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# The Clickable Guard Cell: Electronically Linked Model of Guard Cell Signal Transduction Pathways

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## ABSTRACT

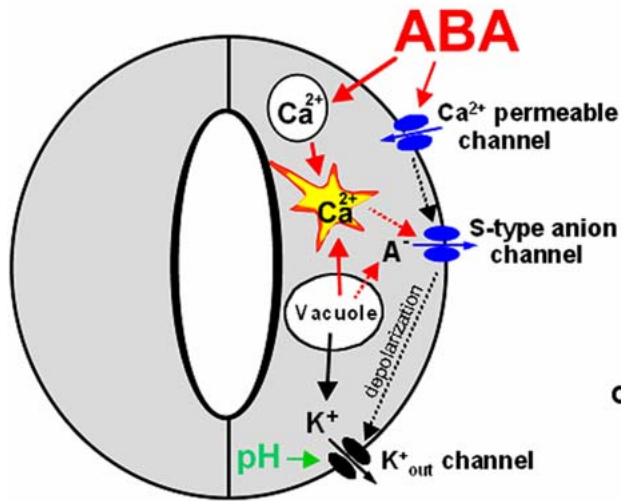
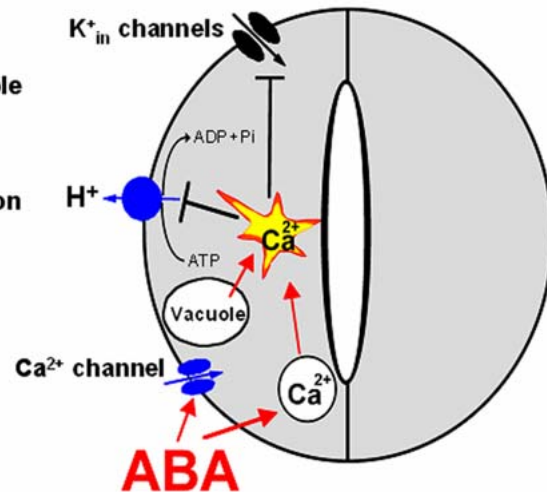
Guard cells are located in the leaf epidermis and pairwise surround stomatal pores, which allow CO<sub>2</sub> influx for photosynthetic carbon fixation and water loss via transpiration to the atmosphere. Signal transduction mechanisms in guard cells integrate a multitude of different stimuli to modulate stomatal aperture. Stomata open in response to light. In response to drought stress, plants synthesize the hormone abscisic acid (ABA) which triggers closing of stomatal pores. Guard cells have become a well-developed system for dissecting early signal transduction mechanisms in plants and for elucidating how individual signaling mechanisms can interact within a network in a single cell. Previous reviews have described pharmacological modulators that affect guard cell signal transduction. Here we focus on mechanisms for which genes and mutations have been characterized, including signaling components for which there is substantial biochemical evidence such as ion channels which represent targets of early signal transduction mechanisms. The fully interactive clickable electronic version of this publication can be accessed at the following web site: <http://www-biology.ucsd.edu/labs/schroeder/clickablegc.html>.

In brief, the interactive clickable version of this report includes the following features: (a) Figure 1 is linked to explanations that appear upon mouse-over. (b) Figures 2 and 3 are clickable and linked to explanatory text for each gene and the respective signaling component. (c) Explanatory background text is an updated version of Schroeder et al. (2001), used with permission of Annual Reviews of Plant Biology. (d) Genes that are discussed within this review are directly linked to electronic databases: Arabidopsis genes are linked to MAtDB, transporter families are linked to PlantsT, genes from other plant species to GenBank. (e) Recent publications are linked to abstracts in PubMed.

## INTRODUCTION

Being rooted in one place plants must respond to changes in environmental conditions and stresses. Interwoven hormone and light signal transduction pathways form complex networks that control plant responses to the environment. In spite of their importance for plant growth and development, these signaling pathways remain "grey boxes", with many major players and their interactions remaining unknown. In guard cells, a network of signal transduction mechanisms integrates water status, hormone responses, light, CO<sub>2</sub> and other environmental conditions to regulate stomatal movements in leaves for optimization of plant growth and survival under diverse conditions. Stomatal guard cells have become one of the well-developed model systems for understanding how various signaling components can interact within a network in a single plant cell. Guard cells allow quantitative dissection of the functions of individual genes and proteins within signaling cascades because:

1. Guard cells respond cell-autonomously to physiological stimulation, allowing cell biological analyses of stomatal opening and closing in response to various stimuli.
2. Guard cells respond to most of the classically known hormonal stimuli in plants, illustrating that presently unidentified but essential plant receptors and early signaling mechanisms function in these cells.
3. A network of ion channels in the plasma membrane and tonoplast of guard cells has been characterized that, together with metabolic responses, controls stomatal movements (see Fig. 1). Being targets of early signaling branches these ion channels provide effective probes to identify and characterize upstream regulators.
4. Stomatal aperture regulates plant CO<sub>2</sub> intake and water loss, thus critically influencing growth and water stress responsiveness. In addition, powerful techniques have recently been developed and adapted for guard cell signal

**ABA mediates stomatal closing****ABA inhibits stomatal opening**

**Figure 1. A model for roles of ion channels in ABA signaling.** Ion channels play a central role in stomatal movements, regulating guard cell turgor as well as controlling  $[Ca^{2+}]_{cyt}$ . The phytohormone abscisic acid (ABA), synthesized in response to drought stress, triggers stomatal closing (left) and inhibits stomatal opening (right). See text and <http://www-biology.ucsd.edu/labs/schroeder/clickablegc.html> for further explanations.

transduction studies, allowing interdisciplinary, molecular genetic, cell biological, second messenger, biophysical, physiological and genomic analyses.

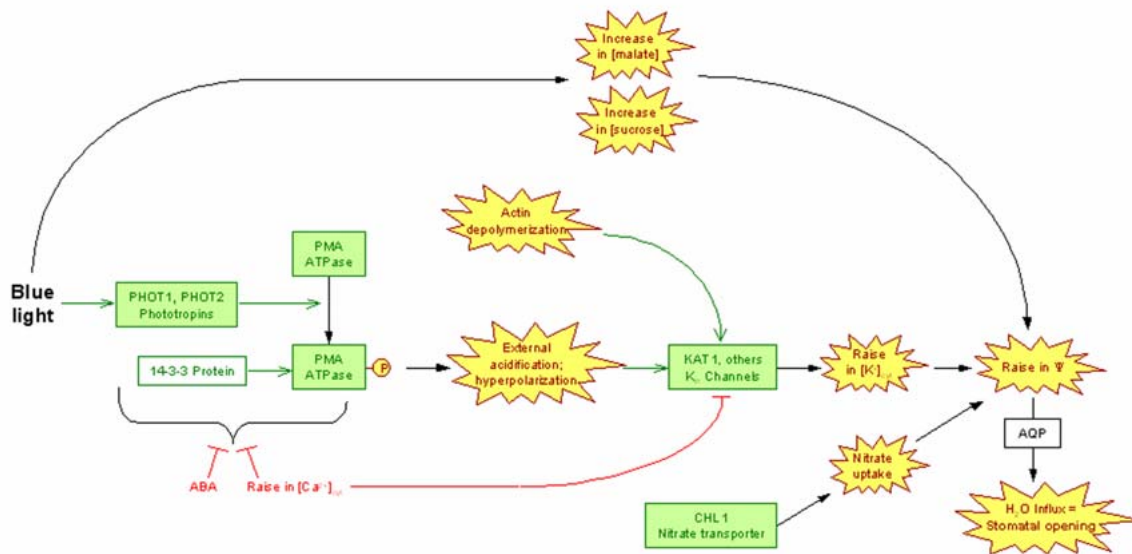
**The roles of ion channels in ABA signaling**

The hormone ABA triggers a signaling cascade in guard cells that results in stomatal closure and inhibits stomatal opening. Stomatal closure is mediated by turgor reduction in guard cells, which is caused by efflux of  $K^+$  and anions from guard cells, sucrose removal, and a parallel conversion of the organic acid malate to osmotically inactive starch (MacRobbie, 1998). Figure 1 shows an extension of early models for the roles of ion channels in ABA-induced stomatal closing (Schroeder and Hedrich, 1989; McAinsh et al. 1990). ABA triggers cytosolic calcium ( $[Ca^{2+}]_{cyt}$ ) increases (McAinsh et al., 1990; Figure 1, left panel).  $[Ca^{2+}]_{cyt}$  elevations activate two different types of anion channels: Slow-activating sustained (S-type; Schroeder and Hagiwara, 1989) and rapid transient (R-type; Hedrich et al., 1990) anion channels. Both mediate anion release from guard cells, causing depolarization (Figure 1, left panel). This change in membrane potential deactivates inward-rectifying  $K^+$  ( $K^+_{in}$ ) channels and activates outward-rectifying  $K^+$  ( $K^+_{out}$ ) channels (Schroeder et al., 1987), resulting in  $K^+$  efflux from guard cells (Figure 1, left panel). In addition, ABA causes an alkalization of

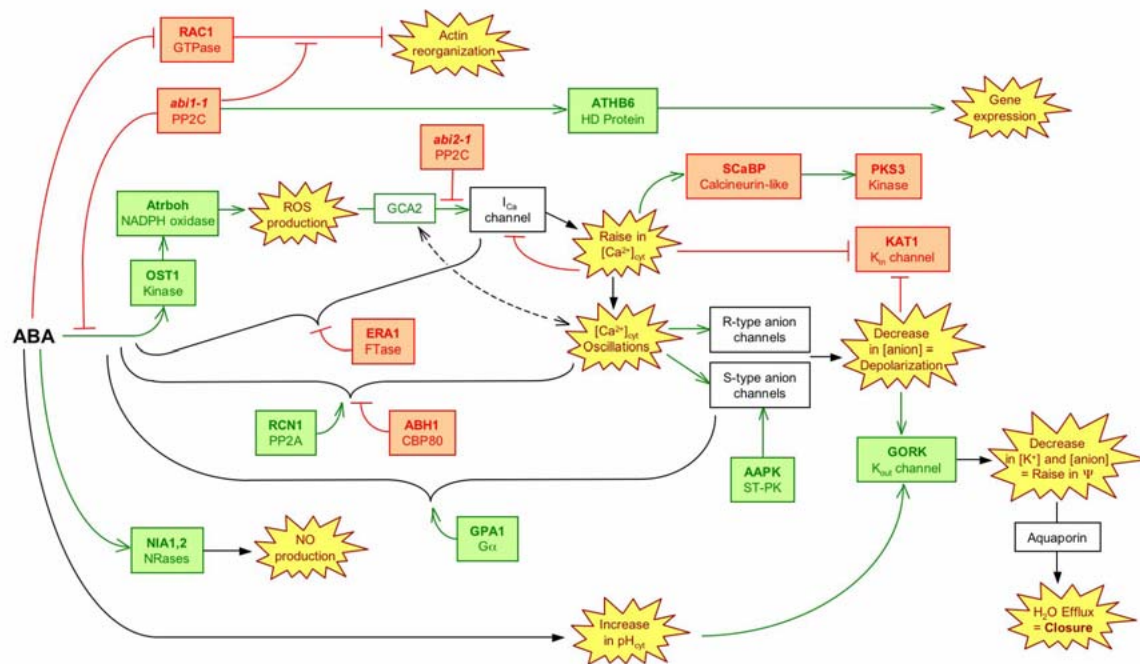
the guard cell cytosol (Blatt and Armstrong, 1993) which directly enhances  $K^+_{out}$  channel activity (Blatt and Armstrong, 1993; Ilan et al., 1994; Miedema and Assmann, 1996) and down-regulates the transient R-type anion channels (Schulz-Lessdorf et al., 1996). The sustained efflux of both anions and  $K^+$  from guard cells via anion and  $K^+_{out}$  channels contributes to loss of guard cell turgor, which leads to stomatal closing (Figure 1).

As vacuoles can take up over 90% of the guard cell's volume, over 90% of the ions exported from the cell during stomatal closing must first be transported from vacuoles into the cytosol (MacRobbie, 1998; MacRobbie, 1995).  $[Ca^{2+}]_{cyt}$  elevation activates vacuolar  $K^+$  (VK) channels proposed to provide a pathway for  $Ca^{2+}$ -induced  $K^+$  release from the vacuole (Ward and Schroeder, 1994). At resting  $[Ca^{2+}]_{cyt}$ ,  $K^+$  efflux from guard cell vacuoles can be mediated by fast vacuolar (FV) channels, allowing for versatile vacuolar  $K^+$  efflux pathways (Allen and Sanders, 1996). The pathways for anion release from vacuoles remain elusive.

Stomatal opening is driven by plasma membrane proton-extruding  $H^+$ -ATPases.  $H^+$ -ATPases can drive  $K^+$  uptake via  $K^+_{in}$  channels (Figure 1, right panel; Kwak et al., 2001). Cytosolic  $Ca^{2+}$  elevations in guard cells down-regulate both  $K^+_{in}$  channels (Schroeder and Hagiwara, 1989) and plasma membrane  $H^+$ -ATPases (Kinoshita et al., 1995), providing a mechanistic basis for ABA and  $Ca^{2+}$  inhibition of  $K^+$  uptake during stomatal opening (Fig. 1, right panel).



**Figure 2. Light-induced stomatal opening.** This figure shows genes that function in stomatal opening and their proposed positions in the blue light signal transduction pathway in *Arabidopsis* guard cells. Positive regulators have green and black arrows, negative ones have red bars; curly braces are used in cases where the exact position of a gene product in the signaling cascade is unknown or when the gene product is an indirect regulator of ABA signaling. Please also see the on-line version of this figure (<http://www-biology.ucsd.edu/labs/schroeder/clickablegc/pages/opening.html>), where it is clickable and linked to explanatory text.



**Figure 3. ABA-induced stomatal closing.** This figure shows genes that function in stomatal closure and their relative positions in the ABA signal transduction pathway in *Arabidopsis* guard cells. Positive regulators have green arrows, negative ones have red bars; curly braces are used in cases where the exact position of a gene product in the signaling cascade is unknown or when the gene product is an indirect regulator of ABA signaling. Please also see the on-line version of this figure (<http://www-biology.ucsd.edu/labs/schroeder/clickablegc/pages/closure.html>), where it is clickable and linked to explanatory text.

### Light-induced stomatal opening

Guard cells respond to a multitude of signals including temperature, partial CO<sub>2</sub> pressure, light, humidity, and hormonal stimuli. For the majority of signals the molecular identity of the sensors is not known, with the notable exception of blue light: The phototropins PHOT1 and PHOT2 were shown to be the blue light receptors in *Arabidopsis* guard cells (Kinoshita et al., 2001). Figure 2 illustrates the signaling cascade from activation of PHOT1,2 to stomatal opening in terms of known genes and their relative position in the signal transduction pathway. For a detailed description of each gene please see the on-line version of this figure (<http://www-biology.ucsd.edu/labs/schroeder/clickablegc/pages/opening.html>) where the figure is clickable and linked to an updated version of Schroeder et al. (2001).

### ABA-induced stomatal closing

One of the best understood plant signaling networks is the one triggered by ABA in guard cells, causing stomatal closure. As shown in Figure 3, many genes encoding for positive as well as negative regulators of guard cell ABA signaling have been identified in *Arabidopsis*. The gene(s) encoding the ABA receptor(s), however, remained elusive. Most of the ABA signal transduction components were found by classic "forward" genetic screens for either increased or reduced sensitivity to ABA. For a detailed description on the experimental evidence and proposed function for each gene, please see the on-line version of this figure (<http://www-biology.ucsd.edu/labs/schroeder/clickablegc/pages/closure.html>) where the figure is clickable and linked to an updated version of Schroeder et al. (2001).

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