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Review

Gasotransmitters are emerging as new guard cell signaling molecules and regulators of leaf gas exchange

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ABSTRACT

Specialized guard cells modulate plant gas exchange through the regulation of stomatal aperture. The size of the stomatal pore is a direct function of the volume of the guard cells. The transport of solutes across channels in plasma membrane is a crucial process in the maintenance of guard cell water status. The fine tuned regulation of that transport requires an integrated convergence of multiple endogenous and exogenous signals perceived at both the cellular and the whole plant level. Gasotransmitters are novel signaling molecules with key functions in guard cell physiology. Three gasotransmitters, nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H_2S) are involved in guard cell regulatory processes. These molecules are endogenously produced by plant cells and are part of the guard cells responses to drought stress conditions through ABA-dependent pathways. In this review, we summarize the current knowledge of gasotransmitters as versatile molecules interacting with different components of guard cell signaling network and propose them as players in new paradigms to study ABA-independent guard cell responses to water deficit.

Contents

1. Introduction

Communications between cells can be achieved through either electrical signals or through chemical substances such as hormones and transmitters. Hormones require receptors for the canonical signal transduction processes. The binding of hormones to their receptors is the essential triggering step that generates a second wave of signals through cellular second messengers.

Among the transmitters, the gasotransmitters are an emerging type of biological active molecules that can be grouped according to the following general criteria [1,2]:

1) they are small molecules of gas;
2) they can freely cross cell membranes;
3) their effect do not rely on receptors;
4) they are enzymatically generated and their production is regulated;
5) their functions can be mimicked by exogenous application; and
6) their cellular effects may or may not be mediated by second messengers but should have specific cellular and molecular targets.

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The concept of gasotransmitters has been recently established. Future investigations concerning the: (i) interaction between gasotransmitters themselves; (ii) the use of common cell target molecules by different gasotransmitters, (iii) the elucidation of the site of production and the site of cellular action, among others, will surely add relevant insights to understanding the relevance of this emergent players in signal transduction in biological systems.

New discoveries could, eventually, lead to modifying the criteria described above for classifying the gasotransmitters. For instance, the traditional dogma says that small gases diffuse through all membranes simply by dissolving in the lipid phase of the membrane. However, recent findings in animal cells point to the fact that gases can be transported through membranes with the help of transporter proteins [3,4]. The water channel aquaporin-1 transports low molecular weight gases in addition to water, and it is expressed in cells that produce or are the targets of nitric oxide (NO) [3]. Aquaporins are also able to specifically transport other gases such as CO_2 and NH_3 [4].

In this review we summarize the current and basic knowledge of gasotransmitters functions in plants. We will focus our overview on the functions of the gasotransmitters nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H_2S) in the stomatal guard cells, the specialized cells responsible of the gas exchange between plants and the environment. Other gases such as O_2 and CO_2 play equally critical functions in cell physiology, however, NO, CO and H_2S are thought to exert precise modulator effects controlling and influencing many signaling events and intracellular processes. Finally, we will discuss the present and future of the studies needed to understand the functions of gasotransmitters in guard cell signaling. In particular, we propose that gasotransmitters may have a critical role in the control of plant responses to water deficit and leaf gas exchange through the modulation of ion channels activity and guard cell physiology in an ABA-independent pathway.

2. Nitric oxide: synthesis and functions

Nitric oxide (NO) is a small diatomic free radical gas that freely diffuses across biological membranes. The unpaired electron in its 2p-π antibonding orbital allows NO to alternate between three different oxidative species, the radical ($NO^{\bullet}$), the nitrosonium cation (NO^+), and nitroxyl anion (NO^-), which makes NO an extremely reactive molecule [5].

NO was first identified in animal systems as an endothelium derived relaxed factor (EDRF) [6]. The discovery of the biological role of NO has been a breakthrough in the modern study of signal transduction. Today, NO is known to function as a neurotransmitter and regulator of immunological responses, blood pressure muscle relaxation, oxygen sensing, ATP production through respiration, among others [7,8].

Later, fascinating discoveries on NO functions in plant growth, development and stress physiology captured the attention of plant biologists. Since the end of eighties, NO has been found to be involved in processes such as seed germination and dormancy, root growth and development, regulation of cell cycle progression, plant–pathogen interaction, respiration, responses to drought stress and many other abiotic and biotic stresses. NO acts as a second messenger downstream of hormones in many signaling cascades involving Ca^{2+}, cGMP, MAPK, CDPK, TFs, among other cellular regulators [9–11].

The pathways of NO synthesis in plants have been recently reviewed in detail [12]. The different NO biosynthesis pathways in plants can be classified as either oxidative or reductive. The enzymatic production of NO can be divided between a nitrate/nitrite-(reductive) and an L-Arg-dependent (oxidative) with an about

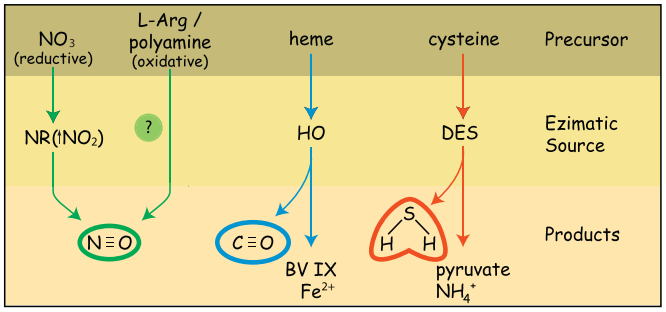

Fig. 1. Gasotransmitter synthesis in plants. Simplified scheme showing the precursors, enzymes and products of the biosynthetic pathways leading to the generation of the gasotransmitters nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H_2S) in plants. Question mark, unknown or incompletely known intermediates and enzymatic sources.

equal number of reports of either one or the other in guard cells [13–16] (Fig. 1). L-Arg-dependent biosynthesis was the first pathway proposed for NO synthesis in guard cells [17]. Almost at the same time, nitrate reductase was reported to be the enzyme responsible for NO biosynthesis in guard cells [13]. NO synthesis *via* nitrate reductase has been observed both *in vitro* and *in vivo* [18,19], and was genetically confirmed using the Arabidopsis nitrate reductase knockout mutant *nia-1/nia-2*. NIA mutant lines have reduced production of endogenous NO as well as a low sensitivity to ABA [13,14,20]. The phenotype was rescued by the addition of exogenous NO [20]. However, *nia* mutants have dramatic changes in aminoacid metabolism and composition with low levels of L-Arg [21]. Therefore, conclusions from experiments performed with *nia* mutants to address the NO generating system in plants should be taken with caution.

L-Arg-dependent NO synthesis in plants is far more complex. Animals have three characterized isoforms of NO synthase that produces NO and citrulline from L-Arg in the presence of O_2. There is no homolog of the mammalian NO synthase in higher plants. The only NO synthase gene found in photosynthetic organisms was recently described in the unicellular marine microalgae *Ostreococcus tauri* [22]. However, NO production in higher plants is extremely sensitive to mammalian NO synthase inhibitors and to non-hydrolysable homologs of L-arg [13,16,17].

Additionally, NO can be produced non-enzymatically from NO_2 at low pH (<5.0) [23]. Even though there is no report of non-enzymatic NO production in guard cells so far, it remains to be explored if high concentrations of NO_2 and the low pH in the guard cell vacuole could be a favorable environment for non enzymatic production of NO.

3. Carbon monoxide: synthesis and functions

Carbon monoxide (CO) is a small diatomic gas with low water solubility. CO is a stable non radical molecule and does not alternate between different redox species [24]. CO binds to iron in its reduced state (Fe^{2+}), and has little biochemical reactivity with non-iron compounds. The physiological importance of CO lays in its ability to bind to heme proteins. CO is produced in animals by the degradation of heme to biliverdin and CO in an enzymatic reaction catalyzed by heme oxygenase (EC 1.14.99.3) [25]. Three isoforms of heme oxygenase are known in animals (HO 1-3). HO-1, the only inducible form, responds to a broad range of physical and chemical agents [25,26].

CO emission by plants was first reported over half a century ago [27]. CO in plants, as in animals, is synthesized *via* heme oxygenase activity [28,29]. Heme oxygenase catalyses the conversion of heme to biliverdin IX releasing CO and Fe^{2+} (Fig. 1) [30].

Arabidopsis thaliana contains one major biochemically characterized heme oxygenase HY1 and three additional putative heme oxygenases (HO2, HO3 and HO4). All proteins are encoded in the nucleus and contain chloroplast transit peptides at their N-termini as demonstrated with green fluorescent protein reporter gene fusions [31]. The four proteins are organized into two subfamilies: HO1 and HO2. All members of the HO1 subfamily (HY1, HO3 and HO4) are active as monomeric heme oxygenases and can convert heme to biliverdin and CO using spinach ferredoxin and ascorbate as electron donors [30,31].

CO binds to hemoglobin heme iron forming carboxyhemoglobin (CO-Hb) (CO has a 200 times greater affinity for heme iron than does O_2). CO not only competes with O_2 for the four binding sites of hemoglobin, but also the partial occupation of hemoglobin (*e.g.* occupancy of two binding sites with CO) inhibits the release of O_2 from the remaining sites reducing hemoglobin O_2-carrying capacity and generating asphyxiation [25]. The discovery of the endogenous production of CO by different mammalian tissues in the 1950s was a milestone for the knowledge about the role of CO in biological systems.

CO production in higher plants together with NO has been implicated in adventitious and lateral root formation in cucumber [32], *Medicago sativa* [33] and rice [34]. CO induces stomatal closure in *Vicia faba* [35] and confers tolerance to heavy metals in *Chlamydomonas reinhardtii* [36].

4. Hydrogen sulfide: synthesis and functions

Hydrogen sulfide (H_2S) is a small flammable colorless gas with a characteristic odor of rotten eggs. It is soluble both in polar and non polar solvents, although, it is solubility is 5 times greater in lipophilic solvents than in water [1]. H_2S is readily oxidized to sulfur dioxide, sulfates, or elemental sulfur. This oxidation undergoes two dissociation steps: first the ionization of a single proton to give the hydrosulfide anion (Eq. (A)); and then a second proton dissociation from the hydrosulfide anion to give sulfide ion (Eq. (B)). The pK_a of these two reactions are 7 and 12 for equations A and B respecitvely. In aqueous solutions at physiological pH (pH 7.4) about one third of the H_2S exists in the undissociated form and most of the rest as the hydrosulfide anion (HS^-) [37]. The active form of H_2S in biological systems is not yet known; therefore H_2S usually stands for H_2S/HS^-.

$$H^2S \rightleftharpoons H^+ + HS^- \quad (A)$$

$$HS^- \rightleftharpoons H^+ + S^{2-} \quad (B)$$

H_2S can also generate sulfhydryl radical $HS^\bullet$ by hydrogen abstraction or electron transfer (Eq. (C)). $HS^\bullet$ can react in aqueous solutions with HS^- to form $HSSH^{\bullet -}$ [38].

$$HO^\bullet + H_2S \rightleftharpoons H_2O + HS^\bullet \quad (C)$$

H_2S, similarly to NO and CO, participates in plant systems in controlling root growth and development, and modulating many adaptive responses to different biotic and abiotic stresses, including the regulation of stomatal movement in response to drought stress [39–48].

H_2S in mammalian systems is mainly synthesized by two pyridoxal-5′-phosphate (PLP)-dependent enzymes: cystathionine ß-synthase (CBS, EC4.2.1.22) and cystathionince γ-lyase (CSE, EC 4.4.1.1) *via* desulfhydration of L-cysteine (L-Cys) [49] catalyzing the formation of H_2S, pyruvate and ammonia. Alternatively, both enzymes can produce H_2S through the transulfuration of L-methionine [50]. L-Cys desulfhydrase (DES, E.C. 4.4.1.1) has been known for three decades in plants (Fig. 1) [51], however the first and only desulfhydrase characterized to date is the *Arabidopsis thaliana* L-cysteine desulfhydrase *DES1* [52]. Two independent knock out mutations of *DES1*, *des1-1* and *des1*-2, decreased cysteine desulfhydrase activity and increased cysteine content in Arabidopsis leaves [52]. Cysteine desulfhydrase activity was reduced by 20 to 25% in the Arabidopsis mutants suggesting that there are other endogenous H_2S sources in plants. Before the characterization of cysteine desulfhydrase DES1, a group of Cys degrading enzymes were suggested as being putative H_2S sources in Arabidopsis, especially some members of the AtNFS-like family [53]. These are pyridoxal phosphate-dependent enzymes with L-Cys desulfurase activity that catalyze the breakdown of L-Cys to Ala and S [54]. Members of this protein family are localized to mitochondria, chloroplasts and the cytosol and are of great importance for Fe-S cluster biosynthesis, and also for the synthesis of other cofactors such as NAD and molybdenum cofactor [55]. H_2S was also reported to be produced specifically from D-Cys in an enzymatic reaction catalyzed by a D-cysteine desulfhydrase (EC 4.4.1.115) even if D-Cys has yet to be identified in plants. This activity has been reported in Arabidopsis and several plant species [53,56]. An alternative mitochondrial H_2S source in plants is the detoxification of HCN. In this reaction catalyzed by ß-cyanoalanine synthase (ß-CAS; 4.4.1.9), cyanide reacts with L-Cys forming H_2S and ß-cyanoalanine [57,58].

5. Gasotransmitters in guard cell signaling

About 30% of the total precipitation (32 trillion tons per year) is vapourized by the transpiration stream of terrestrial plants through leaf stomata [59]. The size of the stomatal pore is regulated through changes in the volume of the guard cells driven by osmotic rearrangements. The regulation of stomatal aperture is a permanent trade off between allowing CO_2 in for photosynthesis and limiting water loss as water vapour, and is controlled by a complex signaling network [59].

ABA is, by far, the most studied regulator of stomatal opening. ABA not only induces stomatal closure and inhibits stomatal opening, but also regulates the expression and/or activity of many of the effectors in the signaling cascade. This knowledge was taken to a new dimension with the characterization of the PYL/PYR/RCAR ABA receptor complex [60–64].

It has been demonstrated that the exogenous addition of any of the gasotransmitters NO, CO or H_2S induces stomatal closure *per se* [39,65,66]. However, all the three gasotransmitters are part of the ABA-dependent signaling network. Even if NO biosynthesis is induced by ABA, an increase of the production of this gasotransmitter occurs independently of ABA during the transition from day to night [67,68]. Since sequestering NO results in inhibition of dark-induced stomatal closure [66,67], an ABA-independent gasotransmitter action on guard cell signaling might be postulated.

5.1. Nitric oxide

The role of NO regulating ABA-induced downstream signals leading to stomatal closure is perhaps one of the best characterized examples of NO function in plant responses to abiotic stresses. The ABA-NO crosstalk driving signaling cascades leading to stomatal closure is summarized in Fig. 2.

ABA-induced H_2O_2 production in *A. thaliana* guard cells leads to a rise of endogenous NO synthesis [14,15]. Arabidopsis *atrbohD/F* mutants lack guard cell NADPH oxidase activity resulting in decreased ROS production and NO generation in response to extracellular calmodulin. This highlights the close interaction between NO and ROS in the guard cell signaling pathway [15]. The increase of NO production results in: (i) a cyclic ADP ribose (cADPR)-dependent increase of cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) [69], (ii) a phosphatidic acid increase derived from phospholipases C and D activities [70] and (iii) a Ca^{2+}-dependent activation of

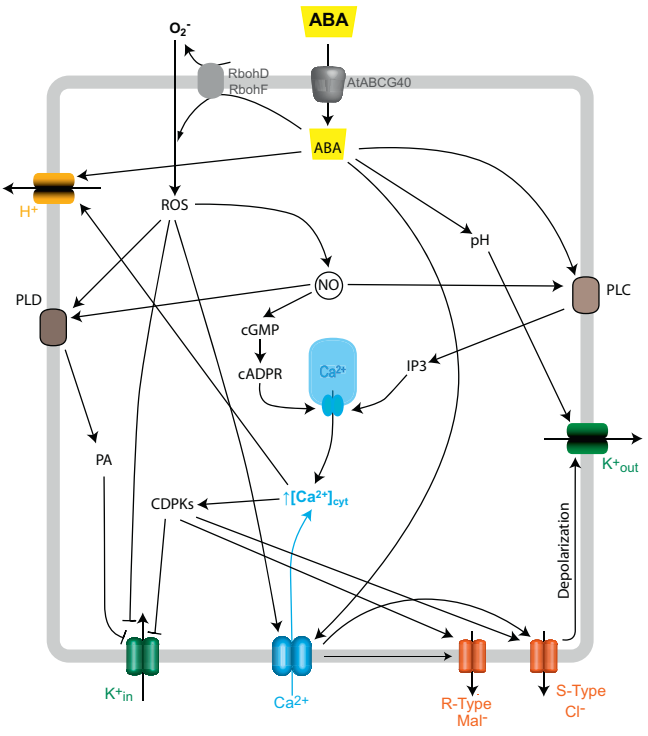

Fig. 2. Simplified schematic model showing the signaling events occurring during ABA-dependent stomatal closure. ABA is internalized by the ABC transporter AtABCG40. ABA promotes an increase of the cytosolic Ca^{2+} concentration $[Ca^{2+}]_{cyt}$ through signaling components such as inositol-3-phosphate (IP3) and nitric oxide (NO). Extrusion of anions through slow and rapid anion channels (S-Type and R-Type respectively) triggers the depolarization of the plasma membrane and generation of the gating voltage required for the extrusion of K^+ through the outward-rectifying K^+ channels (K^+_{out}). Besides, the inhibition of inward-rectifying K^+ channels (K^+_{in}) mediated by Ca^{2+}, H_2O_2, NO and phosphatidic acid (PA) impairs the uptake of K^+ contributing to a net loss of solutes that produces the loss of water that leads to the closure of the stomatal pore. Signaling components: Ca^{2+} membrane channels (Ca^{2+}), anion channels (A^-), malate (Mal^-), chloride (Cl^-), multidrug resistant protein 5 as an anion and calcium channel regulator (MRP5), cyclic GMP (cGMP), cyclic ADPR (cADPR), calcium dependent protein kinases (CDPKs), transcription factors (TFs), respiratory burst oxidase homologue D/F (RbohD/RbohF), phospholipase C (PLC), phospholipase D (PLD), reactive oxygen species (ROS). Pointed arrows, activation; blunt arrows, inhibition. Not all the interactions reported in the bibliography have been indicated.

Cl^- efflux and inhibition of the K^+_{in} channels activity contributing to a net loss of ions from the guard cells and stomatal closure [69] (Fig. 2). Additionally, cGMP-regulated pathways as well as S-nitrosylation posttranscriptional modifications are NO-dependent events in the ABA-induced guard cell responses [69,71]. NO inhibits stomatal opening and participates alone or downstream ABA in this process [72,73]. As stated above and proposed in Fig. 3, gasotransmitters could enter guard cells from surrounding cells by either freely crossing cell membranes or being transported by aquaporins. They could also come from the environment, and then reach molecular targets and influence stomatal aperture through an ABA-independent pathway.

5.2. Carbon monoxide

The biology of CO effects on guard cell signaling is still in its infancy. The exogenous application of hematin as a CO donor, or the application of gaseous CO to aqueous solutions induces stomatal closure in a dose dependent manner in *Vicia faba* [35,65]. Moreover, the CO-mediated regulation of stomatal closure relies on H_2O_2 generation in guard cells [37]. It has been proposed that, heme oxygenase produces CO in response to ABA in *Vicia* guard cells, and that this increase of CO concentration causes stomatal closure in a NO- and cGMP-dependent manner (Fig. 3) [65]. In addition, CO-mediated dark-induced stomatal closure requires NADPH oxidase activity to increase the H_2O_2 level and NO production in guard cell [35].

Endogenous CO production can be regulated by other gasotransmitters. NO can bind to the Fe^{2+} (ferrous) of the heme group of human heme oxygenase HO-1 *in vitro*, inhibiting its CO producing activity [74]. Even if there is no *in vivo* evidence of this interaction in plants, such a possibility should be considered as a putative negative feedback mechanism that controls the biological activity of gasotransmitters through the inhibition of their synthesis.

5.3. Hydrogen sulfide

Exposing maize, spinach and pumpkins plants to atmospheric H_2S did not cause changes in transpiration rates [75]. However, long term exposure of H_2S resulted in a decrease in photosynthetic CO_2 fixation [76]. Treating epidermal peels of different plant species with H_2S donors resulted in contrasting effects on stomatal aperture [39,46,47]. H_2S induced stomatal closure in *Vicia faba*, *Arabidopsis thaliana* and *Impatiens walleriana* [42], while H_2S caused stomatal opening in *Arabidopsis thaliana* and *Capsicum annuum* [49,50]. The ATP binding cassette (ABC) transport inhibitor glibenclamide blocked H_2S-induced stomatal closure, as did propargylglycine, which inhibits L-DES activity [42]. Stomatal aperture assays showed that ABA-induced stomatal closure is partially impaired in presence of L-cysteine desulfydrase inhibitors suggesting that H_2S participates in the ABA signaling pathway in guard cells [39]. The contrasting studies [46,47] propose that H_2S induces stomatal opening through its NO scavenging activity. This is supported by the evidence showing a H_2S-mediated decrease of NO concentration detected in guard cells using the specific fluorescent probe DAF2-DA [46]. More recently it was reported that ethylene-induced stomatal closure requires H_2S and the Arabidopsis mutant *Atl-cdes* does not close the stomata in response to ethylene or NO [41], suggesting that ethylene and NO act upstream of H_2S. The locus mutated in *Atl-cdes* corresponds to the mitochondrial cysteine desulfurase (AtL-CDES; At5g 65720) proposed as a H_2S-releasing enzyme [53]. Taken together, these results suggest that there is an interaction between H_2S and NO in ethylene- and ABA-mediated induction of stomatal movement. Future work should elucidate the exact role of each component and the exact relationship between each of them in controlling and maintaining plant water status.

NO and H_2S in animal systems can regulate the metabolism of each other, or even react together [74]. However, the molecular nature of these interactions is still unclear and has yet to be elucidated. H_2S can increase the release of NO from nitrosothiols such as nitrosogluthathione and therefore regulate the bioavailability of NO *in vivo* [77]. These two gases can also react to each forming a new nitrosothiol species [78]. In some cases, the simultaneous presence of both gases is required for certain physiological responses [79].

No data are yet available regarding H_2S and ROS interaction in guard cell signaling, even though H_2S interacts with ROS in plant responses to some abiotic stresses [43,80]

6. Gasotransmitters and ion channel regulation

The modulator effects of gasotransmitters on ion channel activity have biological consequences at both physiological and pathophysiological levels. Ion channels emerged as targets of gasotransmitters since the beginning of the study of NO as a signaling molecule, and with it the beginning of the history of gasotransmitters. The first ion channels reported as targets of NO were the cGMP-dependent Ca^{2+}-activated K^+ channels (K_{Ca}) [81]. These channels were first reported to be activated by NO *via* the

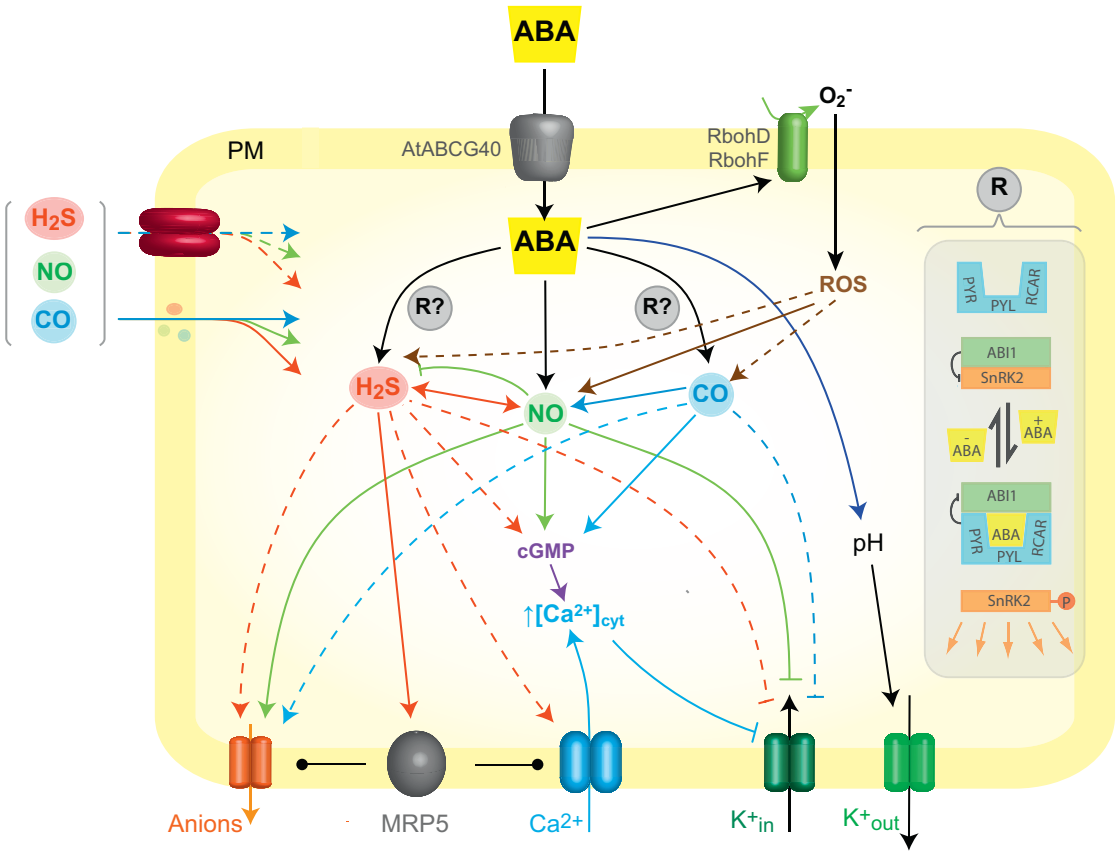

Fig. 3. Gasotransmitters in guard cell signaling. Schematic diagram showing gasotransmitter participation in ABA-dependent and–independent signaling leading to stomatal closure. ABA-dependent: ABA enters to the guard cells through the ABC transporter AtABCG40 [87]. Once sensed by the PYR/PYR/RCAR receptor (R) it binds to the negative regulator PP2C/ABI1 releasing the SnRK2 kinases that remains phosphorylated and amplifies the signal. Nitric oxide (NO) accumulates in ABI1 knock out mutants [15], therefore the pathway between ABA and NO does not has the receptor in between; ABA-independent: Gasotransmitters coming from the neighboring cells or from the apoplast can freely diffuse through plasma membrane into the guard cells or enter through "gas channels" (aquaporins, AQ) and modulate ion channels activity directly or indirectly through Ca^{2+}- or cGMP-dependent pathways. Signaling components: Inward-rectifying potassium channels (K^+_{in}), outward-rectifying potassium channels (K^+_{out}), Ca^{2+} membrane channels (Ca^{2+}), anion channels (A^-), multidrug resistant protein 5 is an anion and calcium channel regulator (MRP5), cyclic GMP (cGMP). Filled lines, reported pathways; dashed lines; potential pathways, question marks, unknown interactions.

guanylate cyclase (GC)/cGMP pathway [81], but shortly after were also found to be directly activated by NO (S-nitrosylation) in a GC/cGMP-independent manner [82]. Channels such as the cyclic nucleotide gated channels, L-type Ca^{2+} channels, ryanodine receptor Type 1, transient receptor potential and the rectifier potassium channel KCNQ1 are regulated by NO either *via* GC/cGMP or by S-nitrosylation [83]. NO was proposed to be a Ca^{2+} homeostasis regulator nearly two decades ago [84].

The story of ion channel regulation by CO [85] and H_2S [86] is more recent. The differentially CO-regulated ion channels in animals are an example of a calcium-activated K^+, voltage-activated K^+ and Ca^{2+} channel (L-type) family [85]. It appears that specific amino acid residues as well as cellular redox state are involved in CO-controlled molecular mechanisms acting on ion channel proteins [85]. The first target of H_2S reported in animal systems was a K^+-ATP channel, which is activated by H_2S [87]. The active form of this channel is a complex of two proteins, the sulfonyl urea receptors SUR1or SUR2, and the Kir6.2 channel protein [86]. In plants, AtMRP5 is a Ca^{2+} and anion channel regulator in the plasma membrane of guard cells [88] that has high homology with sulfonylurea receptor proteins and binds to the sulfonylurea receptor protein blocker glibenclamide [89]. Interestingly, the stomata of Arabidopsis mutant *atmrp5-1* do not fully respond to ABA [90].

The study of ion channels in plants has received a strong contribution from research on regulation of guard cell ion channels and its effects on changes in the stomatal pore aperture. As the regulation of stomatal volume is controlled by the uptake or release of water driven by massive and fast redistribution of ions (mainly K^+) and osmotically active solutes (mainly malate), the fine tuned regulation of ion channel activity is crucial for this key physiological process.

The activation of H^+-ATPases hyperpolarizes the plasma membrane and generates the electrochemical gradient required for K^+ uptake through inward-rectifying K^+ channels that results in stomatal opening. Conversely, the inactivation of H^+-ATPase induces the membrane depolarization that inactivates K^+_{in} and activates K^+_{out} channels. This results in a net loss of solutes in guard cells and the consequent loss of water and stomatal closure (Fig. 3). NO inactivates K^+_{in} and activates K^+_{out} channels [69,91,92], anion channels (including Slac1) [69,93] and both endomembrane and plasma membrane Ca^{2+} channels [69,91], which makes it a modulator of almost all the ion channels involved in the ABA-dependent signaling cascade in guard cells. It has been suggested that CO and H_2S regulate guard cell ion channels, but there is still no direct evidence [39].

It is necessary to determine the molecular mechanisms by which gasotransmitters modulate ion channel activity to be able to fully understand gasotransmitter action in guard cells. Direct protein S-nitrosylation (S-NO), sulfhydration (S-SH) and cellular redox state modulation are mechanisms that may regulate these channels. The regulation of ion channel activity might also potentially include the effects mediated by the interactions of all gasotransmitters

themselves (NO, CO and H_2S), as well as indirect effects through the regulatory involvement of channel-regulating kinases and vesicle recycling processes involved in endocytic protein channel insertion in membranes [94,95].

7. Integrating gasotransmitters and ABA in guard cells signaling

The first suggestion that ABA is involved in water management came from observations that the wilty tomato *flacca* mutant could be rescued by exogenous addition of ABA and was indeed deficient in ABA [96]. It was then confirmed that ABA treatment induced stomatal closure [97,98]. After the emergence of NO as a signaling molecule in plants, this gasotransmitter was linked to guard cell signaling [66] and was found to have an active role in ABA-signaling [17,99]. Now, one decade later, more than 100 reports have been published on the participation of NO in stomatal movement regulation. NO can either induce stomatal closure or inhibit light induced stomatal opening, acting within or independently of the signaling network triggered by ABA [72,99,100].

The second gasotransmitter reported to participate in guard cell ABA-signaling was CO [65]. Although, CO physiology in guard cell signaling has not been fully explored, ABA induces heme oxygenase HO-1-dependent CO production and CO probably interacts with NO and H_2O_2 during the induction of stomatal closure [65,101] (Fig. 3). There are no reports on ABA-independent CO action inducing stomatal closure, but again, like NO and H_2S, CO can potentially diffuse from surrounding cells and/or from the environment and reach molecular targets in guard cells and influence stomatal aperture independently of ABA.

The third gasotransmitter, H_2S is also involved in guard cell signaling [39,46,47] as described above. However little is known about the target(s) of H_2S in general and in guard cells in particular. As stated above, H_2S may be acting through the ABC transporter MRP5, which is an anion and calcium channel regulator [39] (Fig. 3). Overall, increasing evidence supports the participation of gasotransmitters in the regulation of stomatal movement.

8. Concluding remarks and perspectives

For more than 40 years, ABA is considered to be the plant hormone that functions as key regulator of guard cell responses. During that time, evidence has accumulated supporting the presence of an ABA-independent in addition to an ABA-dependent control of stomatal movement.

The gasotransmitters are both generated and perceived by plant cells, including guard cells. They act in downstream signaling events triggered by ABA, but also they are able to potentially drive ABA-independent responses. They function in combination with ABA, but they also possess the capability of crossing cell membranes and function as alternative and autonomous molecules by-passing ABA as part of the plant responses that synchronize heterogeneous environmental signals with endogenous stimuli.

Guard cells have no plasmodesmata. Thereby, the presence of gasotransmitters as signal molecules that can easily cross cell membranes (or be transported by aquaporins) renders them especially relevant for the rapid transmission of information between guard cells and the surrounding mesophyll cells.

Biological systems possess multiple molecular pathways, some of them converging to the same end point. Redundant pathways appear unnecessary from an energetic point of view. However, when a road way becomes blocked, an alternative road can make the difference that rescues the organism from death. We speculate that this could be the case of the gasotransmitters that seem to share some common molecular targets influencing guard cell physiology. The three gasotransmitters NO, CO and H_2S bind to and inhibit mitochondrial cytochrome c oxidase and mitochondrial respiration with an inhibition constant (Ki) of 0.2 nM, 0.3 μM and 0.2 μM respectively (Ki for NO is 0.2 nM and 28 nM for the reduced and oxidized cytochrome c oxidase, respectively) [102]. Cytochrome c oxidase is in complex IV of the mitochondrial electron transport chain contributing to the H^+ extrusion and keeping the ATP formation by complex. As a consequence, increases in the gasotransmitter concentration generate partial or total inhibition of the mitochondrial cytochrome c oxidase. This results in a decrease of ATP formation and the activation of the alternative oxidase pathway that dissipates the high energy accumulated by electrons that cannot escape. Another consequence of respiratory mitochondrial inhibition by gasotransmitters is the overproduction of ROS, which directly effects on the redox status and physiology of the guard cells, probably leading to rapid stomatal closure. It would be interesting in the future to know the effect of a depressed mitochondrial ATP formation and decreased energy production in guard cells. An interesting challenge would be to know how the perception of the ATP status influences the physiology, ion channel activity and water status in guard cells, providing the fine tuned regulation of stomatal aperture. We have a hidden attraction to thinking that the gas exchange valve in plants, the stomatal pore, is also gas regulated.

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References

[1] R. Wang, Two's company, three's a crowd: can H_2S be the third endogenous gaseous transmitter? FASEB J. 16 (2002) 1792–1798.
[2] R. Wang, Signal Transduction and the Gasotransmitters: NO, CO, and H_2S in Biology and Medicine, Humana Press Inc., New Jersey, 2004.
[3] M. Herrera, N.J. Hong, J.L. Garvin, Aquaporin-1 transports NO across cell membranes, Hypertension 48 (2006) 157–164.
[4] W.F. Boron, Sharpey-Schafer lecture: gas channels, Exp. Physiol. 95 (2010) 1107–1130.
[5] J.S. Stamler, D.J. Singel, J. Loscalzo, Biochemistry of nitric oxide and its redox-activated forms, Science 258 (1992) 1898–1902.
[6] L.J. Ignarro, G.M. Buga, K.S. Wood, R.E. Byrns, G. Chaudhuri, Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide, Proc. Natl. Acad. Sci. U.S.A. 84 (1987) 9265–9269.
[7] U. Berchner-Pfannschmidt, S. Tug, M. Kirsch, J. Fandrey, Oxygen-sensing under the influence of nitric oxide, Cell. Signal. 22 (2010) 349–356.
[8] L.J. Ignarro, Nitric Oxide Biology and Pathobiology, Academic Press, San Diego, 2000.
[9] A. Besson-Bard, A. Pugin, D. Wendehenne, New insights into nitric oxide signaling in plants, Annu. Rev. Plant Biol. 59 (2008) 21–39.
[10] M. Delledonne, NO news is good news for plants, Curr. Opin. Plant Biol. 8 (2005) 390–396.
[11] L. Lamattina, C. García-Mata, M. Graziano, G. Pagnussat, Nitric oxide: the versatility of an extensive signal molecule, Annu. Rev. Plant Biol. 54 (2003) 109–136.
[12] K.J. Gupta, A.R. Fernie, W.M. Kaiser, J.T. van Dongen, On the origins of nitric oxide, Trends Plant Sci. 16 (2011) 160–168.
[13] R. Desikan, R. Griffiths, J. Hancock, S. Neill, A new role for an old enzyme: nitrate reductase-mediated nitric oxide generation is required for abscisic acid-induced stomatal closure in *Arabidopsis thaliana*, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 16314–16318.
[14] J. Bright, R. Desikan, J.T. Hancock, I.S. Weir, S.J. Neill, ABA-induced NO generation and stomatal closure in Arabidopsis are dependent on H_2O_2 synthesis, Plant J. 45 (2006) 113–122.
[15] J.-H. Li, et al., A signaling pathway linking nitric oxide production to heterotrimeric G protein and hydrogen peroxide regulates extracellular calmodulin induction of stomatal closure in Arabidopsis, Plant Physiol. 150 (2009) 114–124.
[16] N. Srivastava, V.K. Gonugunta, M.R. Puli, A.S. Raghavendra, Nitric oxide production occurs downstream of reactive oxygen species in guard cells during stomatal closure induced by chitosan in abaxial epidermis of *Pisum sativum*, Planta 229 (2009) 757–765.

[17] S.J. Neill, R. Desikan, A. Clarke, J.T. Hancock, Nitric oxide is a novel component of abscisic acid signaling in stomatal guard cells, Plant Physiol. 128 (2002) 13–16.
[18] H. Yamasaki, Y. Sakihama, Simultaneous production of nitric oxide and peroxynitrite by plant nitrate reductase: *in vitro* evidence for the NR-dependent formation of active nitrogen species, FEBS Lett. 468 (2000) 89–92.
[19] P. Rockel, Regulation of nitric oxide (NO) production by plant nitrate reductase in vivo and in vitro, J. Exp. Bot. 53 (2002) 103–110.
[20] J. Lozano-Juste, J. León, Enhanced abscisic acid-mediated responses in *nia1nia2noa1-2* triple mutant impaired in NIA/NR- and AtNOA1-dependent nitric oxide biosynthesis in Arabidopsis, Plant Physiol. 152 (2010) 891–903.
[21] L.V. Modolo, et al., Decreased arginine and nitrite levels in nitrate reductase-deficient *Arabidopsis thaliana* plants impair nitric oxide synthesis and the hypersensitive response to *Pseudomonas syringae*, Plant Sci. 171 (2006) 34–40.
[22] N. Foresi, et al., Characterization of a nitric oxide synthase from the plant kingdom: NO generation from the green alga *Ostreococcus tauri* is light irradiance and growth phase dependent, Plant Cell. 22 (2010) 3816–3830.
[23] P.C. Bethke, M.R. Badger, R.L. Jones, Apoplastic synthesis of nitric oxide by plant tissues, Plant Cell 16 (2004) 332–341.
[24] C.L. Hartsfield, Cross talk between carbon monoxide and nitric oxide, Antioxid. Redox Signal. 4 (2002) 301–307.
[25] S.W. Ryter, J. Alam, A.M.K. Choi, Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications, Physiol. Rev. 86 (2006) 583–650.
[26] L. Wu, Carbon monoxide: endogenous production, physiological functions, and pharmacological applications, Pharmacol. Rev. 57 (2005) 585–630.
[27] S.S. Wilks, Carbon monoxide in green plants, Science 129 (1959) 964–966.
[28] W. Xuan, S. Xu, X. Yuan, W. Shen, Carbon monoxide: a novel and pivotal signal molecule in plants? Plant Signal. Behav. 3 (2008) 381–382.
[29] G.S. Shekhawat, K. Verma, Haem oxygenase (HO): an overlooked enzyme of plant metabolism and defence, J. Exp. Bot. 61 (2010) 2255–2270.
[30] E. Baudouin, P. Frendo, M. Le Gleuher, A. Puppo, A *Medicago sativa* haem oxygenase gene is preferentially expressed in root nodules, J. Exp. Bot. 55 (2004) 43–47.
[31] B. Gisk, Y. Yasui, T. Kohchi, N. Frankenberg-Dinkel, Characterization of the haem oxygenase protein family in *Arabidopsis thaliana* reveals a diversity of functions, Biochem. J. 425 (2010) 425–434.
[32] W. Xuan, et al., Nitric oxide is involved in hemin-induced cucumber adventitious rooting process, J. Plant Physiol. 169 (2012) 1032–1039.
[33] Y. Han, et al., Exogenous hematin alleviates mercury-induced oxidative damage in the roots of *Medicago sativa*, J. Integr. Plant Biol. 49 (2007) 1703–1713.
[34] Y.-H. Chen, Y.-Y. Chao, Y.Y. Hsu, C.-Y. Hong, C.H. Kao, Heme oxygenase is involved in nitric oxide- and auxin-induced lateral root formation in rice, Plant Cell Rep. 31 (2012) 1085–1091.
[35] X.-P. She, X.-G. Song, Carbon monoxide-induced stomatal closure involves generation of hydrogen peroxide in *Vicia faba* guard cells, J. Integr. Plant Biol. 50 (2008) 1539–1548.
[36] Y.Y. Wei, Q. Zheng, Z.P. Liu, Z.M. Yang, Regulation of tolerance of *Chlamydomonas reinhardtii* to heavy metal toxicity by heme oxygenase-1 and carbon monoxide, Plant Cell Physiol. 52 (2011) 1665–1675.
[37] R.O. Beauchamp, J.S. Bus, J.A. Popp, C.J. Boreiko, D.A. Andjelkovich, A critical review of the literature on hydrogen sulfide toxicity, Crit. Rev. Toxicol. 13 (1984) 25–97.
[38] T.N. Das, R.E. Huie, P. Neta, S. Padmaja, Reduction potential of the sulfhydryl radical: pulse radiolysis and laser flash photolysis studies of the formation and reactions of ·SH and HSSH· – in aqueous solutions, J. Phys. Chem. A 103 (1999) 5221–5226.
[39] C. García-Mata, L. Lamattina, Hydrogen sulphide, a novel gasotransmitter involved in guard cell signalling, New Phytol. 188 (2010) 977–984.
[40] Z.-G. Li, M. Gong, H. Xie, L. Yang, J. Li, Hydrogen sulfide donor sodium hydrosulfide-induced heat tolerance in tobacco (*Nicotiana tabacum* L) suspension cultured cells and involvement of Ca^{2+} and calmodulin, Plant Sci. 185-186 (2012) 185–189.
[41] J. Liu, L. Hou, G. Liu, X. Liu, X. Wang, Hydrogen sulfide induced by nitric oxide mediates ethylene-induced stomatal closure of *Arabidopsis thaliana*, Chin. Sci. Bull. 56 (2011) 3547–3553.
[42] H. Zhang, et al., Hydrogen sulfide promotes wheat seed germination under osmotic stress, Russ. J. Plant Physiol. 57 (2010) 532–539.
[43] H. Zhang, et al., Hydrogen sulfide protects soybean seedlings against drought-induced oxidative stress, Acta Physiol. Plant. 32 (2010) 849–857.
[44] H. Zhang, et al., Hydrogen sulfide promotes root organogenesis in *Ipomoea batatas, Salix matsudana* and *Glycine max*, J. Integr. Plant Biol. 51 (2009) 1086–1094.
[45] H. Zhang, et al., Hydrogen sulfide stimulates alpha-amylase activity during early stages of wheat grain germination, Plant Signal. Behav. 5 (2010) 556–567.
[46] M. Lisjak, et al., A novel hydrogen sulfide donor causes stomatal opening and reduces nitric oxide accumulation, Plant Physiol. Biochem. 48 (2010) 931–935.
[47] M. Lisjak, et al., Hydrogen sulfide effects on stomatal apertures, Plant Signal. Behav. 6 (2011) 1444–1446.
[48] R. Desikan, Sniffing stomata? New Phytol. 188 (2010) 910–913.
[49] R. d'Emmanuele di Villa Bianca, et al., Hydrogen sulfide as a mediator of human corpus cavernosum smooth-muscle relaxation, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 4513–4518.
[50] L. Li, et al., Anti-inflammatory and gastrointestinal effects of a novel diclofenac derivative, Free Radic. Biol. Med. 42 (2007) 706–719.
[51] H.M. Harrington, I.K. Smith, Cysteine metabolism in cultured tobacco cells, Plant Physiol. 65 (1980) 151–155.
[52] C. Alvarez, L. Calo, L.C. Romero, I. García, C. Gotor, An O-acetylserine(thiol)lyase homolog with L-cysteine desulfhydrase activity regulates cysteine homeostasis in Arabidopsis, Plant Physiol. 152 (2010) 656–669.
[53] J. Papenbrock, A. Riemenschneider, A. Kamp, H.N. Schulz-Vogt, A. Schmidt, Characterization of cysteine-degrading and H_2S-releasing enzymes of higher plants – from the field to the test tube and back, Plant Biol. 9 (2007) 582–588.
[54] E.A.H. Pilon-Smits, et al., Characterization of a NifS-like chloroplast protein from Arabidopsis. Implications for its role in sulfur and selenium metabolism, Plant Physiol. 130 (2002) 1309–1318.
[55] D. Van Hoewyk, M. Pilon, E.A.H. Pilon-Smits, The functions of NifS-like proteins in plant sulfur and selenium metabolism, Plant Sci. 174 (2008) 117–123.
[56] H. Rennenberg, P. Filner, Stimulation of H_2S emission from pumpkin leaves by inhibition of glutathione synthesis, Plant Physiol. 69 (1982) 766–770.
[57] I. García, et al., Mitochondrial beta-cyanoalanine synthase is essential for root hair formation in *Arabidopsis thaliana*, Plant Cell. 22 (2010) 3268–3279.
[58] K. Grossmann, A role for cyanide, derived from ethylene biosynthesis, in the development of stress symptoms, Physiol. Plant. 97 (1996) 772–775.
[59] A.M. Hetherington, F.I. Woodward, The role of stomata in sensing and driving environmental change, Nature 424 (2003) 901–908.
[60] Y. Ma, et al., Regulators of PP2C phosphatase activity function as abscisic acid sensors, Science 324 (2009) 1064–1068.
[61] S.-Y. Park, et al., Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins, Science 324 (2009) 1068–1071.
[62] J. Santiago, et al., Modulation of drought resistance by the abscisic acid receptor PYL5 through inhibition of clade A PP2Cs, Plant J. 60 (2009) 575–588.
[63] T. Umezawa, et al., Type 2C protein phosphatases directly regulate abscisic acid-activated protein kinases in Arabidopsis, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 17588–17593.
[64] H. Fujii, et al., In vitro reconstitution of an abscisic acid signalling pathway, Nature 462 (2009) 660–664.
[65] Z. Cao, et al., Involvement of carbon monoxide produced by heme oxygenase in ABA-induced stomatal closure in *Vicia faba* and its proposed signal transduction pathway, Chin. Sci. Bull. 52 (2007) 2365–2373.
[66] C. García-Mata, L. Lamattina, Nitric oxide induces stomatal closure and enhances the adaptive plant responses against drought stress, Plant Physiol. 126 (2001) 1196–1204.
[67] X. She, X. Song, J. He, Role and relationship of nitric oxide and hydrogen peroxide in light/dark-regulated stomatal movement in *Vicia faba*, Acta Botan. Sinica 46 (2004) 1292–1300.
[68] L. Klepper, Comparison between NO_x evolution mechanisms of wild-type and nr_1 mutant soybean leaves, Plant Physiol. 93 (1990) 26–32.
[69] C. Garcia-Mata, et al., Nitric oxide regulates K^+ and Cl^- channels in guard cells through a subset of abscisic acid-evoked signaling pathways, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 11116–11121.
[70] A.M. Distéfano, C. García-Mata, L. Lamattina, A.M. Laxalt, Nitric oxide-induced phosphatidic acid accumulation: a role for phospholipases C and D in stomatal closure, Plant Cell Environ. 31 (2008) 187–194.
[71] S. Sokolovski, M.R. Blatt, Nitric oxide block of outward-rectifying K^+ channels indicates direct control by protein nitrosylation in guard cells, Plant Physiol. 136 (2004) 4275–4284.
[72] C. Garcia-Mata, L. Lamattina, Abscisic acid (ABA) inhibits light-induced stomatal opening through calcium- and nitric oxide-mediated signaling pathways, Nitric Oxide: Biol. Chem. 17 (2007) 143–151.
[73] X. Zhang, A. Takemiya, T. Kinoshita, K. Shimazaki, Nitric oxide inhibits blue light-specific stomatal opening via abscisic acid signaling pathways in *Vicia* guard cells, Plant Cell Physiol. 48 (2007) 715–723.
[74] M. Kajimura, R. Fukuda, R.M. Bateman, T. Yamamoto, M. Suematsu, Interactions of multiple gas-transducing systems: hallmarks and uncertainties of CO, NO, and H_2S gas biology, Antioxid. Redox Signal. 13 (2010) 157–192.
[75] L.J. Kok, K. Stahl, H. Rennenberg, Fluxes of atmospheric hydrogen sulphide to plant shoots, New Phytol. 112 (1989) 533–542.
[76] F.M. Maas, E.N. Loo, P.R. Hasselt, Effect of long-term H_2S fumigation on photosynthesis in spinach. Correlation between CO_2 fixation and chlorophyll a fluorescence, Physiol. Plant. 72 (1988) 77–83.
[77] K. Ondrias, et al., H_2S and HS- donor NaHS releases nitric oxide from nitrosothiols, metal nitrosyl complex, brain homogenate and murine L1210 leukaemia cells, Pflugers Archiv. 457 (2008) 271–279.
[78] M. Whiteman, et al., Evidence for the formation of a novel nitrosothiol from the gaseous mediators nitric oxide and hydrogen sulphide, Biochem. Biophys. Res. Commun. 343 (2006) 303–310.
[79] C. Coletta, et al., Hydrogen sulfide and nitric oxide are mutually dependent in the regulation of angiogenesis and endothelium-dependent vasorelaxation, Proc. Natl. Acad. Sci. U.S.A. 109 (2012) 9161–9166.
[80] H. Zhang, et al., Hydrogen sulfide counteracts chlorophyll loss in sweetpotato seedling leaves and alleviates oxidative damage against osmotic stress, Plant Growth Regul. 58 (2009) 243–250.
[81] B.E. Robertson, R. Schubert, J. Hescheler, M.T. Nelson, cGMP-dependent protein kinase activates Ca-activated K channels in cerebral artery smooth muscle cells, Am. J. Physiol. 265 (1993) C299–C303.
[82] V.M. Bolotina, S. Najibi, J.J. Palacino, P.J. Pagano, R.A. Cohen, Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle, Nature 368 (1994) 850–853.

[83] E. Murphy, M. Kohr, J. Sun, T. Nguyen, C. Steenbergen, S-nitrosylation: a radical way to protect the heart, J. Mol. Cell. Cardiol. 52 (2012) 568–577.
[84] E. Clementi, J. Meldolesi, The cross-talk between nitric oxide and Ca^{2+}: a story with a complex past and a promising future, Trends Pharmacol. Sci. 18 (1997) 266–269.
[85] W.J. Wilkinson, P.J. Kemp, Carbon monoxide: an emerging regulator of ion channels, J. Physiol. 589 (2011) 3055–3062.
[86] C. Peers, C.C. Bauer, J.P. Boyle, J.L. Scragg, M.L. Dallas, Modulation of ion channels by hydrogen sulfide, Antioxid. Redox Signal. 17 (2012) 95–105.
[87] W. Yang, G. Yang, X. Jia, L. Wu, R. Wang, Activation of KATP channels by H_2S in rat insulin-secreting cells and the underlying mechanisms, J. Physiol. 569 (2005) 519–531.
[88] S.J. Suh, et al., The ATP binding cassette transporter AtMRP5 modulates anion and calcium channel activities in Arabidopsis guard cells, J. Biol. Chem. 282 (2007) 1916–1924.
[89] E. Martinoia, et al., Multifunctionality of plant ABC transporters – more than just detoxifiers, Planta 214 (2002) 345–355.
[90] M. Klein, et al., Disruption of AtMRP4, a guard cell plasma membrane ABCC-type ABC transporter, leads to deregulation of stomatal opening and increased drought susceptibility, Plant J. 39 (2004) 219–236.
[91] L. Zhang, X. Zhao, Y.-J. Wang, X. Zhang, Crosstalk of nitric oxide with Ca^{2+} in stomatal movement in *Vicia faba* guard cells, Acta Agron. Sin. 35 (2009) 1491–1499.
[92] X. Zhao, X.-R. Qiao, J. Yuan, X.-F. Ma, X. Zhang, Nitric oxide inhibits blue light-induced stomatal opening by regulating the K^+ influx in guard cells, Plant Sci. 184 (2012) 29–35.
[93] T. Vahisalu, et al., SLAC1 is required for plant guard cell S-type anion channel function in stomatal signalling, Nature 452 (2008) 487–491.
[94] M.C. Lombardo, L. Lamattina, Nitric oxide is essential for vesicle formation and trafficking in Arabidopsis root hair growth, J. Exp. Bot. 63 (2012) 4875–4885.
[95] J.-U. Sutter, et al., Abscisic acid triggers the endocytosis of the arabidopsis KAT1 K^+ channel and its recycling to the plasma membrane, Curr. Biol. 17 (2007) 1396–1402.
[96] M. Tal, D. Imber, C. Itai, Abnormal stomatal behavior and hormonal imbalance in flacca, a wilty mutant of tomato: I. Root effect and kinetin-like activity, Plant Physiol. 46 (1970) 367–372.
[97] R.J. Jones, T.A. Mansfield, Suppression of stomatal opening in leaves treated with abscisic acid, J. Exp. Bot. 21 (1970) 714–719.
[98] D. Imber, M. Tal, Phenotypic reversion of *flacca*, a wilty mutant of tomato, by abscisic acid, Science 169 (1970) 592–593.
[99] C. García-Mata, L. Lamattina, Nitric oxide and abscisic acid cross talk in guard cells, Plant Physiol. 128 (2002) 790–792.
[100] A. Kulik, I. Wawer, E. Krzywińska, M. Bucholc, G. Dobrowolska, SnRK2 protein kinases-key regulators of plant response to abiotic stresses, OMICS 15 (2011) 859–872.
[101] X. Song, X. She, B. Zhang, Carbon monoxide-induced stomatal closure in *Vicia faba* is dependent on nitric oxide synthesis, Physiol. Plant. 132 (2008) 514–525.
[102] C.E. Cooper, G.C. Brown, The inhibition of mitochondrial cytochrome oxidase by the gases carbon monoxide, nitric oxide, hydrogen cyanide and hydrogen sulfide: chemical mechanism and physiological significance, J. Bioenerg. Biomembr. 40 (2008) 533–539.