REVIEW

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Control of stomatal aperture
A renaissance of the old guard

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Stomata, functionally specialized small pores on the surfaces of leaves, regulate the flow of gases in and out of plants. The pore is opened by an increase in osmotic pressure in the guard cells, resulting in the uptake of water. The subsequent increase in cell volume inflates the guard cell and culminates with the opening of the pore. Although guard cells can be regarded as one of the most thoroughly investigated cell types, our knowledge of the signaling pathways which regulate guard cell function remains fragmented. Recent research in guard cells has led to several new hypotheses, however, it is still a matter of debate as to whether guard cells function autonomously or are subject to regulation by their neighboring mesophyll cells. This review synthesizes what is known about the mechanisms and genes critical for modulating stomatal movement. Recent progress on the regulation of guard cell function is reviewed here including the involvement of environmental signals such as light, the concentration of atmospheric CO_2 and endogenous plant hormones. In addition we re-evaluate the important role of organic acids such as malate and fumarate play in guard cell metabolism in this process.

Introduction

Stomata are formed by pairs of specialized epidermal guard cells which are generally surrounded by subsidiary cells. They serve as major gateways both for CO_2 influx from the atmosphere and transpirational water loss of plants.[1] Due to the impermeable wax layer on the epidermal surface, the stomatal complex, plays a major role in controlling gas exchange between plant and the surrounding atmosphere.[2] Stomata are, therefore, important regulators of the global atmospheric environment,[3] and the developmental fate of epidermal cells to differentiate or not into a pair of mature guard cells is also intimately linked to environmental cues.[3,4] Moreover, environmental cues provided by the light intensity, concentration of atmospheric carbon dioxide or endogenous plant hormonal stimuli control stomatal aperture and development.[3]

Bearing the importance of guard cells in mind it is perhaps unsurprising that considerable research effort has been expended to better comprehend the structure, development and the physiology of stomata.[1,5-8] Given the relative ease of the isolation of guard cells, which gate stomata, our understanding of these cells exceeds that of many of the other 40 cell types described thus far in plants.[9,10] Although guard cells can be regarded as one of the most thoroughly investigated cell types, our knowledge of the signaling pathways that regulate guard cell function is still incomplete.[1,11,12] Despite the anatomical simplicity of the stomatal valve, there is little consensus on how stomata sense and respond to their extrinsic and intrinsic environment.[13] One largely unresolved question is how environmental signals such as light intensity, the concentration of atmospheric CO_2 and endogenous plant hormones are able to modulate basal stomatal movements and gas exchange in accordance to prevailing environmental conditions. While it is well known that opening responses are achieved by coordination of light signaling, light-energy conversion, membrane ion transport, and metabolic activity in guard cells[14] and current data suggest that light and carbon dioxide play major roles in the control of stomatal movement[1,14] the exact mechanisms which underlie this strict mechanistic control are yet to be unravelled.

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Overview of Guard Cell Players and their Functions

Metabolism of guard cells continuously interacts with the environment, sensing biotic and abiotic stimuli and signals coming from both the atmosphere and from roots and responding with appropriate changes in pressure turgor. By this mechanism guard cells control stomata aperture in accordance with changes in the plants environment, in order to preserve water and CO_2 assimilation. Several environmental factors affect stomata opening, including hormone, light quality and intensity, air humidity, atmospheric CO_2 concentration, biotic and abiotic stresses.[3,11,14-17] Recent research progress has revealed that the guard cell response is co-ordinated by a complex of signal transduction networks including input from CO_2, abscisic acid (ABA) and Ca^{2+}.[1,11,18] The signal transduction network appears to be intermediated by kinase/phosphatase regulation, secondary metabolites and the regulation of ion channels.[1,11,18] However, given their importance, some of the principal components of the signaling pathways are described in the following sections.

CO_2 Sensing and Signaling

The effects of CO_2 concentration upon stomata aperture has been known for decades.[19-21] At lower atmospheric partial pressure of

CO_2 higher stomata aperture is observed in attempt to keep a minimum of CO_2 available in the sub-stomatal chambers and in order to maintain carbon assimilation in the mesophyll cells through operation of photosynthetic machinery. By contrast the opposite response is observed at high CO_2 concentrations in the intercellular spaces which lead to stomatal closure without reductions in the carbon assimilation. Recently CO_2-induced stomatal closure has been studied in detail and plant mutants with affected CO_2 stomatal response have been identified.[22-26]

The physiological mechanism by which elevated CO_2 regulates stomatal aperture has been extensively documented.[11,27,28] In brief, high atmospheric CO_2 concentrations activate anion channels and K^+ out efflux channels in guard cells,[29,30] and promote chloride release from guard cells and membrane depolarization.[31] Indeed considerable experimental evidence has been accrued that a ion Ca^{2+} is required for CO_2-induced stomatal closure.[32,33] It has been reported that elevated CO_2 activates plasma membrane anion channels of guard cells.[27] Interestingly it was found that after a rise in CO_2 from 150 to 800 μmol mol^{-1}, it took a few minutes before the apoplastic Cl^- increased.[31] The authors suggested that the accumulation of an intermediate effector is necessary to activate anion channels. Malate has been proposed as such an effector that mediates between CO_2 and anion channel activation.[34] These authors reported that changes in the CO_2 concentration modify the apoplastic malate concentration and that elevated malate enhanced the probability of opening of anion channels by shifting their activation potentials. Although the nature of the intermediate effector that drives anion efflux is unresolved, its accumulation may increase with increasing CO_2 concentration, which induces concentration-dependent anion efflux and stomatal closure.

Despite the fact that the mechanism by which CO_2 regulates stomatal closure is relatively well accepted, the cell types wherein CO_2 is sensed is still a matter of discussion. Potentially, CO_2 could be directly sensed by the guard cells[22,35] or remotely via communication from the mesophyll cells.[15,36,37] It is additionally possible that both cells types could be directly involved in the stomatal CO_2 response.[1] Nevertheless the recent study of Araújo and colleagues[37] clearly indicate that mesophyll derived malate plays an important role in controlling stomatal aperture.

Several attempts have recently been made to identify the proteins responsible for binding CO_2 and thus triggering a signal transduction pathway which could control stomatal responses. One of these studies allowed the isolation of Arabidopsis mutants (high leaf temperature 1; *ht1-1* and *ht1-2*) with altered stomatal movements in response to CO_2.[25] Further analyses demonstrated that *HT1* is a protein kinase expressed mainly in guard cells and suggested that it is important in the control of stomatal movement with its function being more evident in response to CO_2 than to ABA or light. A recent study demonstrated that carbonic anhydrases (CA) can be involved in CO_2 sensing without alteration in ABA and light responses.[24] This study additionally characterised a double-mutant ß-carbonic anhydrases ßCA1 and ßCA4 Arabidopsis plant which was affected in both stomatal movement and density but did not display alterations in either ABA or blue light responses.[24] Moreover it was demonstrated that ßCA-mediated stomatal movements were not linked to leaf photosynthesis. In summary this study suggests that CO_2-binding carbonic anhydrase proteins might function early in CO_2 signaling pathway, controlling gas exchange between plants and their surrounding atmosphere.

Elevated atmospheric CO_2 concentration is known to promote activity of anion channels which mediate efflux of the osmoregulatory anions, Cl^- and $malate^{2-}$, during stomatal closure.[29] For this reason several efforts have recently been made to identify genes encoding guard cell anion channels. However, only recently the first guard cell anion channel was identified in *Arabidopsis thaliana*. The gene SLAC1 (Slow Anion Channel Associated 1), which encodes a protein homologue to bacterial and fungal dicarboxylate/$malate^{2-}$ transporter protein, localized specifically in the plasma membrane of guard cells was isolated.[23,38] Recent evidence however demonstrated by functional expression in *Xenopus laevis* oocytes that guard cell-expressed Arabidopsis *SLAC1* encodes a weak voltage-dependent, anion-selective plasma membrane channel rather than a malate transporter.[39] Analyses of the *slac*1 mutants suggest that SLAC1 protein function as a mediator of the CO_2 sensitivity and regulation of stomatal closure. Further analysis revealed that SLAC1 belongs to a protein family of four related members with the same plasma membrane localization but different tissue specificity expression patterns.[23] Additional experiments suggest that the SLAC1-protein family function is essential to maintain organic/inorganic anion homeostasis on the cellular level[23] and is required for stomatal closure in response ABA, ozone, light/dark transitions, humidity change, calcium ions, hydrogen peroxide and nitric oxide.[38] The lack in SLAC1 protein was shown to impair slow (S-type) anion channel currents that are activated by cytosolic Ca^{2+} and ABA, but do not affect rapid (R-type) anion channel currents or Ca^{2+} channel function. Taken together these results associated with the permeability of S-type anion channels to malate[40] suggest that SLAC1 plays an important role in the function of S-type anion channels. Additionally three malate transporters have been recently cloned that are responsible for cytosol to vacuole and cytosol to apoplast exchange[22,41,42] and their role is discussed in detail in the section "On the role of organic acids as a component of the stomata signaling cascade."

CO_2 and Stomatal Development

By adjusting stomatal distribution and density plants can respond to environmental factors such as CO_2 and water and light availability.[7,8,43] Thus, in addition to the control that CO_2 concentrations have upon regulation of stomatal aperture, the mechanisms controlling the abundance of stomatal complexes are also crucial in determining photosynthetic performance and water use efficiency. Correlation of guard cell size, stomatal density and conductance with atmospheric CO_2 has been assessed over millions of years[44] and it suggests a mechanism connecting long-term CO_2 change with the ecological radiation of land plants and increasing gas-exchange capacity of land plants over geologic time. During their millions-of-years history, plants have been exposed to large variations in environmental conditions that prompted genetic

adaptations toward mechanisms that optimize individual fitness. Over this period, plant adaptation to CO_2 is apparent as periods with high CO_2 favored species with few relatively large stomata and low stomatal conductance (g_s), whereas periods with low CO_2 (as at present) favored species with many relatively small stomata and higher g_s.[44]

Changes in stomatal development have been observed under high CO_2 atmospheric conditions, where decreases in stomatal numbers are observed in parallel with increases in atmospheric CO_2 concentration.[8,45] Particularly interesting results have been found for the relationship between stomatal densities and atmospheric CO_2 concentrations. It has been demonstrated that a wide range of species show a reduction in stomatal density with CO_2 enrichment[46] and that several tree species respond to atmospheric CO_2 concentrations by an alteration in stomatal density.[47,48] Furthermore, responses of stomatal development to light intensity or CO_2 concentration have been shown to involve signaling from mature leaves to the developing leaves.[43] Molecular evidences have been provided which corroborate that CO_2 levels are indeed linked to stomatal development.[49] A high carbon dioxide (*HIC*) gene has been isolated from Arabidopsis which represents a negative regulator of stomatal development that is responsive to the prevailing CO_2 concentration. This gene encodes a putative 3-keto acyl coenzyme A synthase, which is involved in the synthesis of very-long-chain fatty acids.[49] Further studies also performed with *Arabidopsis thaliana*, have revealed that stomatal development and patterning is regulated by responsive mitogen-activated protein kinases (MPK),[50] and the stress-responsive AtMPK3, along with AtMPK6 was shown to play a key role in regulating stomatal development and patterning in addition to its role in biotic and abiotic stress signaling. Interestingly, recent evidence suggests that density and development of stomata on the *Arabidopsis thaliana* leaves depend, in part, on the microRNA-mediated regulation of Agamous-like16 (AGL16) gene.[51] AGL16 is a member of the MADS box protein family, which mRNA is targeted for sequence-specific degradation by the microRNA miR824. Additionally several potential quantitative trait loci have been also identified in poplar[52,53] and thus it seems reasonable to assume that leaf traits may be robust enough to be developed as markers for accelerated breeding programs, offering another research approach to increase crop yield.

ABA Sensing and Signaling

ABA is involved in broad of physiological process, such as regulating seed germination and seedling growth and it is also essential in processes that lead to plant resistance upon biotic and abiotic stresses.[18] Under drought and high salinity conditions the levels of ABA increase in the roots which is perceived by the guard cells where it triggers changes in ion fluxes leading to stomata closure and inhibiting stomatal opening.[38,54,55] Altogether these effects minimize water loss and allow plants to cope with drought episodes.

Several efforts have been devoted to identifying ABA receptors in guard cells and the findings of candidates for ABA receptors have been reported. A candidate for ABA-binding protein (ABAR) was identified recently.[56] In this study the H subunit of Mg-chelatase (CHLH), which is a key component in both chlorophyll biosynthesis and plastid-to-nucleus signaling, was shown to bind ABA and function as a positive regulator in seed germination, post-germination growth and stomatal movement in Arabidopsis. Further analysis revealed that ABAR/CHLH is a ubiquitous protein, which indicates that it might be able to perceive the ABA signal at the whole-plant level.[56] In another study homologs for a G-protein coupled receptors (GPCRs), known as a conserved mechanism for the extracellular signal perception at the plasma membrane in eukaryotic organisms,[57] were found Arabidopsis plants. It has been shown that a G-protein coupled receptor 2 (GCR2) encoding a putative GPCR is localized in the plasma membrane and that it interacts with the G protein α-subunit GPA1 to mediate ABA responses. This protein receptor binds ABA with high affinity at physiological concentration.[58] However, it is important to mention that the characterization of GCR2 as a GPCR and ABA receptor is still a matter of discussion.[59-61] More recently, other GPCR-like proteins (GTG1 and GTG2) were identified and its characterization revealed that the GTG proteins represent a novel class of proteins with topology similar to GPCRs but with classic GTP-binding/GTPase activity.[62] Further analysis providing biochemical and phenotypic evidences suggest that GTG1 and GTG2 proteins are redundantly involved in G protein-coupled ABA signaling and are, or are parts of, ABA receptor complexes[62] and their detailed characterization will likely clarify the mechanism underlying the ABA response.

Additional studies to identify key players in ABA signaling pathway are ongoing. It includes the type 2C protein phosphatases (PP2Cs) ABI1 and ABI2, that act negatively regulating ABA responses.[63] In this study, interaction partner of ABI1 and ABI2, named as regulatory components of ABA receptor (RCARs) were identified. RCARs proteins belong to a family with 14 members in Arabidopsis and one of its members, RCAR1, was shown to bind ABA, to mediate ABA-dependent inactivation of ABI1 or ABI2 in vitro, and to antagonize PP2C action in plants.[63] Due to the possibility that ABA receptors may be molecular and functionally redundant or required for vital processes[64] chemical genetic approaches have been also used to identify ABA receptors in plants.[65] In this study, a synthetic growth inhibitor called pyrabactin that function as a selective ABA agonist and acts through PYRABACTIN RESISTANCE 1 (PYR1), a founding member of START protein family, called PYR/PYLs, is required for both pyrabactin and ABA signaling in vivo. It was shown that ABA binds to PYR1, which in turn binds to and inhibits PP2Cs protein. It is assumed that PYR/PYLs are ABA receptors functioning at the apex of a negative regulatory pathway that controls ABA signaling by inhibiting PP2Cs. In brief, these studies provide in vitro evidence that perception of ABA signaling by the PYR/PYL/RCAR proteins shuts down negative regulation of ABA signaling by PP2Cs.[1]

Cross Talk between ABA and the "Clock"

Several studies reported that ABA is regulated by the circadian clock[66,67] and ABA-related genes controlled by the clock have also

been reported in references 68–70. Evidence of a feedback loop between cADPR and the circadian oscillator suggests a mechanism by which the circadian coordination of ABA-transducing components might be involved in clock-mediated regulation of plant responses to ABA.[69] The influence of the clock is not limited to gene expression but is also evidenced in the circadian regulation of physiological processes controlled by ABA such as drought stress where ABA induces the closure of the stomatal pore, and this closure was found to be gated by the clock.[66,67] Evidence of a feedback mechanism that links circadian clock with plant responses to drought was recently provided.[67] In this study, TOC1 (timing of CAB expression 1), a key clock component binds to the promoter of the ABA-related gene (ABAR/CHLH/GUN5) and controls its circadian expression. In agreement with the importance of both ABA and its regulation by genes related to the clock pathway it is generally assumed that the circadian clock is able to confer an advantage to plants, but the nature of that advantage was until recently unresolved. However, recent reports have demonstrated the importance of circadian transcription translation loops for regulating C assimilation and starch metabolism for optimizing plant growth.[71,72] In addition, it seems that transported product(s) of photosynthesis, such as sugars, are important inputs for regulating circadian transcription translation loops.[73,74] This explains why plants gain advantage from circadian control.

Light Regulation of Stomatal Movement

Plants have evolved multiple photoreceptor systems to monitor light quality, quantity and direction. It is well known that stomatal morphogenesis is controlled by genetic as well as environmental factors and in general, an increase in light intensity results in an increase in stomatal index and a systemic signal from mature leaves determine the response.[43,75-78] Light is perceived by various photoreceptors,[79,80] and stomatal movements are regulated by both blue and red light.[14] The blue-light response of stomata appears to be strongly affected by red light, and the underlying mechanisms in the interaction between blue-light signaling and guard cell chloroplasts were recently reviewed in reference 14.

It is shown that blue light-induced stomatal opening is mediated by the blue light receptor phototropins (PHOT1 and PHOT2) and cryptochromes (CRY1 and CRY2).[81] Recent findings suggest that the light control of stomatal development is mediated through a crosstalk between the cryptochrome-phytochrome-COP1 signaling system and the mitogen-activated protein kinase signaling pathway.[82] Blue light is required for the activation of phototropins, plant-specific Ser/Thr autophosphorylating kinases, and the activated phototropins transmit the signal to the plasma membrane H^+-ATPase for its activation.[80,83] Activation of the H^+-ATPase is caused by the phosphorylation of a Thr residue in the C terminus with subsequent binding of a 14-3-3 protein to the Thr residue.[84,85] Since phototropins are Ser/Thr protein kinases, it might be possible that phototropins directly phosphorylate the H^+-ATPase. However, this has been shown not to be the case since it was demonstrated that protein phosphatase 1 (PP1), a major member of the PPP family of Ser/Thr protein phosphatases, mediates the signaling between phototropins and H^+-ATPase in guard cells.[86] Therefore, ABA is likely to inhibit the signaling molecule(s), including phototropins, PP1, H^+-ATPase and other unidentified components.

It has also been suggested that the guard cell response to red light is in part an indirect response to red-light-driven intercellular CO_2 uptake in the mesophyll.[87] For example, it has been shown that chloroplast-containing guard cells in albino sections of variegated leaves do not respond to photosynthetically active radiation, but are sensitive to blue light and CO_2, bringing into question a direct role of guard cell photosynthesis on red-light-mediated stomatal opening in intact leaves.[35]

On the Role of Organic Acids as a Component of the Stomata Signaling Cascade

The importance of organic acids such as malate and fumarate on the regulation of stomatal aperture has been recently addressed.[37,88,89] Nevertheless the molecular regulatory hierarchy underlying this highly specialized cell type is as yet not fully understood and is discussed bellow.

Malate has long been discussed as an important regulator of stomatal opening and is thought to be an integral part of the mechanism by which guard cells adjust their action in response to external CO_2 concentrations.[30,34,36,87,90] Recently a plasma membrane ABC malate uptake transporter was identified in guard cells. The gene encoding the ABC transporter family member ABCB14 (ABC transporter B family member 14), was identified and characterized in Arabidopsis plants.[22] This study demonstrated that AtABCB14 protein modulates the stomatal closure on transition to higher atmospheric CO_2 concentration environment. High CO_2 levels accelerates stomatal closure in plants lacking AtABCB14 protein and exogenously applied malate mimics the high CO_2 levels responses in AtABCB14 protein deficient plants. Thus it represents a negative regulator of CO_2-induced stomatal closure in Arabidopsis plants and plays its function by transporting malate from apoplast into guard cells elevating the osmotic pressure. It is however clear that many other components of the CO_2 signal transduction pathway still needs to be identified.

Additional evidence for the role of organic acids in the regulation of stomatal movement has been recently provided. The antisense inhibition of the iron-sulphur subunit of succinate dehydrogenase (SDH) in tomato plants resulted in an increased photosynthesis (25%) and biomass at the whole plant level via an organic acid-mediated effect on stomatal aperture.[37] Furthermore, measurement of apoplastic and protoplastic organic acid concentrations in these transformants, and also in transformants with deficient stomatal efficiency functioning,[91] revealed a negative correlation between the concentrations of fumarate and gas exchange through the stomata. Altogether, these information alongside that recently obtained for *At*ABCB[22] and potentially also SLAC1,[23,38] coupled with the transport of malate by the vacuolar aluminum-activated malate transporter (ALMT6),[92] provide

strong evidence to support that modulation of guard cell malate and fumarate concentration can greatly influence stomatal function. It has also been shown that fumarate is present mainly in photosynthetically active tissue, its content rises with plant age and light intensity with the total fumarase activity remains constant throughout the diurnal cycle[93] as well as is present in high levels in phloem exudates[94] and these results are consistent with the observation that fumarate has similar effects as malate during stomatal closure.[22] Given that malate is physiologically present in the apoplast at higher concentrations, it would seem likely that it exerts greater influence on stomatal aperture than fumarate does in vivo. When considered alongside the recent identification of transporters which import malate (or fumarate), into the guard cell,[22] or have been assumed to export it,[23,38] these studies thus provide a mechanism by which these organic acids can influence stomatal function. Moreover they provide strong support of the theory of Mott (2009) that guard cells are not autonomously regulated and strongly support the contention of Mott and others that the mesophyll harbors significant control over guard cell function.[6,22,89]

Despite the fact that these data clearly document the importance of organic acids in guard cell function, the experiments discussed thus far do not, in their own right, rule out interaction with other well characterized mechanisms of guard cell regulation such as those controlled by ABA, potassium or calcium.[11,12,28,95,96]

Most plant species (except onion, which does not accumulate starch in guard cells) accumulate malate preferentially over other anions. A close correlation has been obtained between stomatal opening and malate accumulation in guard cells.[27,37] It is well known that malate can be produced through the degradation of starch in guard cells under blue light.[14] Interestingly, the opening of stomata by blue light was severely impaired in Arabidopsis phosphoglucomutase mutant plants, which did not accumulate starch in guard cell chloroplasts.[97] However, this blue-light response was restored in the presence of high concentrations of Cl^-, possibly because Cl^- replaced malate as a necessary counterion for K^+ highlighting the complexity of the mechanisms responsible for the stomatal control.

Conclusion and Future Perspective

Stomatal opening is induced by low CO_2 concentrations, high light intensity and high humidity. By contrast closing is promoted by high CO_2 concentrations, darkness, drought and the plant hormone ABA. It is evident that significant progress has been made towards the elucidation of the cellular and molecular processes involved in stomatal patterning and aperture. Nevertheless it is clear that further experimentation is required to tease out the molecular hierarchy shared between the mechanisms involved in stomatal movement. It seems likely that the relative importance of each regulatory mechanism will vary with circumstances; however, a fuller understanding of this is surely required to engineer sustainable increases in crop yield. Finally, it is interesting to note that although neither malate nor fumarate exert their effects on stomata by affecting ABA, the phytohormone could, conditionally, act upstream of the organic acids, given that a recent study in Arabidopsis revealed the SDH2-3 gene to be upregulated by ABA.[98] It will be important to establish the functional significance of this observation in future studies. Further genetic, molecular biological and biochemical analyses will be required to identify other components of the signal transduction pathway(s) and the corresponding effectors that are involved in the stomatal responses in order to obtain a deeper understanding of the biochemical mechanisms and the precise factors and environmental cues underlying this phenomenon. Finally, from an applied perspective and since water is likely to be at premium in the coming decades[99] it will be interesting to ascertain whether the increased yield observed in SDH antisense plants can also be obtained under conditions of supra-optimal water supply.

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References

1. Kim TH, Böhmer M, Hu H, Nishimura N, Schroeder JI. Guard cell signal transduction network: advances in understanding abscisic acid, CO_2 and Ca^{2+} signaling. Annu Rev Plant Biol 2010; 61:561-91.
2. Sirichandra C, Wasilewska A, Vlad F, Valon C, Leung J. The guard cell as a single-cell model towards understanding drought tolerance and abscisic acid action. J Exp Bot 2009; 60:1439-63.
3. Hetherington AM, Woodward FI. The role of stomata in sensing and driving environmental change. Nature 2003; 424:901-8.
4. Gray J. Guard cells: transcription factors regulate stomatal movements. Curr Biol 2005; 15:593-5.
5. Berry JA, Beerling DJ, Franks PJ. Stomata: key players in the earth system, past and present. Curr Opin Plant Biol 2010; 13:232-9.
6. Mott KA. Opinion: Stomatal responses to light and CO_2 depend on the mesophyll. Plant Cell Environ 2009; 32:1479-86.
7. Bergmann DC, Sack FD. Stomatal development. Annu Rev Plant Biol 2007; 58:163-81.
8. Casson SA, Hetherington AM. Environmental regulation of stomatal development. Curr Opin Plant Biol 2010; 13:90-5.
9. Martin C, Bhatt K, Baumann K. Shaping in plant cells. Curr Opin Plant Biol 2001; 4:540-9.
10. Fernie AR. The future of metabolic phytochemistry: larger numbers of metabolites, higher resolution, greater understanding. Phytochemistry 2007; 68:2861-80.
11. Israelsson M, Siegel RS, Young J, Hashimoto M, Iba K, Schroeder JI. Guard cell ABA and CO_2 signaling network updates and Ca^{2+} sensor priming hypothesis. Curr Opin Plant Biol 2006; 9:654-63.
12. Hetherington AM, Brownlee C. The generation of Ca^{2+} signals in plants. Annu Rev Plant Biol 2004; 55:401-27.
13. Brodribb TJ, McAdam SAM. Passive origins of stomatal control in vascular plants. Science 2011; 331:582-5.
14. Shimazaki Ki, Doi M, Assmann SM, Kinoshita T. Light regulation of stomatal movement. Annu Rev Plant Biol 2007; 58:219-47.
15. Mott KA, Sibbernsen ED, Shope JC. The role of the mesophyll in stomatal responses to light and CO_2. Plant Cell Environ 2008; 31:1299-306.
16. Fan LM, Zhao Z, Assmann SM. Guard cells: a dynamic signaling model. Curr Opin Plant Biol 2004; 7:537-46.
17. Acharya B, Assmann S. Hormone interactions in stomatal function. Plant Mol Biol 2009; 69:451-62.
18. Raghavendra AS, Gonugunta VK, Christmann A, Grill E. ABA perception and signaling. Trends Plant Sci 2010; 15:395-401.
19. Jones RJ, Mansfield TA. Increases in the diffusion resistances of leaves in a carbon dioxide-enriched atmosphere. J Exp Bot 1970; 21:951-8.
20. Mott KA. Do stomata respond to CO_2 concentrations other than intercellular. Plant Physiol 1988; 86:200-3.
21. Mott KA. Sensing of atmospheric CO_2 by plants. Plant Cell and Environ 1990; 13:731-7.
22. Lee M, Choi Y, Burla B, Kim YY, Jeon B, Maeshima M, et al. The ABC transporter AtABCB14 is a malate importer and modulates stomatal response to CO_2. Nat Cell Biol 2008; 10:1217-23.
23. Negi J, Matsuda O, Nagasawa T, Oba Y, Takahashi H, Kawai-Yamada M, et al. CO_2 regulator SLAC1 and its homologues are essential for anion homeostasis in plant cells. Nature 2008; 452:483-6.
24. Hu H, Boisson-Dernier A, Israelsson-Nordstrom M, Bohmer M, Xue S, Ries A, et al. Carbonic anhydrases are upstream regulators of CO_2-controlled stomatal movements in guard cells. Nat Cell Biol 2010; 12:87-93.

25. Hashimoto M, Negi J, Young J, Israelsson M, Schroeder JI, Iba K. Arabidopsis HT1 kinase controls stomatal movements in response to CO_2. Nat Cell Biol 2006; 8:391-7.
26. Young JJ, Mehta S, Israelsson M, Godoski J, Grill E, Schroeder JI. CO_2 signaling in guard cells: Calcium sensitivity response modulation, a Ca^{2+}-independent phase and CO_2 insensitivity of the gca2 mutant. Proc Natl Acad Sci USA 2006; 103:7506-11.
27. Vavasseur A, Raghavendra AS. Guard cell metabolism and CO_2 sensing. New Phytol 2005; 165:665-82.
28. Assmann SM. The cellular basis of guard cell sensing of rising CO_2. Plant Cel Environ 1999; 22:629-37.
29. Brearley J, Venis MA, Blatt MR. The effect of elevated CO_2 concentrations on K^+ and anion channels of *Vicia faba* L. guard cells. Planta 1997; 203:145-54.
30. Raschke K. Alternation of the slow with the quick anion conductance in whole guard cells effected by external malate. Planta 2003; 217:651-7.
31. Hanstein SM, Felle HH. CO_2-triggered chloride release from guard cells in intact fava bean leaves. Kinetics of the onset of stomatal closure. Plant Physiol 2002; 130:940-50.
32. Schwartz A, Ilan N, Grantz DA. Calcium effects on stomatal movement in *Commelina communis* L.: use of EGTA to modulate stomatal response to light, KCl and CO_2. Plant Physiol 1988; 87:583-7.
33. Webb AAR, McAinsh MR, Mansfield TA, Hetherington AM. Carbon dioxide induces increases in guard cell cytosolic free calcium. Plant J 1996; 9:297-304.
34. Hedrich R, Marten I, Lohse G, Dietrich D, Winter H, Lohaus G, et al. Malate-sensitive anion channels enable guard-cells to sense changes in the ambient CO_2 concentration. Plant J 1994; 6:741-8.
35. Roelfsema MRG, Konrad KR, Marten H, Psaras GK, Hartung W, Hedrich R. Guard cells in albino leaf patches do not respond to photosynthetically active radiation, but are sensitive to blue light, CO_2 and abscisic acid. Plant Cell and Environ 2006; 29:1595-605.
36. Hedrich R, Marten I. Malate-induced feedback regulation of plasma membrane anion channels could provide a CO_2 sensor to guard cells. EMBO J 1993; 12:897-901.
37. Araujo WL, Nunes-Nesi A, Osorio S, Usadel B, Fuentes D, Nagy R, et al. Antisense inhibition of the iron-sulphur subunit of succinate dehydrogenase enhances photosynthesis and growth in tomato via an organic acid-mediated effect on stomatal aperture. Plant Cell 2011; 23:600-27.
38. Vahisalu T, Kollist H, Wang YF, Nishimura N, Chan WY, Valerio G, et al. SLAC1 is required for plant guard cell S-type anion channel function in stomatal signaling. Nature 2008; 452:487-91.
39. Geiger D, Scherzer S, Mumm P, Stange A, Marten I, Bauer H, et al. Activity of guard cell anion channel SLAC1 is controlled by drought-stress signaling kinase-phosphatase pair. Proc Natl Acad Sci USA 2009; 106:21425-30..
40. Schmidt C, Schroeder JI. Anion selectivity of slow anion channels in the plasma membrane of guard cells (large nitrate permeability). Plant Physiol 1994; 106:383-91.
41. Emmerlich V, Linka N, Reinhold T, Hurth MA, Traub M, Martinoia E, et al. The plant homolog to the human sodium/dicarboxylic cotransporter is the vacuolar malate carrier. Proc Natl Acad Sci USA 2003; 100:11122-6.
42. Meyer S, Mumm P, Imes D, Endler A, Weder B, Al-Rasheid KAS, et al. AtALMT12 represents an R-type anion channel required for stomatal movement in Arabidopsis guard cells. Plant J 2010; 63:1054-62.
43. Lake JA, Quick WP, Beerling DJ, Woodward FI. Plant development: Signals from mature to new leaves. Nature 2001; 411:154.
44. Franks PJ, Beerling DJ. Maximum leaf conductance driven by CO_2 effects on stomatal size and density over geologic time. Proc Natl Acad Sci USA 2009; 106:10343-7.
45. Casson SA, Gray JE. Influence of environmental factors on stomatal development. New Phytol 2008; 178:9-23.
46. Woodward FI, Kelly CK. The influence of CO_2 concentration on stomatal density. New Phytol 1995; 131:311-27.
47. Woodward FI, Bazzaz FA. The responses of stomatal density to carbon dioxide partial pressure. J Exp Bot 1988; 39:1771-82.
48. McElwain JC, Chaloner WG. Stomatal density and index in fossil plants track atmospheric carbon dioxide in the palaeozoic. Ann Bot 1995; 76:389-95.
49. Gray JE, Holroyd GH, van der Lee FM, Bahrami AR, Sijmons PC, Woodward FI, et al. The HIC signaling pathway links CO_2 perception to stomatal development. Nature 2000; 408:713-6.
50. Wang H, Ngwenyama N, Liu Y, Walker JC, Zhang S. Stomatal development and patterning are regulated by environmentally responsive mitogen-activated protein kinases in Arabidopsis. Plant Cell 2007; 19:63-73.
51. Kutter C, Schöb H, Stadler M, Meins F, Si-Ammour A. MicroRNA-mediated regulation of stomatal development in Arabidopsis. Plant Cell 2007; 19:2417-29.
52. Ferris R, Long L, Bunn SM, Robinson KM, Bradshaw HD, Rae AM, et al. Leaf stomatal and epidermal cell development: identification of putative quantitative trait loci in relation to elevated carbon dioxide concentration in poplar. Tree Physiol 2002; 22:633-40.
53. Rae AM, Ferris R, Tallis MJ, Taylor G. Elucidating genomic regions determining enhanced leaf growth and delayed senescence in elevated CO_2. Plant Cell Environ 2006; 29:1730-41.
54. Levchenko V, Konrad KR, Dietrich P, Roelfsema MRG, Hedrich R. Cytosolic abscisic acid activates guard cell anion channels without preceding Ca^{2+} signals. Proc Natl Acad Sci USA 2005; 102:4203-8.
55. Siegel RS, Xue S, Murata Y, Yang Y, Nishimura N, Wang A, et al. Calcium elevation-dependent and attenuated resting calcium-dependent abscisic acid induction of stomatal closure and abscisic acid-induced enhancement of calcium sensitivities of S-type anion and inward-rectifying K^+ channels in Arabidopsis guard cells. Plant J 2009; 59:207-20.
56. Shen YY, Wang XF, Wu FQ, Du SY, Cao Z, Shang Y, et al. The Mg-chelatase H subunit is an abscisic acid receptor. Nature 2006; 443:823-6.
57. Pierce KL, Premont RT, Lefkowitz RJ. Seven-transmembrane receptors. Nat Rev Mol Cell Biol 2002; 3:639-50.
58. Liu X, Yue Y, Li B, Nie Y, Li W, Wu WH, et al. A G Protein-coupled receptor is a plasma membrane receptor for the plant hormone abscisic acid. Science 2007; 315:1712-6.
59. Illingworth CJR, Parkes KE, Snell CR, Mullineaux PM, Reynolds CA. Criteria for confirming sequence periodicity identified by Fourier transform analysis: Application to GCR2, a candidate plant GPCR? Biophys Chem 2008; 133:28-35.
60. Guo J, Zeng Q, Emami M, Ellis BE, Chen JG. The *GCR2* gene family is not required for ABA control of seed germination and early seedling development in Arabidopsis. PLoS One 2008; 3:2982.
61. Gao YJ, Zeng QN, Guo JJ, Cheng J, Ellis BE, Chen JG. Genetic characterization reveals no role for the reported ABA receptor, GCR2, in ABA control of seed germination and early seedling development in Arabidopsis. Plant J 2007; 52:1001-13.
62. Pandey S, Nelson DC, Assmann SM. Two Novel GPCR-Type G proteins are abscisic acid receptors in Arabidopsis. Cell 2009; 136:136-48.
63. Ma Y, Szostkiewicz I, Korte A, Moes Dl, Yang Y, Christmann A, et al. Regulators of PP2C phosphatase activity function as abscisic acid sensors. Science 2009; 324:1064-8.
64. McCourt P, Creelman R. The ABA receptors—we report you decide. Curr Opin Plant Biol 2008; 11:474-8.
65. Park SY, Fung P, Nishimura N, Jensen DR, Fujii H, Zhao Y, et al. Abscisic Acid Inhibits Type 2C Protein Phosphatases via the PYR/PYL Family of START Proteins. Science 2009; 324:1068-71.
66. Robertson F, Skeffington A, Gardner M, Webb A. Interactions between circadian and hormonal signaling in plants. Plant Mol Biol 2009; 69:419-27.
67. Legnaioli T, Cuevas J, Mas P. TOC1 functions as a molecular switch connecting the circadian clock with plant responses to drought. EMBO J 2009; 28:3745-57.
68. Covington MF, Harmer SL. The circadian clock regulates auxin signaling and responses in Arabidopsis. PLoS Biol 2007; 5:222.
69. Dodd AN, Gardner MJ, Hotta CT, Hubbard KE, Dalchau N, Love J, et al. The Arabidopsis circadian clock incorporates a cADPR-based feedback loop. Science 2007; 318:1789-92.
70. Mizuno T, Yamashino T. Comparative transcriptome of diurnally oscillating genes and hormone-responsive genes in *Arabidopsis thaliana*: Insight into circadian clock-controlled daily responses to common ambient stresses in plants. Plant Cell Physiol 2008; 49:481-7.
71. Graf A, Schlereth A, Stitt M, Smith AM. Circadian control of carbohydrate availability for growth in Arabidopsis plants at night. Proc Natl Acad Sci USA 2010; 107:9458-63.
72. Dodd AN, Salathia N, Hall A, Kévei E, Tóth Rk, Nagy F, et al. Plant circadian clocks increase photosynthesis, growth, survival and competitive advantage. Science 2005; 309:630-3.
73. Bläsing OE, Gibon Y, Günther M, Höhne M, Morcuende R, Osuna D, et al. Sugars and circadian regulation make major contributions to the global regulation of diurnal gene expression in Arabidopsis. Plant Cell 2005; 17:3257-81.
74. James AB, Monreal JA, Nimmo GA, Kelly CnL, Herzyk P, Jenkins GI, et al. The circadian clock in Arabidopsis roots is a simplified slave version of the clock in shoots. Science 2008; 322:1832-5.
75. Gay AP, Hurd RG. The influence of light on stomatal density in the tomato. New Phytol 1975; 75:37-46.
76. Schoch PG, Zinsou C, Sibi M. Dependence of the stomatal index on environmental factors during stomatal differentiation in Leaves of *Vigna sinensis* L. J Exp Bot 1980; 31:1211-6.
77. Thomas PW, Woodward FI, Quick WP. Systemic irradiance signaling in tobacco. New Phytol 2004; 161:193-8.
78. Coupe SA, Palmer BG, Lake JA, Overy SA, Oxborough K, Woodward FI, et al. Systemic signaling of environmental cues in Arabidopsis leaves. J Exp Bot 2006; 57:329-41.
79. Franklin KA, Larner VS, Whitelam GC. The signal transducing photoreceptors of plants. Int J Dev Biol 2005; 49:653-64.
80. Christie JM. Phototropin blue-light receptors. Annu Rev Plant Biol 2007; 58:21-45.
81. Mao J, Zhang YC, Sang Y, Li QH, Yang HQ. A role for Arabidopsis cryptochromes and COP1 in the regulation of stomatal opening. Proc Natl Acad Sci USA 2005; 102:12270-5.
82. Kang CY, Lian HL, Wang FF, Huang JR, Yang HQ. Cryptochromes, phytochromes and COP1 regulate light-controlled stomatal development in Arabidopsis. Plant Cell 2009; 21:2624-41.
83. Kinoshita T, Doi M, Suetsugu N, Kagawa T, Wada M, Shimazaki K-i. phot1 and phot2 mediate blue light regulation of stomatal opening. Nature 2001; 414:656-60.
84. Kinoshita T, Shimazaki K-i. Blue light activates the plasma membrane H^+-ATPase by phosphorylation of the C-terminus in stomatal guard cells. EMBO J 1999; 18:5548-58.
85. Emi T, Kinoshita T, Shimazaki K-i. Specific binding of vf14-3-3a isoform to the plasma membrane H^+-ATPase in response to blue light and fusicoccin in guard cells of broad bean. Plant Physiol 2001; 125:1115-25.

86. Takemiya A, Kinoshita T, Asanuma M, Shimazaki Ki. Protein phosphatase 1 positively regulates stomatal opening in response to blue light in *Vicia faba*. Proc Natl Acad Sci USA 2006; 103:13549-54.
87. Roelfsema MRG, Hanstein S, Felle HH, Hedrich R. CO_2 provides an intermediate link in the red light response of guard cells. Plant J 2002; 32:65-75.
88. Meyer S, De Angeli A, Fernie AR, Martinoia E. Intra- and extra-cellular excretion of carboxylates. Trends Plant Sci 2010; 15:40-7.
89. Fernie AR, Martinoia E. Malate. Jack of all trades or master of a few? Phytochemistry 2009; 70:828-32.
90. Van Kirk CA, Raschke K. Release of malate from epidermal strips during stomatal closure. Plant Physiol 1978; 61:474-5.
91. Nunes-Nesi A, Carrari F, Gibon Y, Sulpice R, Lytovchenko A, Fisahn J, et al. Deficiency of mitochondrial fumarase activity in tomato plants impairs photosynthesis via an effect on stomatal function. Plant J 2007; 50:1093-106.
92. Meyer S, Scholz-Starke J, De Angeli A, Kovermann P, Burla B, Gambale F, et al. Malate transport by the vacuolar AtALMT6 channel in guard cells is subject to multiple regulation. Plant J 2011; 67:247-57. DOI: 10.1111/j.365-313X.2011.04587.x.
93. Gibon Y, Bläsing OE, Palacios-Rojas N, Pankovic D, Hendriks JHM, Fisahn J, et al. Adjustment of diurnal starch turnover to short days: depletion of sugar during the night leads to a temporary inhibition of carbohydrate utilization, accumulation of sugars and post-translational activation of ADP-glucose pyrophosphorylase in the following light period. Plant J 2004; 39:847-62.
94. Chia DW, Yoder TJ, Reiter WD, Gibson SI. Fumaric acid: an overlooked form of fixed carbon in Arabidopsis and other plant species. Planta 2000; 211:743-51.
95. Mori IC, Murata Y, Yang Y, Munemasa S, Wang YF, Andreoli S, et al. CDPKs CPK6 and CPK3 function in ABA regulation of guard cell S-Type anion- and Ca^{2+}-permeable channels and stomatal closure. Plos Biol 2006; 4:327.
96. Kwak JM, Mäser P, Schroeder JI. The clickable guard cell, version II: Interactive model of guard cell signal transduction mechanisms and pathways. Chang C, Graham I, Last R, McClung M, Weinig C, Eds. The Arabidopsis Book. American Society of Plant Biologists 2008; 1-17.
97. Lascève G, Leymarie J, Vavasseur A. Alterations in light-induced stomatal opening in a starch-deficient mutant of *Arabidopsis thaliana* L. deficient in chloroplast phosphoglucomutase activity. Plant Cell Environ 1997; 20:350-8.
98. Roschzttardtz H, Fuentes I, Vasquez M, Corvalan C, Leon G, Gomez I, et al. A nuclear gene encoding the iron-sulfur subunit of mitochondrial complex II is regulated by B3 Domain Transcription Factors during seed eevelopment in Arabidopsis. Plant Physiol 2009; 150:84-95.
99. Cominelli E, Galbiati M, Tonelli C, Bowler C. Water: the invisible problem. EMBO Rep 2009; 10:671-6.