From milliseconds to millions of years: guard cells and environmental responses
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During the past year, significant advances have been made in our understanding of stomatal development and its response to climate change, and in our knowledge of how guard cell Ca2+ oscillations encode environmental signals. Recent studies on (de)phosphorylation mechanisms have provided new information on how guard cells respond to abscisic acid and blue light.

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Introduction
Stomata, located in the plant epidermis, consist of a pair of guard cells and the pore they enclose (Figure 1a,b). Stomata open and close through turgor changes driven by massive ion fluxes, which occur mainly through the guard cell plasma membrane and tonoplast.

The guard cell has become a model system for the study of signal transduction. Guard cell signaling is considered to be intriguing, not only because stomates play an important role in the plant’s response to its variable environment but also because the guard cell is mechanically separate from the surrounding cells, and because guard cells have sensitive reversible reactions to internal and external stimuli. A detailed analysis of guard cell signal transduction may be useful for studies of other plant signaling systems such as, pulvinar movement, root gravitropism and pollen-tube growth.

Almost every kind of ion transporter known to function in plants has been described in the guard cell (Figure 1c,d) [1–3]. In the plasma membrane, H+-ATPases pump H+ out of the cytosol. The plasma membrane is thus hyperpolarized, which drives ion influx by other transporters. Inward K+ channels and a presumed Cl− transporter are responsible for ion influx during stomatal opening. Sucrose uptake, likely occurring via a H+-sucrose co-transport mechanism, also appears to play a significant role in osmotic build-up under some stomatal opening conditions [4–6]. Although the guard cell inward K+ channel, KAT1, was identified several years ago, sensitive reverse transcription polymerase chain reaction techniques have also shown expression of three other inwardly rectifying K+ channels — AKT1, AKT2/3, and KAT2 — in guard cells. KAT1 may not play as dominant a role in K+ uptake as previously believed [7•].

Outward K+ channels [8] and anion channels are the main conduits for passive ion efflux from the cytosol to the apoplast during stomatal closure. Evidence is accumulating that the guard cell S-type anion channel may consist of, or be regulated by, an ATP-binding cassette (ABC)-type transporter [9•]. In the tonoplast, anion channels, fast-vacuolar channels (FV), vacuolar K+-selective channels (VK), slow-vacuolar channels (SV), and vacuolar voltage-dependent Ca2+ channels (VV) have been characterized (Figure 1) [2,3].

How so many ion transporters are organized in response to various stimuli over both the short term and the long term is a key question in stomatal physiology. This review will provide an overview of some of the advances in this area over the past 18 months. Other valuable recent reviews covering various aspects of stomatal physiology are also available [1,10••–12••,13].

Stomata are modified by environmental factors
The stomatal signaling system has been moulded by environmental factors over both long (millions of years) and short (hours, minutes, or even seconds) timescales. Considerable progress has been made in the elucidation of environmental perception and ion transport mechanisms occurring over the short timescale. Our understanding of what has taken place over the developmental and evolutionary timescales remains limited, but several papers have broken new ground. In 1987, Woodward [14] pointed to evidence in the fossil record that plants have responded to post-industrial increases in atmospheric CO2 levels with decreases in stomatal density. This response may have been selected for because it allowed reduced water loss, while photosynthesis was maintained due to the increased availability of carbon dioxide. Conversely, an increase in stomatal density can lead to increased water loss, accompanied by increases in leaf cooling.

In 2001, it has been hypothesized that the evolution of the broad lamina typical of most present-day leaves was made possible by an increase in stomatal density that occurred...
Ion transport in guard cells. (a) Stomatal complex of Vicia faba. Scale bar = 10 μm. (b) Stomatal complex of Arabidopsis thaliana. Scale bar = 10 μm. (c) Ion channels and transporters discussed in this article that reside at the guard cell plasma membrane. The Cl– transporter and the sucrose transporter are inferred from physiological experiments but have yet to be characterized by molecular or electrophysiological techniques. (d) Ion channels and transporters discussed in this article that reside at the guard cell tonoplast. FV, fast-vacuolar channel; SV, slow-vacuolar channel; VK, vacuolar K+ -selective channel; VV, vacuolar voltage-dependent Ca2+ channel.

following a drastic decrease in atmospheric CO2 levels that occurred in the late Paleozoic, 410–363 million years ago. It is speculated that this increased stomatal density promoted evaporative cooling, which in turn kept broader undissected leaves with their attendant thicker boundary layers from overheating [15•,16].

Over a much shorter developmental timescale, some plant species also show an acclimatory response to atmospheric CO2, with a decrease in stomatal density observed under elevated CO2 conditions [17]. Recently, Gray et al. [18••] demonstrated the expression in guard cells of the gene HIC (HIGH CARBON DIOXIDE). In plants in which the HIC gene was disrupted by insertion of the β-glucuronidase gene, the stomatal index increased upon doubling of experimental CO2 levels. The hic mutants thus exhibited a reversal of the normal inverse correlation between stomatal index and ambient CO2 concentration. On the basis of nucleotide and derived peptide sequence homologies, HIC encodes a putative 3-keto acyl coenzyme A synthase. Enzymes of this type are involved in the synthesis of very-long-chain fatty acids. In plants, long-chain fatty acids are found in waxes and cutins, which are constituents of the cuticle, as well as in glycerolipids and sphingolipids. Some substance that normally diffuses from pre-existing guard cells under high CO2 concentrations, and prevents neighboring cells from differentiating into guard cells, may be absent in the hic mutants or may be immobilized as a result of alterations in the composition of the cuticular layer [18••,19].

Other hic mutants also lend support to the idea that disruption of the extracellular matrix leads to a change in stomatal index: eceriferum-1 and eceriferum-6 mutants of Arabidopsis [20], both of which have alterations in wax composition on the leaf surface, exhibit higher stomatal indices than do wild-type plants.

The effect of the hic mutation differs from that of other stomatal mutants such as too many mouths (tmm) [21] and stomatal density and distribution1 (sdd1) [22•], in that the stomata of the hic mutants show increased density but do not show abnormal clustering. TMM has not yet been cloned, but SDD1 encodes a subtilisin-like serine protease, suggesting that the processing of a proteinaceous signal may be involved in stomatal patterning. It is interesting to speculate that TMM, SDD1, or other gene products implicated in stomatal patterning may, like HIC, prove sensitive to environmental factors that regulate stomatal movements, such as light, drought, air pollutants, and humidity. Some studies have already shown that long-term drought stress and abscisic acid (ABA) treatment result in increased stomatal density and reduced guard cell size ([23] and references therein). The ABA effect on cell size indicates that not only stomatal patterning but also guard cell development is influenced by the environment. Normal differentiation of the stomatal complex involves cytoskeletal reorganization, and some studies have implicated a sensitivity of guard cell actin filament and microtubules arrays to signals such as ABA and light [24,25].

**The role of Ca2+ in guard cell responses to ABA, cold, and oxidative stress**

Guard cells can sense and rapidly respond to many signals, including not only external stimuli but also internal stimuli such as ABA and auxin [26,27]. This area of study is advancing rapidly, and this past year has seen particular advances in our understanding of the inter-relationships between ABA and Ca2+ signaling.

Changes in cytosolic calcium are elicited by many of the signals to which plants respond, and cytosolic calcium has been proposed to be a universal second messenger. How is the specificity and reliability of Ca2+ signaling achieved? Perhaps a given external stimulus could produce a specific type of Ca2+ response that amplifies the signal upstream of Ca2+, such as signals from receptors and G proteins, and then signals to Ca2+-regulated downstream molecules, for example, calcium-dependent protein kinases [28], inward K+ channels and anion channels.

Along these lines, recent studies have utilized proteinaceous Ca2+ reporters to measure Ca2+ oscillations in guard cells, with particular reference to ABA signaling. Allen et al.
transformed a calcium reporting green fluorescent protein construct or ‘cameleon’ into Arabidopsis, and assessed guard cell Ca2+ responses to several distinct stimuli. They found that ABA caused both repetitive cytosolic transients and more prolonged oscillations. Cold stimulated small, repetitive cytosolic Ca2+ transients, whereas H2O2 induced one or two separate Ca2+ transients. Thus, each stimulus led to a Ca2+ response with distinct features. Even for a single signal, Ca2+ responses may vary in a manner that reflects signal strength. Staxen et al. [30] found that different ABA concentrations administered to Commelina communis epidermis led to guard cell Ca2+ oscillations of different periods, with higher concentrations of the hormone eliciting lower frequency oscillations. This phenomenon may arise from differential contributions of Ca2+ uptake and internal Ca2+-release pathways at different ABA concentrations [12••].

How are Ca2+ oscillations produced?

Ca2+ oscillations (periodic Ca2+ concentration changes over time) and Ca2+ waves (spatial changes in calcium concentration) are produced through the integration of Ca2+ influx, release of Ca2+ from the endomembrane system, Ca2+ efflux from the cytosol to the apoplast, and Ca2+ sequestration into intracellular stores. Different stimuli presumably use different Ca2+ sources or releasing pathways to establish characteristic Ca2+ responses, and this hypothesis was recently lent credence by two studies. Wood et al. [31•] used aequorin to report Ca2+ responses to several stimuli in the presence of Ca2+ channel blockers and the Ca2+ chelator EGTA. They concluded that mechanical and ABA signaling in guard cells rely heavily on Ca2+ release from intracellular stores, whereas low-temperature signaling relies heavily on Ca2+ uptake from the apoplasm. Allen, Schroeder, and colleagues [29••] studied Ca2+ oscillations in the Arabidopsis det3 mutant. This mutant exhibits reduced expression of the C-subunit of the V-type ATPase, accompanied by reduced endomembrane energization. They found that the det3 mutation changed the Ca2+ oscillations observed when stomata were challenged with external Ca2+ and H2O2, but they found no detectable changes in response to ABA and cold stimuli [29••].

The result with cold treatment is consistent with the results of Wood et al. [31•]. However, it is perhaps unexpected that the det3 mutation affected the H2O2 response but not the ABA response, as Schroeder’s group has also implicated H2O2 as a secondary messenger for ABA action (see below) [32•]. The guard cell may utilize multiple redundant pathways, thus ensuring adequacy of the vital ABA response. In fact, this idea of redundancy is consistent with the multiplicity of Ca2+ pathways implicated in guard cells, as described below.

Ca2+ release and re-uptake pathways in guard cells

One mechanism for increasing cytosolic Ca2+ levels is via Ca2+ uptake from the apoplast. In 2000, two laboratories characterized plasma membrane channels in Vicia faba and Arabidopsis that mediate Ca2+ uptake and are activated by ABA during hyperpolarization [32•,33•]. In Vicia faba, these Ca2+ channels show some hyperpolarization-induced activity in the absence of ABA. In Arabidopsis, it was demonstrated that ABA activation of the channels occurred via a H2O2 secondary messenger. It will be interesting to ascertain the extent to which reactive oxygen species regulate this channel type in Vicia faba and in other plant species.

Endomembrane Ca2+ efflux in response to ABA has been studied extensively and several secondary messengers have been implicated in this response. Previous studies have shown that ABA stimulates inositol 1,4,5-trisphosphate (IP3) production in guard cells and that IP3 induces
cytosolic Ca\(^{2+}\) elevation and partial stomatal closure [34–36]. IP\(_{3}\) may also serve as a precursor to IP\(_{6}\) (inositol hexakis phosphate), which has also been linked to Ca\(^{2+}\)-dependent ABA responses [37]. Staxen et al. [30] characterized ABA-induced Ca\(^{2+}\) oscillations in detail and found that these oscillations and stomatal closure were both partially inhibited by U-73122 (1-[6-\{17\β-3-methoxyestra-1,3,5(10)-trien-17-yl\}amino\}hexyl]-1H-pyrole-2,5-dione), an inhibitor of phosphoinositide-specific phospholipase C (PLC).

PLC and another phospholipase, PLD, which cleaves the phospholipid headgroup to yield phosphatidic acid, both show transient increases in activity following ABA application. PLD seems to function in the same pathway as PLC: inhibitors of PLC and PLD each partially oppose ABA-induced stomatal closure, but simultaneous application of both inhibitors does not result in enhanced attenuation of ABA-induced closure [38•]. The relative positions of the two phospholipases in their signaling chain remains unknown, but do seem that PLD activation is downstream of — or independent of — Ca\(^{2+}\) release because the direct product of PLD action, phosphatidic acid, does not elicit cytosolic Ca\(^{2+}\) increases in guard cells.

The lack of complete reversal of ABA-induced stomatal closure by combined PLC and PLD inhibitors implicates other Ca\(^{2+}\) release pathways in the ABA response, and a parallel pathway appears to be that mediated by cyclic ADP-ribose (cADPR). In contrast to the situation with PLC and PLD, simultaneous application of PLD and cADPR antagonists does increase the extent to which ABA-induced stomatal closure is reduced. For example, simultaneous application of nicotinamide, an inhibitor of cADPR action, and 1-butanol, an inhibitor of phosphatidic acid production, results in an almost complete block of ABA-induced stomatal closure in \textit{Vicia faba} epidermal peels [38•]. Patch clamping of isolated guard cell vacuoles confirms that cADPR activates Ca\(^{2+}\) release from this organelle via Ca\(^{2+}\)-selective channels, and microjection of cADPR into guard cells elicits cytosolic Ca\(^{2+}\) increases and stomatal closure [39].

One of the most recent calcium-mobilizing molecules to be identified is sphingosine 1-phosphate (SIP). In \textit{Commelina communis}, ABA-induced stomatal closure is opposed by a competitive inhibitor of sphingosine kinase, the enzyme responsible for producing SIP from sphingosine. Application of SIP to epidermal peels induces Ca\(^{2+}\) oscillations in guard cells and stomatal closure. An increase in SIP levels occurs in whole leaves following drought stress, and it will be important to ascertain whether or not such responses also occur in guard cells [40••]. SIP is unusual in that it can serve as an intracellular signal or, if located extracellularly, interact with outwardly facing receptors [41]. The idea of a mesophyll messenger that coordinates photosynthetic and stomatal functions has intrigued stomatal physiologists for years [42]. Could SIP play such a role?

Yet another Ca\(^{2+}\) mobilization pathway has yet to be assayed in guard cells, although it has been detected in other types of plant cells. Navazio et al. [43] demonstrated that endoplasmic reticulum vesicles from cauliflower respond to nicotinic acid adenine dinucleotide phosphate (NAADP), a Ca\(^{2+}\)-releasing molecule recently described in both vertebrates and invertebrates. In contrast to the IP\(_{3}\) and cADPR pathways, NAADP did not elicit Ca\(^{2+}\) release from tonoplast vesicles. The results with NAADP thus highlight the importance of the endoplasmic reticulum as a Ca\(^{2+}\) store in plants. Given the plethora of Ca\(^{2+}\) release pathways described in guard cells to date, one would predict that NAADP will also be found to mobilize Ca\(^{2+}\) in this cell type.

**Ca\(^{2+}\)-independent pathways**

The existence of multiple Ca\(^{2+}\) mobilization pathways and Ca\(^{2+}\) release sites undoubtedly contributes to the generation of stimulus-specific Ca\(^{2+}\) signals. It should be kept in mind, however, that Ca\(^{2+}\)-independent pathways also exist in the guard cell. ABA can inhibit K\(^{+}\) channels involved in stomatal opening and in some circumstances elicit stomatal closure without detectable calcium increases in guard cells [31•,39,44]. Protons have been implicated as one of the other secondary messengers in the guard cell ABA response. First, ABA also induces pH increases prior to stomatal closure; and second, elevated pH activates the plasma membrane outward K\(^{+}\) channel through which K\(^{+}\) efflux occurs during stomatal closure. This channel is Ca\(^{2+}\)-insensitive [45]. The activities of FV and SV channels, which release K\(^{+}\) and Ca\(^{2+}\) to the cytosol (Figure 1) [2] are also stimulated by alkaline cytosolic pH, although the VK current is inhibited under these conditions [3,46]. These data imply that changes in pH function alongside changes in pCa to modulate stomatal movement.

**Nonionic signaling in guard cell responses to stress**

The guard cell not only uses Ca\(^{2+}\) oscillations and pH changes to establish specific responses but also employs nonionic signaling molecules. Evidence that guard cell pathways have novel features not found universally in all plant cells has been provided recently by two studies. Sutton et al. [47] found that co-injection of oocytes with guard cell protoplast mRNA along with cRNA for KAT1 resulted in the expression of an inward K\(^{+}\) channel that was inhibited upon ABA application to the oocyte. However, oocytes co-injected with mesophyll mRNA and KAT1 cRNA produced inward K\(^{+}\) currents that were not inhibited by ABA [47]. These results show that the mesophyll ABA signaling pathway is distinct from, and cannot substitute for, the guard cell ABA signaling pathway.

One guard-cell-specific component may be AAPK (ABA-activated protein kinase), a serine-threonine protein kinase whose biochemical activity has only been detected in guard cells to date [48]. On the basis of sequencing of two peptides from AAPK using tandem mass spectrometry,
Li et al. [49**] cloned the AAPK cDNA from *Vicia faba* guard cells. Mutating Lys43 in AAPK to an alanine residue resulted in a kinase with apparent dominant-negative effects, and guard cells transformed with AAPK K43A no longer closed in response to ABA application to epidermal peels. Guard cells expressing the mutant version of the kinase also failed to activate plasma membrane anion channels, which play a central role in ABA-induced stomatal closure.

Protein phosphorylation is reversed by protein phosphatases. *abi1-1* and *abi2-1* are two ABA-insensitive dominant mutant alleles of the protein phosphatase 2C (PP2C) genes *ABI1* and *ABI2*. Both of these mutations confer a wilt phenotype and ABA insensitivity of stomatal closure. Recently, Gosti et al. [50**] isolated seven recessive alleles of *ABI1* as intragenic revertants of the *abi1-1* mutation. These revertants show hypersensitive responses to ABA in terms of the inhibition of seed germination and seedling root growth. When water is withheld, the revertants also show enhanced drought tolerance. The recessive nature of the revertants suggests that a loss of function in *ABI1* is responsible for the ABA-hypersensitive phenotype.

Consistent with the above hypothesis, recombinant proteins derived from the intragenic revertant alleles do not exhibit detectable PP2C activity. In the same assay, the PP2C encoded by the dominant, possibly gain-of-function, *abi1-1* allele exhibits residual phosphatase activity [50**]. Taken together, the evidence suggests that wild-type *ABI1* phosphatase negatively regulates ABA responses, and it is tempting to speculate that it reverses the action of the ABA-activated kinase, AAPK.

Guard cells respond not only to drought stress but also to stresses such as salinity and air pollution [51]. In addition to their previously implicated roles in cell division and organ development, polyamines have been found to accumulate upon plant exposure to such stresses [52]. Liu et al. thus addressed the hypothesis that polyamines are involved in stomatal action and, indeed, found that application of all of the natural polyamines inhibited stomatal opening and induced stomatal closure. Polyamines also inhibited whole-cell inward K⁺ currents, plausibly acting on the KAT1 inward K⁺ channel. This inhibition of inward K⁺ channels was not observed in isolated membrane patches, suggesting that the response also requires cytoplasmic factors [53]. These data suggest that polyamines may be components of stress-induced signal transduction in guard cells, and the next step will be to document the extent to which guard cell polyamine concentrations change in response to stress signals.

**Blue light and stomatal opening**

Stomatal closure in response to ABA and stomatal opening in response to light comprise two major areas of investigation by stomatal physiologists. Stomatal opening is triggered by both red light — acting through chlorophyll — and blue light. Low-intensity blue light is much more effective in stimulating stomatal opening than comparable fluence rates of red light, pointing to a sensitive, non-chlorophyllous photoreceptor as the starting point for this photoreponse. Identification of this photoreceptor is still under investigation. Zeiger and colleagues have provided photobiological evidence that zeaxanthin, a carotenoid intermediate in the xanthophyll cycle, is the specific blue-light photoreceptor [54]. Guard cells from the *npq1* mutant of *Arabidopsis*, which lacks zeaxanthin, show reduced stomatal opening in response to blue light when assayed in isolated epidermal peels [54]. Gas exchange experiments measuring blue-light-induced transpiration from whole leaves of the *npq1* mutant, however, show a wild-type stomatal response to blue light. The same is true for all other blue-light photoreceptor mutants assayed to date (i.e. *nph1*, *cry1*, and *cry2*) [55,56]. Moreover, in *Vicia faba* at least, the ultraviolet action spectrum for stomatal opening does not match the absorption spectrum of bulk (trans) zeaxanthin, and is instead consistent with that of either a cis-carotenoid (including cis-zeaxanthin) or a flavin [57]. If zeaxanthin is indeed the guard cell blue-light photoreceptor, then the whole-leaf experiments with *npq1* imply that there must be a mesophyll blue-light photoreceptor that senses and reports the presence of blue light to the zeaxanthin-less guard cells of *npq1*.

Although the early stages of the blue-light photoreponse remain mysterious, the ultimate targets of that signal transduction chain are becoming clearer. Recently, two studies [58**,59] have lent support to the idea that it is an H⁺-ATPase, rather than a redox pathway, that mediates the proton pumping that hyperpolarizes the plasma-membrane drive K⁺ uptake and stomatal opening in response to blue light [60]. Shimazaki’s group used biochemical techniques to demonstrate that blue-light activation of the H⁺-ATPase involves blue-light-dependent phosphorylation of the carboxyl terminus of the H⁺-ATPase [58**]. This phosphorylation increases the affinity of the H⁺-ATPase for a 14-3-3-protein (14-3-3 proteins are positive regulators of H⁺-ATPases). Three 14-3-3 cDNAs were identified in a *Vicia faba* guard cell cDNA library, and analysis of the native 14-3-3 protein co-immunoprecipitated with the H⁺-ATPase indicates that just one of the 14-3-3 isomers, 14-3-3a, specifically associates with the H⁺-ATPase following blue-light irradiation [61]. These elegant experiments have thus identified 14-3-3a as a key player in the stomatal response to blue light.

**Conclusions**

Massive progress has been made since the first successful patch clamp recordings from guard cells in 1984. A number of important signaling elements have been identified that function in guard cells on the timescale of milliseconds to minutes, although the ordering and interactions of these signaling molecules remain unknown in many cases. A few key genes have also been identified that play a role in
guard cell development. However, on the intermediate timescale of the life-time of a single mature guard cell or a single leaf, we still know very little about environmental effects on guard cell physiological function and gene expression [62,63].

Many of the most telling experiments carried out over the past few years have been conducted on isolated epidermal peels or guard cell protoplasts, or on whole leaves monitored with gas-exchange techniques. It has been demonstrated unequivocally, however, that in response to signals such as ABA and atmospheric humidity, not all of the guard cells in a leaf act in unison. There exist domains or ‘patches’ within the leaf that show differential responses to an identical stimulus; furthermore, the boundaries and behavior of such patches fluctuate over time. Such results indicate that stomata in the intact leaf do not function independently but rather are coupled, perhaps hydraulically, within a given patch. This ‘emergent collective behavior’ [64**] adds one more layer of complexity to stomatal function under real-world conditions.

Update

Heterotrimeric G proteins are one of the most important components of animal signaling pathways. Recent studies have shown that the *Arabidopsis* G protein α subunit, GPA1, is also a vital regulator of plant growth and development [74**, 75**], including guard cell signaling. Wang et al. [75**] used plants harbouring T-DNA insertions in GPA1 to demonstrate G protein involvement in ABA-inhibition of stomatal opening and inward K+ channels, and in pH-independent ABA-activation of slow anion channels. *gpa1* mutant plants exhibit greater rates of water loss than their wild-type counterparts [75**]. These results place G proteins in the guard cell ABA signaling network.

Calcium is a universal signaling element. Allen et al. [76**] present elegant data showing that calcium oscillation numbers frequency, and duration all regulate stomatal aperture [76**]. A detailed analysis is presented in Hetherington’s article on pp 415–420 of this issue.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as: **of special interest
*
**of outstanding interest


Genetic disruption of the inward K+ channel KAT1 has no effect on stomatal behavior in the assays reported in this paper. Other inward K+ channels are also expressed in the guard cell and may compensate for the loss of functional KAT1.


ABC (ATP-binding cassette) transporters constitute a large protein family in Arabidopsis. This paper shows that the ABC transporter, AtMRP5, is expressed in guard cells and is involved in stomatal opening.


This excellent review updates current knowledge of guard cell abscisic acid (ABA) responses, summarizing most of the components that have been shown to be involved in this signaling pathway.


This review describes some of the recent developments in stomatal physiology, with particular emphasis on membrane trafficking.


This article provides a comprehensive overview of Ca2+ influx pathways across the plasma membrane and Ca2+-release pathways from endomembranes.


Atmospheric CO2 concentration influences on stomatal density and evaporative cooling may have been key elements in the evolution of the broad leaf lamina found in many present day land plants.


The HIC gene identified in this report may be crucial for stomatal response to global climate change. hic mutants show an unusual increase in stomatal index under elevated CO2 concentrations. HIC is hypothesized to encode an enzyme involved in the synthesis of the leaf cuticle.


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