of 38% ± 4 (n = 4). This is presumably because it is not possible to efficiently inactivate this short peptide by heating. As is apparent from the single experiment and the mean I/V curves of Fig. 7, B and C, the peptide acts by shifting the voltage dependence of the channel to more negative values. For this reason it is difficult to reach full activation of the channel in the Raf 621p-injected oocytes. Only in one experiment (not shown) was it possible to measure the current at full activation, and the measured shift in the activation curve was −12 mV. We can conclude that endogenous 14-3-3s modulate KAT1 channel in oocytes and presumably influence the position of the activation curve of this channel. This finding could partly explain the strong rundown phenomenon observed in excised patches of this channel.

KAT1 Interacts in Vitro with the 14-3-3 Proteins—To test whether the KAT1 protein interacts directly with the 14-3-3 proteins, we have performed overlay experiments with radioactive 14-3-3s on total protein extract obtained from oocytes injected with KAT1 mRNA and tested for KAT1 expression 3 days from injection. Membrane fraction from control or KAT1-expressing oocytes was purified, subjected to SDS-PAGE, and blotted onto nitrocellulose membrane. Membranes were incubated with recombinant 32P-labeled 14-3-3 isoform from maize G14-6. Autoradiography shows (Fig. 8) that the radioactive probe in the crude membrane fraction purified from KAT1-expressing oocytes recognizes only a single band of ~80 kDa (lane 3), corresponding to the expected KAT1 molecular mass, whereas no signal is detected in control membrane purified from oocytes not expressing KAT1 (lane 2). As expected, the 14-3-3 probe efficiently recognizes the H^+-ATPase in the maize plasma membrane fraction loaded as positive control (lane 1).

Control experiments included the addition of Raf 621p peptide to inhibit the interaction of 14-3-3s with KAT1 and the substitution of wild-type 14-3-3 with the non-functional K56E 14-3-3 mutant. In both cases we obtained no interaction (data not shown).

CONCLUSIONS

Recombinant 14-3-3 proteins from maize activate KAT1 channels expressed in X. laevis oocytes. The 14-3-3-induced increase in current is due to a positive shift in the voltage dependence of the channel and not to an increase in the number of channels. This effect is disrupted by mutation K56E in the canonical interaction site of maize 14-3-3s. Excised patch...
experiments and in vitro data suggest a direct interaction between KAT1 and the 14-3-3 proteins. Endogenous 14-3-3 from Xenopus oocytes can control the activation curve of this channel and represent a possible source of variability in this well known expression system for ion channels.

Physiological Considerations—KAT1-like K⁺ channels mediate K⁺ uptake in many plant cells. The activity of these channels is controlled by the proton pump that establishes the negative membrane voltage that opens the channels and thermodynamically favors K⁺ uptake (40). It is also known that the two transport systems operate in concert, as in much as conditions created by the proton pump, such as external acidification, enhance the activity of KAT1 channels and that the activity of the channel is required to depolarize the negative potential that thermodynamically inhibits the proton pump (41). The finding that 14-3-3 proteins, well known as proton pump activators, directly stimulate KAT1 channels constitutes strong molecular evidence that these two transporters can operate in concert in regulating K⁺ uptake.

Acknowledgments—We thank M. I. De Michielis for helpful discussion and for the gift of the Raf621p peptide and Jack Dainty for reading the manuscript.

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35. Deleted in proof
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doi: 10.1074/jbc.M603361200 originally published online September 21, 2006

Access the most updated version of this article at doi: 10.1074/jbc.M603361200

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