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Insights into the Molecular Mechanisms of CO_2-Mediated Regulation of Stomatal Movements

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Plants must continually balance the influx of CO_2 for photosynthesis against the loss of water vapor through stomatal pores in their leaves. This balance can be achieved by controlling the aperture of the stomatal pores in response to several environmental stimuli. Elevation in atmospheric [CO_2] induces stomatal closure and further impacts leaf temperatures, plant growth and water-use efficiency, and global crop productivity. Here, we review recent advances in understanding CO_2-perception mechanisms and CO_2-mediated signal transduction in the regulation of stomatal movements, and we explore how these mechanisms are integrated with other signaling pathways in guard cells.

Introduction

Historically, the Earth's atmospheric CO_2 concentrations [CO_2] remained below 300 parts per million for the better part of the last 800,000 years. However, since the industrial revolution, atmospheric [CO_2] has been rapidly rising, and is presently near ~410 parts per million (https://scripps.ucsd.edu/programs/keelingcurve/). Considerably higher [CO_2] is predicted to have occurred during the Cambrian period about 500 million years ago, but many of today's organisms would not survive these conditions. The present and ongoing elevation in [CO_2] not only causes global warming, but also affects the physiology and development of plants. On the leaf surface of vascular plants, pores called 'stomata' mediate the exchange of gases between the atmosphere and the intracellular spaces of leaves. These pores are formed by pairs of guard cells (Figure 1) that increase and reduce their turgor pressure to control the pore aperture. In the light, photosynthesis causes reduction in the [CO_2] in the intercellular space of leaves (C_i). Low C_i is a signal that causes stomatal opening, increasing influx of CO_2 for further assimilation. In contrast, C_i rises rapidly in darkness, triggering closure of stomatal pores in C3 and C4 plants (Figure 1). Stomatal opening and closing optimally balance CO_2 uptake for photosynthesis and water loss by regulating gas exchange (Figure 1). The ongoing rise in atmospheric [CO_2] further causes an increase in the [CO_2] inside leaves, resulting in a narrowing of stomatal pores globally, even during light periods. Moreover, the continued elevation in [CO_2] negatively regulates stomatal development by decreasing stomatal density in the leaf epidermis of many plant species [1,2].

Reduction of stomatal apertures and density by the continued elevation in atmospheric [CO_2] can reduce gas exchange and increase intrinsic water-use efficiency and plant leaf temperatures, and is predicted to affect crop yields [3,4]. It may be expected that such elevated [CO_2] could increase plant biomass. However, due to the abiotic stresses linked to climate change, including drought or limited soil nutrients, some studies have shown that elevated [CO_2] does not necessarily increase crop yields [4,5]. Investigation of the mechanisms by which CO_2 regulates stomatal movements and how guard cells and leaves sense and respond to changes in [CO_2] will aid in the understanding of how plants are currently processing the increasing CO_2 levels. Insights from such studies can further lead to new approaches for engineering crop plants to adapt to climate change. Advances in understanding CO_2-mediated control of stomatal development have been reviewed recently [1,2] and are not covered here. Rather, in this review we focus on recent advances that are illuminating the CO_2-sensing and signal-transduction mechanisms that control stomatal movements, and we describe questions that should be addressed. Table 1 lists genes and mutants with reported functions in CO_2-mediated regulation of stomatal movements.

CO_2 Signal Perception: The Role of Guard Cells and Mesophyll Cells

The existing literature indicates that both guard cells, which are located within the leaf epidermis, as well as the mesophyll cells in the inner space of leaves possess the capacity to respond to CO_2. These observations led to the present working hypothesis that both cell types contribute to stomatal CO_2 responses [2,6]. We review CO_2-sensory mechanisms in these two cell types separately.

Guard-Cell CO_2-Response Mechanisms

The existence of [CO_2]-responsive sensory mechanisms within guard cells has been documented by studies using isolated guard-cell protoplasts and leaf epidermis that were shown to respond to [CO_2] changes ([2] and references therein). Several genes have been identified and characterized that function in *Arabidopsis* guard cells in CO_2-mediated control of stomatal movements (Table 1 and Figure 2); these encode proteins including the carbonic anhydrases βCA1 and βCA4, the protein kinases MPK12 [7,8], MPK4 [9,10], HT1 [11], CBC1 and CBC2 [12], OST1 [13,14] and GHR1 [7,15], the S-type and R-type anion

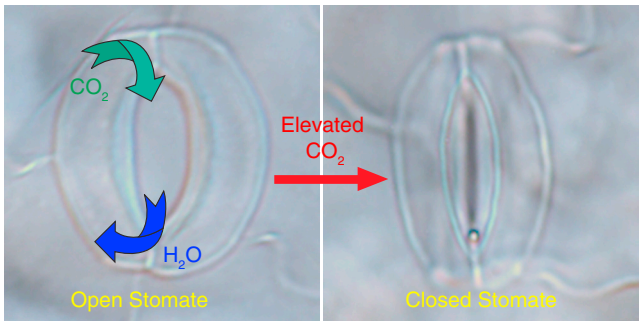

Figure 1. Closing of stomata by elevated CO_2.
Plants control CO_2 exchange and water loss to the atmosphere in response to endogenous and environmental stimuli via stomatal pores, the size of which are controlled by surrounding guard cells via changes in turgor pressure. When the CO_2 concentration within the intracellular spaces of leaves (C_i) rises, a signal transduction network is triggered in both the guard cells and the underlying mesophyll (not shown in the image) that mediates stomatal closure, resulting in reduced water evaporation from leaves.

channels AtSLAC1 and AtALMT12/QUAC1 [16–19], and a MATE-type transporter RHC1 [20]. However, the guard-cell CO_2 sensors remain unknown.

In *Arabidopsis* guard cells, carbonic anhydrases accelerate the catalysis of CO_2 molecules to bicarbonate (HCO_3^-) and protons. Disruption of two carbonic anhydrase genes, *βCA1* and *βCA4*, causes slowing of the stomatal CO_2 response in *Arabidopsis* [21]. Similarly, disruption of rice and maize carbonic anhydrase genes has recently been shown to slow CO_2 control of stomatal movements [22,23]. Moreover, expression of a structurally unrelated human carbonic anhydrase, αCAII, in *Arabidopsis ca1ca4* double-mutant guard cells can restore the wild-type CO_2 response [21], suggesting that βCA1 and βCA4 mediate the stomatal CO_2 response via their catalytic carbonic-anhydrase activity. Combined yeast-split-ubiquitin screening, *in vitro* co-immunoprecipitation, bimolecular fluorescence complementation, and split-luciferase analyses *in planta* showed that βCA4 interacts with the plasma membrane intrinsic protein 2-1 (PIP2;1 aquaporin), and this interaction is proposed to facilitate CO_2 influx and catalysis in guard cells [24] (Figure 2). The *Arabidopsis* genome contains 6 βCAs and 13 PIP genes. An intact CO_2 response in *pip2;1* single mutants [24] supports the notion that redundancy among PIP2s is likely in *Arabidopsis.*

Studies have suggested that intracellular HCO_3^- ions activate S-type anion channels [14,20], which mediate Cl^- and NO_3^- efflux across the guard-cell plasma membrane and function in stomatal closing [16,17]. Moreover, direct microinjection of HCO_3^- enables an enhancement of SLAC1-mediated anion-channel currents in *Xenopus* oocytes [24], leading to the hypothesis that intracellular HCO_3^- might directly regulate SLAC1 channel activity. Furthermore, the SLAC1 transmembrane region has been shown to function in the stomatal CO_2 response [25]. These studies suggest that the S-type anion channel SLAC1 might function as one of the CO_2/HCO_3^- sensors in plant guard cells. Investigation of this hypothesis by accelerated molecular dynamics modeling, testing of SLAC1 mutants *in vitro*, and expression of an HCO_3^--insensitive isoform of SLAC1 in guard cells supports the model that SLAC1 itself can function as a direct secondary HCO_3^- sensor in CO_2 control of stomatal movements [26]. Based on these findings, a model of guard-cell CO_2 sensing can be proposed in which CO_2/HCO_3^- are sensed by two mechanisms operating in parallel: in the first, the CO_2/HCO_3^- signal is perceived, leading to activation of upstream protein kinases required for activation of S-type anion channels in guard cells (as discussed later), and in the second, intracellular HCO_3^- ions directly enhance SLAC1 channel activity.

Effects of Mesophyll on the CO_2 Response

In addition to the above-described signal transduction mechanisms within guard cells (Figure 2), several studies support a role of mesophyll cells in stomatal conductance regulation in response to [CO_2] changes [6,27]. These studies showed that the stomatal response to CO_2 in isolated leaf epidermis was significantly less pronounced than that observed when mesophyll tissues were placed back onto the epidermis. Experiments with placing various sized cellophane and polyethylene spacers between the epidermis and mesophyll [27] supported the existence of a mesophyll-derived signal that diffuses towards the epidermis to regulate stomatal aperture. These signals have been suggested to be of an aqueous (water soluble) nature or, alternatively, of a vapor phase (gaseous) nature [27]. Several gaseous plant molecules have been suggested to be involved in the regulation of stomatal conductance, including methyl jasmonate [28], reactive oxygen species, nitric oxide [29] and hydrogen sulfide [30]. However, water-soluble molecules have also been shown to control stomatal conductance via the apoplast, the aqueous phase of the cell wall space, including malate and sucrose [31]. Furthermore, apoplastic malate has been shown to regulate voltage-dependent properties of R-type anion channels in the guard-cell plasma membrane [32], suggesting a potential role of apoplastic malate in CO_2 signal transduction from mesophyll to guard cells [31,32]. Whether and which of these molecules may originate from the mesophyll in response to [CO_2] changes remains unknown and requires investigation. Mutations in proteins that function in mesophyll cells for the stomatal CO_2 response need to be identified and will aid in understanding the underlying role of mesophyll cells in CO_2 regulation of stomatal movements. Furthermore, the relative contributions of guard cells and mesophyll cells to the CO_2 response remain to be determined, and this will require the genetic identification of CO_2-insensitive mutations in both cell types.

CO_2 Signal Transduction in Guard Cells

Guard-cell photosynthesis conducted by guard-cell chloroplasts does not directly control CO_2-induced stomatal closing [2,33]. As described above, the upstream mediators of CO_2-controlled stomatal movements involve carbonic anhydrases [24] that catalyze the conversion of CO_2 into protons and HCO_3^-. Research has shown that physiological [CO_2] shifts do not result in measurable pH shifts in the cytoplasm of guard cells (for example [14]). Patch-clamp analyses at defined free CO_2, HCO_3^-, and proton concentrations suggest that HCO_3^- acts as an intracellular signaling molecule in CO_2 signal transduction [14].

Recently it was demonstrated that, downstream of CO_2, the mitogen activated protein kinases MPK12 and MPK4 form a node that is essential for the stomatal response to changes in [CO_2] [7–9]. The importance of MPK4 for CO_2-induced stomatal regulation was initially found in research that showed that silencing of MPK4 in tobacco plants resulted in impaired

Table 1. Components reported to control stomatal aperture in response to CO_2-mediated signaling.

Protein name	Locus ID	Protein function	Phenotype in response to [CO_2] change	Reference
Protein kinases				
HT1 (HIGH LEAF TEMPERATURE 1)	AT1G62400	Protein kinase	Impaired stomatal opening response, leaf temperature elevated	[11]
[a]NtMPK4 (MITOGEN-ACTIVATED PROTEIN KINASE 4)	LOC107794128	Mitogen-activated protein kinase	Impaired stomatal closure	[10]
OST1 (OPEN STOMATA 1)	AT4G33950	SnRK2 kinase	Impaired stomatal closure, lower leaf temperature	[14]
MPK12 (MITOGEN-ACTIVATED PROTEIN KINASE 12)	AT2G46070	Mitogen-activated protein kinase	Altered stomatal response kinetics	[8]
CBC1 (CONVERGENCE OF BLUE LIGHT AND CO_2 1)	AT3G01490	Protein kinase superfamily protein	Altered stomatal response to CO_2 (*cbc1 cbc2* double mutant exhibits impaired stomatal opening response)	[12]
CBC2 (CONVERGENCE OF BLUE LIGHT AND CO_2 2)	AT5G50000	Protein kinase superfamily protein	Altered stomatal response to CO_2 (*cbc1 cbc2* double mutant exhibits impaired stomatal opening response)	[12]
KIN7 (leucine-rich repeat protein KINase family protein 7)	AT3G02880	Leucine-rich repeat protein kinase family protein	Impaired stomatal closure	[36]
GHR1 (GUARD CELL HYDROGEN PEROXIDE-RESISTANT 1)	AT4G20940	Transmembrane receptor-like protein	Altered stomatal response kinetics	[7,15]
Protein phosphatases				
ABI1 (ABA INSENSITIVE 1)	AT4G26080	Protein phosphatase 2C	Conditionally impaired stomatal CO_2 response; impaired abscisic acid response	[37]
ABI2 (ABA INSENSITIVE 2)	AT5G57050	Protein phosphatase 2C	Conditionally impaired stomatal CO_2 response; impaired abscisic acid response	[37]
Transporter and channels				
SLAC1 (SLOW ANION CHANNEL 1)	AT1G12480	Anion channel	Impaired stomatal response, cool leaf temperature	[16,17]
QUAC1 (QUICK-ACTIVATING ANION CHANNEL 1)/ALMT12 (ALUMINUM-ACTIVATED MALATED TRANSPORTER 12)	AT4G17970	Aluminum-activated, malate transporter	Altered stomatal response kinetics	[18,19]
RHC1 (RESISTANT TO HIGH CO_2)	AT4G22790	MATE-type transporter	Impaired stomatal response	[20]
AtABCB14 (ATP-BINDING CASSETTE B 14)	AT1G28010	ABC transporter	Altered stomatal response kinetics	[29]
TPK1 (TWO PORE K CHANNEL 1)	AT5G55630	Two-pore potassium channel	Impaired stomatal closure	[36]
AtPIP2;1 (PLASMA MEMBERANE INTRINSIC PROTEIN 2;1)	AT3G53420	Aquaporin	βCA4 interactor, *in vitro* reconstitution of CO_2 regulation of SLAC1	[24]
Enzymes and other protein functions				
HT2 (HIGH LEAF TEMPERATURE 2)/ PATROL 1	AT5G06970	Munc13-like protein involved in mediating H^+-ATPase translocation	Impaired stomatal opening response, leaf temperature elevated	[34]
BIG	AT3G02260	Calossin-like protein required for polar auxin transport	Cool leaf, impaired stomatal closure and altered CO_2 regulation of stomatal density	[35]

(*Continued on next page*)

Table 1. *Continued*

Protein name	Locus ID	Protein function	Phenotype in response to $[CO_2]$ change	Reference
βCA1 (β-CARBONIC ANHYDRASE 1)	AT3G01500	Carbonic anhydrase	Slow stomatal CO_2 response and higher stomatal density (*ca1 ca4* double mutant)	[2,21]
βCA4 (β-CARBONIC ANHYDRASE 4)	AT1G70410	Carbonic anhydrase	Slow stomatal CO_2 response and higher stomatal density (*ca1 ca4* double mutant)	[2,21]
[a]OsβCA1 (β-CARBONIC ANHYDRASE 1)	LOC_Os1g45274	Carbonic anhydrase	Slow stomatal CO_2 response in rice	[22]
[a]ZmCA1 (CARBONIC ANHYDRASE 1)	GRMZM2g121878	Carbonic anhydrase	Slow stomatal CO_2 response (ca1 ca2 double mutant) in maize	[23]
[a]ZmCA2 (CARBONIC ANHYDRASE 2)	GRMZM2g348512	Carbonic anhydrase	Slow stomatal CO_2 response (ca1 ca2 double mutant) in maize	[23]
GCA2 (GROWTH CONTROL BY ABSCISIC ACID 2)	Not available	Not available	Impaired stomatal closure	[38]

[a]Nt, *Nicotiana tabacum*; Os, *Oryza sativa*; Zm, *Zea mays*. All others are from *Arabidopsis thaliana*.

CO_2-induced stomatal closure but did not alter the stomatal closing response to abscisic acid, another regulator of stomatal function [10]. *Arabidopsis* plant lines lacking the *MPK12* gene displayed increased transpiration and partial defects in stomatal movements in response to high and low $[CO_2]$ shifts [8]. However, light-induced stomatal opening — as well as stomatal closure induced by reduced air humidity and ozone — were intact in *mpk12* mutant plants [7,8], demonstrating the specificity of MPK12 in the $[CO_2]$ response. When MPK4 was silenced in guard cells of homozygous MPK12-deficient plants, the double mutant lines abolished stomatal closure and opening in response to $[CO_2]$ shifts [9], but again retained an intact abscisic-acid-induced stomatal closure. This finding suggested that MPK4 and MPK12 function synergistically to regulate CO_2-induced stomatal movements [9].

The RAF-like MAP kinase kinase kinase HT1 negatively regulates high $[CO_2]$-induced stomatal closing [11,34]. The activity of HT1 is down-regulated by MPK12 *in vitro* [7,8]. In turn, the HT1 protein kinase down-regulates SLAC1 activity in *Xenopus* oocytes [7,20] and HT1 is a negative regulator of CO_2 activation of S-type anion channels in guard cells [14,20]. Presently, phosphorylation sites in SLAC1 that shut down channel activity remain unknown. High $[CO_2]$-induced down-regulation of HT1 may directly or indirectly enable S-type anion channel activation (Figure 2), which initiates ion efflux from guard cells and triggers stomatal closure in response to elevated $[CO_2]$.

Recently, the CBC1 (CONVERGENCE OF BLUE LIGHT AND CO_2 1) and CBC2 protein kinases were identified and shown to interact with HT1, which phosphorylates CBC1 and CBC2 *in vitro* [12]. The *cbc1/cbc2* double mutant shows constitutively closed stomata that do not respond to $[CO_2]$ changes [12]. This is reminiscent of the $[CO_2]$ phenotype of the strong recessive *ht1-2* allele [11]. Indeed, the *cbc1/cbc2/ht1-9* triple mutant shows the same stomatal phenotype as *ht1-9* and *cbc1/cbc2* mutants. Altogether, these results indicate that CBC1, CBC2 and HT1 function in the same pathway [12] (Figure 2). However, whether CBC1 and CBC2 directly suppress SLAC1 by phosphorylation or inhibit protein kinases that activate SLAC1, such as OST1, remains unknown (Figure 2).

A new signaling component implicated in high $[CO_2]$-mediated stomatal closing and stomatal development was recently uncovered through an infrared thermal-imaging screen [35]. A single point mutation in the *BIG* gene triggered a partially diminished response in elevated $[CO_2]$-induced stomatal closure and activation of S-type anion channels. *BIG* is also required for the reduction of stomatal density in response to elevated $[CO_2]$ [35]. Interestingly, BIG is not involved in the inhibition of stomatal opening in response to low $[CO_2]$. These data suggest a role for BIG as a crucial signaling component that separates the low $[CO_2]$-induced stomatal opening from stomatal closure promoted by high $[CO_2]$ [35]. Identification of possible mechanisms by which BIG regulates CO_2-induced stomatal closure will be of interest in further research (Figure 2).

Stomatal closing requires K^+ efflux from guard-cell vacuoles via vacuolar K^+ channels encoded by *TPK* genes. A recent study identified a protein kinase, KIN7, that functions in activation of the guard-cell vacuolar K^+ channel encoded by TPK1. *kin7* mutant alleles are disrupted in abscisic-acid- and CO_2-induced stomatal closing [36], suggesting that CO_2- and abscisic-acid-signaling pathways share this mechanism. The trafficking of KIN7 from the plasma membrane to the tonoplast (vacuolar) membrane was reported [36], suggesting a mechanism for CO_2 regulation of vacuolar membrane ion fluxes during stomatal movements.

Although several critical components of stomatal CO_2 signaling have been identified (Table 1 and Figure 2), their interactions and targets remain largely unknown. Furthermore, proteins that act as direct receptors to sense CO_2 and/or HCO_3^- and trigger the underlying phosphorylation events required for CO_2-induced stomatal movements in guard cells remain unknown. Thus, elucidation of the exact mechanisms and sequence of events that mediate CO_2 sensing in regulation of stomatal movements is an important unanswered question.

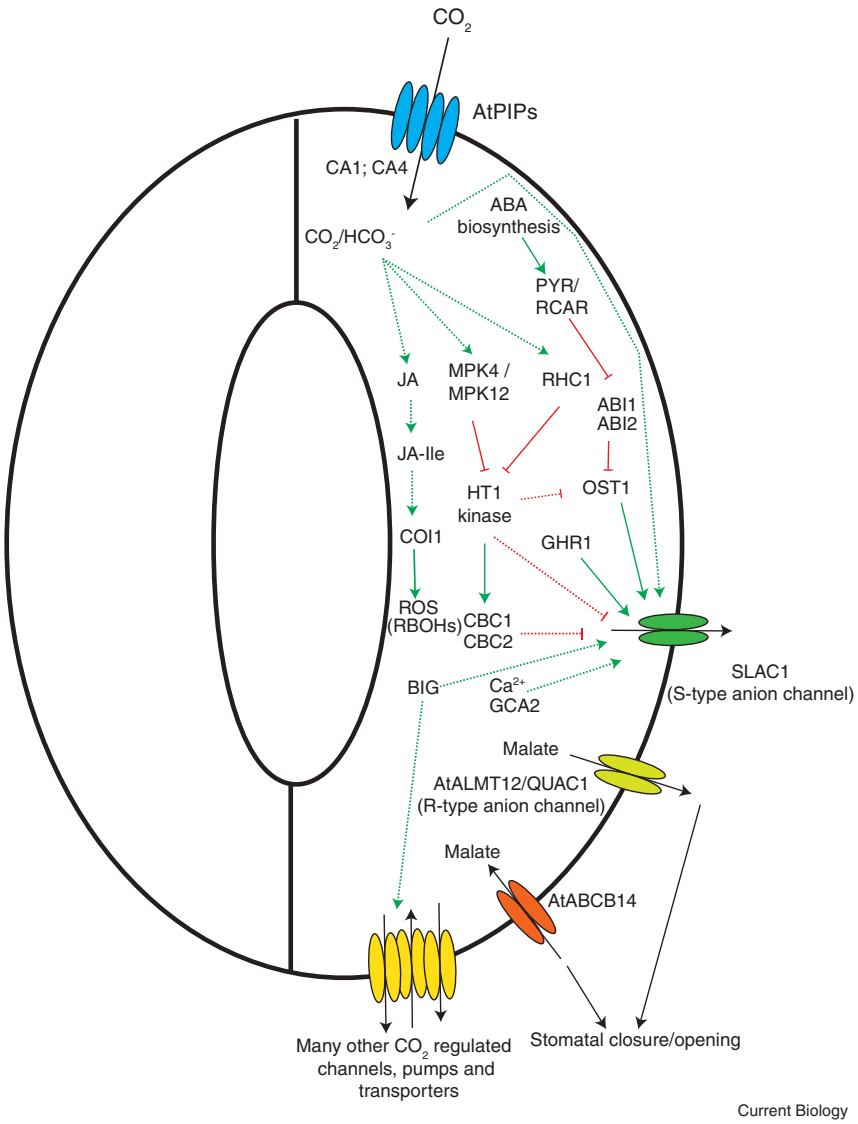

Figure 2. Simplified model for CO_2 signal transduction in regulation of stomatal movements.

CO_2 enters guard cells through aquaporins (AtPIPs, blue ovals). The PIP2;1 aquaporin interacts with the carbonic anhydrase βCA4, and the activity of the carbonic anhydrases leads to accelerated bicarbonate (HCO_3^-) formation. HCO_3^- and/or CO_2 act as signal molecules. Downstream protein kinases (MPKs, HT1, OST1 and CBCs), intracellular calcium ions, ion channels (SLAC1, AtALMT12/QUAC1), ion transporters (AtABCB14) and the membrane protein GHR1 are required for CO_2 control of stomatal closure. Convergence with abscisic acid (ABA) and jasmonate (JA) signaling is also indicated. Although key genetic components have been recently identified, many of the cellular signaling, biochemical, and interaction mechanisms remain to be elucidated. Connections represent positive regulation (green arrows) and negative regulation (red blocks) of high [CO_2]-induced stomatal closing. Regulation pathways are predicted to be direct (lines) or are unknown and remain to be further investigated (dashed lines).

Moreover, abscisic-acid and CO_2 responses share several genetic components, including *SLAC1*, *OST1*, *GCA2*, *AtALMT12/QUAC1* and potassium channels [14,16–19,38]. Of note, elevated [CO_2] and abscisic acid induce increases in guard-cell cytosolic free Ca^{2+} [39], and Ca^{2+} contributes to the stomatal CO_2 and abscisic-acid responses implicating a role for Ca^{2+} in linking these two pathways in guard cells [14,38,39] (Figure 2).

In this context, the hypothesis that high-[CO_2]-induced stomatal closure requires abscisic-acid perception and signaling in guard cells has been investigated [40]. When mutants of the PYR/RCAR abscisic-acid receptors were tested in CO_2 responses, a correlation was observed between the lack of abscisic-acid receptors and the intensity of the CO_2-induced stomatal closure. A quadruple mutant deficient in the abscisic-acid receptors PYR1, PYL1, PYL2 and PYL4 showed a delayed but otherwise full CO_2-induced stomatal closing [14]. In contrast, a more recent study showed that the *pyr1/pyl1/pyl4* triple mutant and the *pyr1/pyl1/pyl2/pyl4* quadruple mutant abolished high-[CO_2]-induced stomatal closure in stomatal-aperture assays [40]. Furthermore, mutants impaired in abscisic-acid biosynthesis were tested using the same assays and were reported to show similar abolishment of responses to [CO_2] in stomatal-aperture assays [40]. These data could be explained in two ways [40]: first, abscisic acid may increase the plant's sensitivity to CO_2. Alternatively, or in addition, high [CO_2] might trigger a fast accumulation of abscisic acid. On the other hand, the abscisic-acid biosynthesis mutants *aba1* and *aba3* did not abolish CO_2-induced stomatal closure in intact plant gas-exchange measurements [41]. Recent research investigating models by which CO_2 elevation interfaces

CO_2 Signaling Pathway Interactions with Other Stimuli

Stomatal aperture is regulated by several environmental stimuli, including light, drought, CO_2, relative humidity, and pathogens [3,29]. Understanding how plants compile and coordinate this multitude of stimuli into proper guard-cell turgor pressure, and thus stomatal aperture, is crucial and is less well understood. Here we discuss external and internal stimuli that, together with CO_2, control stomatal movements.

Abscisic Acid

As mentioned in the previous section, the phytohormone abscisic acid triggers stomatal closing. Interactions between abscisic-acid- and CO_2-mediated signal transduction in guard cells have been observed for many years but have not been fully elucidated. Classic studies reported, over 40 years ago, that stomatal closing could be induced by abscisic acid only in the presence of ambient to high [CO_2] in *Xanthium strumarium* L. The dominant abscisic-acid-insensitive mutants, *abi1-1* and *abi2-1*, conditionally impair CO_2-induced stomatal closing [37].

with abscisic-acid signal transduction in guard cells, including use of a real-time ABA FRET reporter, points to a new model, in which CO_2-mediated signal transduction merges with abscisic-acid signal transduction downstream of the OST1 protein kinase and with parallel basal (background) abscisic-acid signaling and OST1 kinase activity being required for a robust stomatal CO_2 response [42].

Methyl Jasmonate

Jasmonates act as plant hormones and have essential functions in regulating plant growth, development, and defense against pathogens and abiotic stresses. SCF^{COI1} acts as the jasmonate receptor and targets the JASMONATE ZIM-DOMAIN proteins for degradation and ubiquitination, leading to activation of diverse jasmonate-dependent responses. Recent metabolomic analyses of guard cells showed that the concentrations of methyl jasmonate and jasmonoyl-L-isoleucine increased in response to elevated [CO_2] but remained unaltered when plants were grown under low [CO_2] conditions [28]. In addition, epidermal-strip assays found that stomatal apertures of guard cells in epidermal peels did not respond to high [CO_2] in the jasmonoyl-L-isoleucine synthesis mutant, *jasmonate resistant 1* (*jar1*) — or in the signaling mutants *coronatine-insensitive1* (*coi1*) and *jasmonate insensitive 1* (*jin1*, also named *myc2*) — as compared to wild-type plants [28]. Jasmonate was proposed to act downstream of carbonic anhydrases in the CO_2-signaling transduction pathway since jasmonoyl-L-isoleucine metabolites did not change significantly under elevated [CO_2] in the *ca1*/*ca4* double mutant [28].

Light

In addition to low [CO_2], stomatal opening is also induced by both red and blue light. Red-light-induced stomatal opening is likely mediated in large part by a reduction of intercellular [CO_2] via photosynthesis in *Arabidopsis* [43]. Blue light-induced stomatal opening is mediated via a different signaling pathway, and is dependent on inhibition of the plasma-membrane anion channels [44] and activation of plasma-membrane proton pumps [12,29], among other targets. A recent study identified a key molecular basis for blue-light and CO_2 signal transduction convergence. The CBC1 protein kinase is rapidly phosphorylated in response to blue light in *Arabidopsis* guard-cell protoplasts [12]. Interestingly, double-mutant plants in *CBC1* and its close homolog *CBC2* showed not only impaired blue-light-induced stomatal opening but also a lack of stomatal opening in response to low [CO_2] [12]. CBC1 and CBC2 are directly phosphorylated by the blue-light receptor PHOT1. Moreover, as mentioned earlier CBC1 and CBC2 are also phosphorylated by the HT1 protein kinase that is required for low-[CO_2]-induced stomatal opening (Figure 2). Thus, CBC1 and CBC2 function as a key recognized convergence point of the blue-light and CO_2 signal transduction pathways in regulation of stomatal movements. Interestingly, *cbc1cbc2* mutant plants show abscisic-acid-induced stomatal closure [12], suggesting that CBC1 and CBC2 do not directly function in abscisic-acid signal transduction. The target proteins that are phosphorylated by CBC1 and CBC2 remain presently unknown.

Modeling Stomatal Movement Responses

A few studies have modeled stomatal movements in response to abscisic acid by examining signaling networks that incorporate the many known components and parameters [45–47]. These studies are able to model the response of abscisic-acid-induced stomatal closure and can make experimentally testable predictions. In addition, kinetic models that incorporate many components and ion channel/transporter regulation and activity parameters have been developed to model the kinetics of stomatal conductance as a function of abscisic acid and humidity [48,49]. It would be worthwhile to expand these modeling approaches to [CO_2]-mediated signal transduction. Furthermore, it would be interesting to determine whether recent efforts in model reduction that systematically reduce the number of parameters and components [50] can be applied towards predicting properties or functions of additional (currently undiscovered) mechanisms in CO_2 signal transduction.

Conclusions

In recent years, several essential genes and mechanisms that function in guard cells for [CO_2]-mediated control of stomatal movements have been identified (Table 1 and Figure 2). However, knowledge of how they interact and are integrated into a signaling network remains fragmented. Also, the intracellular CO_2 and HCO_3^- sensors remain unknown, and the mechanisms by which intracellular CO_2 and HCO_3^- function as second messengers in guard cells remain unclear. Further research aimed at identification of CO_2 and HCO_3^- sensors and the precise interactions among the newly recognized components in the CO_2-signal-transduction network could involve in-depth biochemical, genetic molecular, cell biological and natural variation analyses, and systematic probing of CO_2-dependent transcriptomic and proteomic resources and systems-biological mining of data sets. Moreover, genetic mutants and leaf cell-wall metabolomic analyses are required to dissect the mechanisms by which an unknown diffusible signal from mesophyll cells regulates CO_2 control of stomatal conductance. A fundamental understanding of how plants control stomatal movements in response to both daily [CO_2] changes inside leaves and the continuing atmospheric rise in [CO_2] will be important for adapting crop plants towards maximizing yield and water-use efficiency in an elevated-[CO_2] world.

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