Knockout of the guard cell K^+out channel and stomatal movements

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The central roles of potassium channels in regulating membrane potential and controlling action potential repolarization are well documented (1). In plants an additional important function of potassium channels in mediating long-term potassium transport during cell movements, turgor changes, and tropisms has been proposed. Two guard cells surround each stomatal pore in leaves and control the opening and closing of their central pore via increases in their solute content during stomatal opening (2) and decreases in solute content during stomatal closing (3). In this issue of PNAS, Hosy *et al.* (4) provide direct genetic evidence that outward rectifying potassium (K^+out) channels in guard cells contribute to stomatal closing in leaves. Guard cells accumulate potassium (K^+), which results in stomatal opening (2) and release K^+, which results in stomatal closing (3). Ion channel characterizations in guard cells, and motor cells that control turgor-driven leaf movements, led to the model that K^+ channels can contribute to the underlying long-term K^+ influx (5, 6) and K^+ efflux (5–7). Biophysical, cell biological, and second messenger regulation analyses by several groups have supported this model.

Hosy *et al.* identified an insertional T-DNA disruption mutant in the *Arabidopsis* guard cell-expressed outward-rectifying K^+out channel gene, *GORK*. Heterologous expression in *Xenopus* oocytes has previously shown that the *GORK* cDNA encodes an outward rectifying K^+ channel activity with properties similar to those described in guard cells and *GORK* is expressed in guard cells (8). Hosy *et al.* further generated a dominant negative GORK mutant by inserting the positively charged amino acid arginine into the K^+ selectivity filter domain of the GORK channel. The T-DNA disruption mutant, *gork-1*, showed no measurable K^+out channel activity in guard cells and dominant negative mutant lines showed <10% of native K^+ channel activity (4).

Stomatal movement analyses in response to abscisic acid and darkness showed that the *gork-1* disruption mutant caused a reduced stomatal closing response, providing genetic evidence for the function of K^+out channels (4). Furthermore, *gork* dominant negative mutants showed slight slowing of stomatal closure compared with WT responses. K^+ channel current activities in guard cells are approximately an order of magnitude larger than physiological K^+ fluxes during stomatal movements (6), which may account for the relatively limited effect of partial dominant negative *gork* repression. Gas exchange and plant water loss analyses also show that *gork-1* disruption reduces the rate of stomatal closing and increases water loss of leaves and whole plants. The *gork-1* mutant also shows slightly enhanced light-induced stomatal opening, which can be explained by the functions of K^+ channels in balancing K^+ efflux and K^+ influx in guard cells (4).

Genetic evidence shows that outward rectifying potassium channels in guard cells contribute to stomatal closing in leaves.

The *gork-1* mutant clearly shows a role for the GORK K^+ channel in stomatal closing. However, a residual abscisic acid (ABA)- and darkness-induced stomatal closing response in *gork-1* further points to redundant parallel turgor reduction mechanisms. Potassium salts and sucrose both have been proposed to function as major intracellular solutes that mediate stomatal opening (9). The *gork-1* mutant provides a model system in which the contribution of sucrose removal to stomatal closing by physiological stimuli such as ABA, CO_2, or darkness could be analyzed.

It is further likely that the *gork-1* mutant shows residual K^+ efflux via mechanisms other than GORK. In *Commelina communis* guard cells radiolabeled Rb^+ flux measurements allow quantification of time-dependent K^+ (Rb^+) transport in guard cells (3). This method has not been established in the smaller *Arabidopsis* guard cells, but if feasible, would be an important tool to compare K^+ efflux rates in *gork* mutants and WT. Analyses of inward-rectifying K^+ (K^+in) channel mutants have shown roles for these K^+in channels in long-term K^+ uptake (10, 11). For example, sodium hexanitrocobaltate (III) K^+ staining has been used to measure changes in *Arabidopsis* guard cell K^+ content during light-induced stomatal opening in these inward-rectifying K^+ uptake channel mutant lines (11) and could be used to analyze *gork* mutants. Elemental x-ray microanalysis can also be used to compare K^+ content in *Vicia faba* and WT and mutant *Arabidopsis* guard cells (2, 11). The characterization of the *gork-1* mutant will allow future analysis of the relative contributions of additional K^+, sucrose, and other possible solute transport and metabolic mechanisms to stimulus-induced stomatal closing.

Hosy *et al.* pose an interesting question of whether redundant solute release mechanisms function directly in parallel to K^+out channels or whether their roles become more prominent in the *gork-1* mutant background. Identification and mutation of other K^+ and solute efflux mechanisms will be needed to answer this question.

Although ABA enhances K^+out current activity (12), and K^+out channels are the predominant K^+ efflux current activity in guard cells (6, 13), other possible K^+ efflux mechanisms have been reported in these cells. In halophyte guard cells a cation-permeable conductance that mainly mediates Na^+ sodium influx has been characterized (14). This conductance is inhibited at elevated Ca^{2+} levels. Furthermore a transiently activated outward-rectifying K^+ current, named I_{AP}, was characterized in *Arabidopsis* guard cells and was predicted to contribute to K^+ efflux (15). Both of these conductances are down-regulated by Ca^{2+} elevations (14, 15). Because the present characterization of K^+ currents in *gork-1* mutants was performed at 20 mM extracellular $CaCl_2$ (4), reducing extracellular Ca^{2+} concentrations may unmask additional K^+ efflux activities.

The finding that the K^+out channel currents do not inactivate even when guard cells are depolarized for long con-

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tinuous periods of 20 min (16) suggests that these channels can mediate K^+ efflux during long-term depolarizations that occur in response ABA (17). However, some guard cells have also been shown to undergo repetitive transient depolarizations (18). Furthermore, ABA is known to cause repetitive cytoplasmic calcium increases (19, 20). Repetitive cytosolic Ca^{2+} elevations have been linked to transient plasma membrane voltage changes (21). The transiently activated outward rectifying K^+ conductance I_{AP} (15) could conceivably contribute to transient K^+ efflux in response to repetitive depolarizations.

Even the nontransient GORK-type K^+out channel would be activated during short-term depolarizations, which in turn would favor repolarization to negative membrane voltages. Therefore, it would also be interesting to determine whether *gork-1* knockout enhances guard cell depolarizations and affects membrane potential and cytoplasmic Ca^{2+} oscillations.

Upon water withdrawal *gork-1* plants showed increased water loss both in leaves and whole plants (4). These findings are consistent with the enhanced stomatal apertures and reduced ABA response in the *gork-1* mutant. Presently only two *Arabidopsis* genes, *SKOR* and *GORK*, have been unequivocally characterized as plant plasma membrane K^+out channel genes (4, 8, 22). A different type of plant K^+ channel gene, the *KCO* class, has been proposed to also contribute to plasma membrane K^+out channel activities (23). It is interesting that the *gork-1* knockout line did not show any further pleiotropic growth or morphological or developmental defects (4). Despite RT-PCR analysis showing expression of *GORK* in root hair cells (24), no root hair phenotype was found for the *gork-1* mutant (4). Further studies may reveal conditional phenotypes and redundancies.

In conclusion, the *gork-1* knockout analysis of Hosy *et al.* provides molecular genetic evidence for the model that K^+out channels function in mediating stomatal closing and turgor reduction in plant cells (6, 7). Furthermore, the availability of a K^+out channel knockout mutant will allow researchers to address important new future questions in stomatal physiology and plant K^+ transport.

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