Review

Stomatal movements and long-distance signaling in plants

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As the nerve-mediated signaling in animals, long-distance signaling in plants is a prerequisite for plants to be able to perceive environmental stimuli and initiate adaptive responses. While intracellular signal transduction has been attracting considerable attentions, studies on long-distance signaling in plants has been relatively overlooked. Stomatal movements are well recognized as a model system for studies on cellular signal transduction. It has been demonstrated that the stomatal movements may be frequently tuned by long-distance signaling under various environmental stimuli. Stomatal movements can not only respond to persistent stress stimuli but also respond to shock stress stimuli. Stomatal responses to drought stress situations may be best characterized in terms of interwoven networks of chemical signaling pathways playing predominant roles in these adaptive processes. In cases of shock stress stimuli, stomatal movements can be more sensitively regulated through the long-distance signaling but with distinctive patterns not observed for drought or other persistent stresses. Here, the fundamental characteristics of stomatal movements and associated long-distance signaling are reviewed and the implications for plant responses to environmental stresses are discussed.

Introduction

Capability to respond to environmental stimuli is a basic essence for a living organism to live and thrive. It appears that the more advanced the organism is, the greater behavioral variability to these responses display. Stimulus-response adaptive behavior is embodied in the way that the living organism is able to take adaptive actions before serious harms occur. Such responses are best demonstrated in animals where the nerve-mediated signaling enable animal to react swiftly and purposefully, thus preserving them from possible harms. The nerve-mediated signaling is characterized by a pattern of long-distance signaling, i.e., when one part is stimulated another distinct part of the body may take corresponding actions. Living in ever-changing environments, plants should have evolved the capability to sense and respond to various stress stimuli. In recent years, with a rapid progress in molecular biology, the molecular mechanisms for stress-resistance in plants have attracted considerable attentions. Studies on cellular signal transduction have revealed that in response to environmental stresses expressions of numerous genes associated with stress tolerances can be regulated, indicating that plant cells are indeed able to sense and respond to environmental stresses.[1-3] Nevertheless, knowledge about the plant adaptation responses associated with long-distance signaling is relatively scarce compared with that on the molecular biology.

Stomata, a delicate cellular structure to control CO_2 uptake and water loss, are capable of responding to various stimuli, such as light, hormone, CO_2, temperature and humidity, becoming a highly developed model system to investigate the signal transduction in plants. Importantly, in the past several decades, more and more studies have suggested that stomatal movements can not only be a model system for researches on cellular signal transduction, but also be a model system for researches on plant long-distance signaling since it has been increasingly documented that the stomatal behavior may be remotely regulated in response to a variety of environmental stresses. Among the researches concerning stomatal movements in relation to long-distance signaling, the best studied are the stomatal movements in relation to root-to-shoot signaling under drought stresses. Abscisic acid, pH, hydraulic signal have been demonstrated to be the dominant signals involved in the long-distance signaling process under drought stress.[4-6] Long-distance delivery of cytokinins, acetylcholine (Ach) and other biologically active substances also play some potential roles in keeping stomata in a normal state under non-stressed condition.[7] In the case of stress condition, they may either directly act as negative signals or possibly as modulators of stomatal sensitivity thus playing important roles in stomatal responses to the stress-induced remote signals. More interestingly, it was recently observed that stomata are capable of responding to local shock stimuli with a signaling pattern not seen in drought or other persistent stresses. With an aim to illustrate the particular roles of the long-distance signaling in plants, this review shall summarize the fundamental characteristics of the stomatal regulation associated with long-distance signaling and discuss its implications for plant responses to environmental stresses.

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Stomatal Movements in Response to Persistent Stimuli of Environemntal Stress

Abscisic acid signal. It is traditionally thought that leaf stomatal conductance is closely correlated with leaf water potential (Ψ_l), such that the decrease in stomatal conductance only occurs when a decrease in the leaf water potential occurs under a soil drying condition.[4] The root-split experiment by Blackman and Davies[8] demonstrated that a decrease in leaf stomatal conductance did occur even no perceptible changes occurred in leaf water potential under soil drying condition.[8] The experiment with a soil pressure chamber also provided strong evidences that leaf stomatal conductance is able to reduce while no decrease in leaf water potential was observed.[9] From these experiments, it was concluded that, in response to soil drying, the leaf stomatal conductance was regulated by root to shoot signals.

It has long been known that abscisic acid (ABA) is able to regulate stomatal movements.[10,11] Therefore, whether ABA can be a root-to-shoot signal has attracted considerable attention. Numerous studies have found that drought stress can induce a dramatic increase in ABA content in both root and xylem sap, and the increase of ABA is closely correlated with a decrease in leaf stomatal conductance.[12-14] Feeding xylem sap collected from drought plants to detached leaves was able to inhibit stomatal movement, and such a inhibitory effect could be relieved when ABA was removed form the xylem sap by a ABA affinity column.[4,15] These studies demonstrated that ABA is able to act as a long-distance signal to remotely regulate stomatal movement in the early stage of drought stress.[4] While the crucial roles of ABA in the root-to-shoot signaling have been well established, it should be noted that the mechanism for the long-distance signaling is complicated and ABA may not be a universal signal that plays a central role in all cases. There are evidences that the remote regulation of stomatal movement by root-derived ABA is dependent on plant species. For instance, root-derived ABA may be able to act as a central signal to sensitively regulate stomatal movement in many plant species,[12,13,16] whereas in some other plant species it may not be able to do so.[17,18] The remote regulation of stomatal movements by root-derived ABA under drought stress has been extensively reviewed.[4-6,19,20]

Except for the case of drought stress, the information on the ABA regulation of stomatal movements in response to other environmental stresses is relatively sparse. Montero et al.,[21] found that salt stress was able to induce an increase in xylem ABA content, and furthermore, that increase in xylem ABA content was highly correlated to a decrease in stomatal conductance in *Phaeolus vugaris.* Likewise, Sauter et al., reported that salt stress was able to induce dramatic increases in both free and conjugated ABA in xylem saps in several plant species, such as barley, maize and *Anastatica hierochuntica.*[22] These studies seem to suggest an important role of ABA in the root-to-shoot signaling under salt stress at least in some plant species. In case of flooding stomatal closure can be observed. ABA was suggested to be implicated in the flooding-induced stomatal closure since flooding frequently results in increases in foliar ABA[23] and ABA deficient mutant of pea (*Pisum sativam*) have impaired stomatal responses to flooding.[23,24] However, flooding is not able to induce an increase in xylem sap ABA, and in contrary, it often induces a decrease in xylem sap ABA. Because of this, flooding-induced stomatal closure is clearly not induced by a delivery of ABA signal from root to shoot. A building up of ABA in leaves was proposed to account for the flooding-induced stomatal closure,[23] but such a hypothesis has not been commonly accepted since the building up of ABA is too slow and short-lived.[25]

pH signal. ABA compartmentalization in plant tissue is commonly thought to be determined by cellular pH, as amounts of root-derived ABA accumulated in its target sites, i.e., the outside of guard cells,[26,27] can be regulated by leaf apoplastic pH. Based on this theory, a change in leaf apoplastic pH should result in a change in the amount of ABA accumulated in its target sites and thus induce a change in the stomatal aperture. This virtually means that pH may be able to act as signal to regulate stomatal movement if a pH change can be triggered by some stimuli. Indeed, it has repeatedly been shown that drought stress is able to induce an increase in xylem sap pH for many plant species.[5,28] It was proposed that the soil drying-induced pH increase in xylem would make apoplast of the leaf more alkaline, which would contribute to a sequestration of more ABA in the apoplast of guard cells, thus promoting the stomatal closure in the presence of ABA.[5,19,28] By feeding artificial xylem sap buffered to different pH to detached leaves of *Commelina communis*, Wilkinson and Davies[27] found that an increase in pH from 6.0 to 7.0 caused a reduction of transpiration rate by about 50% in the presence of low concentration of ABA.[27] More recently, Jia and Davies[28] demonstrated that modulation of apoplastic pH was capable of modulating of stomatal movements in response to root-derived ABA signal.[29] These data strongly suggest that pH can be a signal to coordinately regulate stomatal movement with the ABA signal under drought stress.[5] Nevertheless, other studies have shown that the pH signaling can not be a mechanism universally adopted by all plant species. For instance, there are evidences that drought stress may even lead to a decrease in xylem sap pH in some plant species.[6,28] Except for the case of drought stress, literature on stomatal regulation by pH signaling is rare. Nevertheless, it was occasionally reported that some other stresses, such as flooding, might be involved in pH signaling.[23]

Hydraulic signal. While chemical signals have been increasingly demonstrated to be responsible for root-to-shoot signaling in response to soil drying, there are evidences supporting a hydraulic signaling.[29-33] For example, Fuchs and Livingston[31] found that the soil drying-induced reduction in leaf conductance could be progressively reversed by the pressurization of the root system. In addition, the leaf conductance would return to its pre-pressurization levels within minutes once the pressurization was released. This indicates that hydraulic signal was a predominant regulator of the stomatal behavior. Similar findings were made by Saliendra et al., in woody plant *Betula occidentalis*[33] and Yao et al., in bell pepper.[30]

Reports on both chemical and hydraulic signals seem to be reasonable. How can we understand this conflict? One hypothesis for it is the hydraulic signal contributes to promote stomatal sensitivity to root-derived chemical signals. Such an idea was supported by a study of Tardieu and Davies,[34,35] who found that a relatively lower water potential of *Commelina communis* epidermis would significantly promote ABA-induced stomatal closure although the lower water potential had no direct effect on the stomatal aperture. The same observation was obtained by feeding ABA into the field-grown plants over different ranges of leaf water potential.[34] Besides a direct modulation for the stomatal sensitivity, the hydraulic signal

may also modify the concentration or flux of the chemical message as a function of the changes in water flux.[35] In addition to the explanations above, it is more likely that the chemical and hydraulic signals function in different plant species or at a different stress stage. It appears that hydraulic signaling function more likely in woody plants.[30-33] If both chemical and hydraulic signaling are involved in a specific signaling process, it is important to determine at what stage which signaling pattern plays a predominant role, and at what stage different signaling patterns may synergistically to control the stomatal behavior.

Stomatal Movements in Response to Shock Stimuli of Environmental Stresses

For most environmental stimuli, such as drought, salt stress, flooding or hypoxia, their impacts on plant physiology are relatively persistent. Besides these stresses, there are some other stresses, such as wounding and touch, which are characterized by a sudden impact or the so-called shock stimuli. Compared to studies on drought, salt or flooding stress, studies on stomatal responses in response to shock stimuli are rare. More recently, a study in our laboratory has shown that heat-shock of root is able to produce a strong impact on the stomatal behavior in *Commelina communis L.*[36] In this latter study, heat-shock was induced by immersing only part of the root system into hot water and the leaf stomatal conductance was found to decline to a lowest level within only several tenths of minutes. Surprisingly, after the initial decrease, it rapidly increased to a higher level, and again, went down to another lowest level. After several such cycles, so-called oscillation, the stomatal conductance would be finally stabilized in a relatively lower level. The bigger portion of the root system was exposed to heat stress, or the higher was the shock-temperature, the stronger the oscillation and inhibitory effects were scored. More importantly, the inhibitory effect of heat-shock on stomatal movement is much stronger than that of drought stress.

The phenomenon of stomatal oscillations has been observed long-time ago.[37] It is traditionally believed that these stomatal oscillations result from disturbances of the leaf or xylem water potentials. In keeping with this proposition, in early 60's of the last century, papers by Barrs et al.,[38] and Ehrler et al.,[39] suggested correlations between stomatal oscillations and leaf water potential. Moreover, study of Steppe et al.,[40] reported stomatal oscillations in association with an oscillation in xylem water potential. In recent years, studies on signal transduction in guard cells have demonstrated that Ca^{2+} signaling may play crucial roles in stomatal oscillation.[41-43] More recently, Yang et al.,[44] found that a rapid ion-exchange treatment between Ca^{2+} and K was capable of triggering stomatal oscillations. With a pharmacological method, they have demonstrated that water channel may be involved in the process since water channel inhibitor $HgCl_2$ could completely block the ion-exchange induced stomata oscillations.[44] It is not surprising to find that stomatal oscillations may occur in response to many environmental stimuli, because direct stimuli on guard cells may be able to trigger Ca^{2+} and water channel associated signaling.[41-43] Unlike common environmental stimuli, the stomatal oscillations in response to heat shock on partial root is mediated by long-distance signaling. Further investigation demonstrated that all the well known long-distance signals or factors, such as ABA, pH, leaf water potential and hydraulic, are not involved in the signaling process.[36] Reactive oxygen species (ROS) are well recognized as critical signals in guard cell signal transduction and closely associated with Ca^{2+} signal. It appears that ROS may be in part involved in the signaling process, but it can not account for the effect of stomatal oscillations. The virtual mechanism for root heat shock-induced stomatal oscillations remains unknown.

Since early in the last century, when electric signal was firstly recorded in some sensitive plants such as *Mimosa pudica,* the electric signaling in plants has greatly attracted the attention of many plant scientists. Besides in sensitive plants, electric signaling has also been established to be involved in the signaling in sensitive organs, such as tendrils, of climbing plants.[7,45-47] In common plants, it has been well established that root scorch is able to induce the production of electric signal, which propagates vertically along the vascular bundle to the stem tip and also propagates vertically along the leaf vein.[7] It has been suggested that long-distance electric signaling may be involved in various physiological processes.[45,48-51] It is not known whether heat-shock induced stomatal oscillation is associated with electric signaling. Literature concerning stomatal oscillation in response to long-distance signaling is rare. Further revealing this mechanism will contribute to our understanding of the profound implications of the long-distance signaling.

Stomatal Movements in Relation to the Integration of Multi-Signals

Long-distance delivery of biologically active substances in stomatal movements. Cytokinins and IAA are commonly regarded as antagonists of ABA action on stomata. Because cytokinins are known to be synthesized mainly in roots, special attention has been paid on its long-distance delivery in relation to the stomatal regulation under environmental stresses. There are evidences that external application of cytokinins is capable of maintaining stomata in an open state,[8,52] and that elevated levels of leaf cytokinins is correlate with stomatal opening in some plants.[53] In transgenic tobacco plants, overproducing cytokinins resulted in an increased transpiration,[54] suggesting important roles of cytokinins in the regulation of stomatal behavior. Acetylcholine (Ach) is well known to be a nerve transmitter, which plays crucial roles in animal signaling. Surprisingly, Ach was found to exist and function in many physiological processes in plants.[55-59] Feeding Ach at the proper dosage to the de-rooted plantlet via the cut end, the Lou's research group has found that Ach is able to enhance the transpiration of *Vicia faba* seedling with a much stronger effect than cytokinins. Furthermore, it was also demonstrated that Ach could be produced in roots and delivered to leaf in *Vicia faba* plant.[60] In addition, it was found that neostigmine, an inhibitor of Ach-esterase, could promote the regulatory effect of Ach on stomatal behavior. In contrary, atropine, an inhibitor of Ach receptor, reduces the regulatory effect of Ach on stomatal movement, suggesting the presence of Ach-esterase and receptor in guard cells. More important, an experiment in the Chenghou Lou's laboratory has shown that the stomatal opening at a normal condition requires message delivery from root-to-shoot. In this experiment, the cut end of sweet potato shoot was immersed into water and the water supply to the shoot was fast enough to meet leaf transpiration so that no decrease in the leaf water potential was observed. If all of the adventitious roots in the moist chamber were moved from the plantlet, the stomatal transpiration gradually declined for one day or so, and the stomatal behavior became torpid and reluctantly opened until finally closed under day

light. However, as new adventitious roots appeared in succession, the behavior of stomata would gradually return to its normal state. Further investigations demonstrated that the message responsible for the stomatal opening is likely Ach because application of Ach is capable of replacing the roots for keeping the stomatal behavior normal.[7] Taken together, all these findings suggested a critical role of Ach in the regulation of stomatal behavior.

When discussing stomatal responses in response to long-distance signaling, we normally focus our attention on the characteristics of the long-distance delivered signals. In fact, the question of stomatal sensitivity to the signal is by no means less important than the signal itself. The reason for this is that in many cases plant is not able to respond to long-distance signals regardless of a dramatic increase in the signal intensity induced by stress stimuli.[6,17,18] For example, in wheat plant, it was reported that drought stress could result in an increase in xylem sap ABA for nearly 50 times, but such a dramatic increase of ABA was not able to inhibit stomatal movements.[18] Until now, we do not have a clear idea how the stomatal sensitivity is controlled. As discussed above, cytokinins and Ach have been suggested to play crucial roles in the regulation of stomatal movement in normal conditions. In terms of the topic concerning long-distance signaling in response to environmental stresses, it is important to know whether they contribute to stomatal regulation through long-distance signaling under stress conditions.

A change in the delivery rate/amount of bio-active substances is a prerequisite for them to act as signals. No information is available about whether the long-distance delivery of Ach can change significantly in response to environmental stresses. A few of studies suggested that drought stress may be able to induce a reduction of xylem sap cytokinins in some plant species,[61-64] but this reduction occurs only when drought stress is very severe.[52] Because of this, it is difficult to propose whether cytokinins and Ach may be able to act as long-distance signals to regulate stomatal movement under drought stresses. Nevertheless, cytokinins and Ach are well characterized to be antagonists of ABA action on stomata, hence it can be inferred that the normal delivery of these substances from root to shoot should reduce stomatal responses to root-derived ABA signal, which means that these substances may likely play important roles in modulation of stomatal sensitivity to root signals under drought stress. Therefore, in terms of the importance of stomatal sensitivity, additional attentions should be paid to the roles of cytokinins, Ach and other related antagonists of ABA in the long-distance signaling no matter whether drought stress may be able to induce significant changes in the delivery of the these substances.

Integration of Multi-Signals

We normally think that long-distance signaling is mediated by a major signal. Indeed, in the case of drought stress, ABA has been suggested to play a critical role in the root-to-shoot signaling. However, with more and more information available, it has been increasingly clear that long-distance signaling may actually involve many signals, and integration of these signals is crucial for them to sensitively trigger the corresponding responses. Stomatal movement in relation to root-to-shoot signaling under drought stress can be a good model to illustrate this. Generally speaking, stomatal response in response to drought stress should be dissected from two major

aspects: one is the mechanism for the regulation of signal delivery from root-to-shoot, and another is the mechanism for the modulation of stomatal sensitivity to the root-derived signals. The capability of root signals in stomatal regulation is clearly determined by an integration of the two mechanisms above. In case of drought stress, mechanism for the regulation of long-distance signaling of ABA is complex. Microorganisms in rhizosphere, lateral transport in root cortex across apoplastic barriers, redistribution in the stem, leaf apoplastic pH and rapid catabolism in leaf tissues, all these factors may play crucial roles in the regulation of root-derived ABA accumulation in the action sites of guard cells therefore regulating the stomatal responses to the drought stress stimuli.[6,20,65-68] Among these factors, pH has been paid particular attentions due to its potential roles in controlling ABA accumulation in action sites. It has been well documented that pH is an important signal and in many cases, only when ABA signals are integrated with pH signals, are stomata able to respond adaptively to drought stress. Therefore, pH has now been recognized as a co-signal of the ABA signal.[5,6,19]

More complexly, pH itself is frequently affected by some other factors such as nitrate and ammonium. Accordingly, these factors also have been demonstrated to be important modulators of the stomatal responses to drought stress.[6,28] Direct modulation of stomatal sensitivity to root signals is an important mechanism for the regulation of stomatal movements in response to stress stimuli, but information on this topic is much less abundant compared with that on root to shoot signaling. While hydraulic signals have been demonstrated to play independent roles in some plant species or in some stages of drought stress, there are evidences that hydraulic signals may be able to directly modulate stomatal sensitivity to ABA signal thus modulating the stomatal responses to drought stress.

There are many arguments for roles of cytokinins, Ach and some other biologically active substances in the stomatal regulation. Since these substances are well established to be antagonists of ABA action on stomata, no matter whether drought stress may be able to induce significant changes in their long-distance delivery, theses substance should play potential roles in modulating stomatal responses to root-derived ABA signal. Taken stomatal sensitivity into account, integrations of ABA signaling with long-distance delivery of cytokinins, Ach and other related ABA antagonists should be paid more attentions in dissecting the regulation mechanisms for stomatal responses to drought stress.

Conclusions

Living in an ever-changing environment, plants must have evolved the capabilities to sense and respond to varieties of environmental stimuli. Theses capabilities are not only embodied in the plant cells, being capable of making various molecular or biochemical responses through intracellular signal transduction in response to direct stimuli, but also initiate systemic responses through long-distance signaling in response to local stimuli. The essence of the long-distance signaling is to initiate the responses when a stimulus is applied on only part of individual plants. This is clearly a major characteristic of the reaction. Stomatal movements in response to drought stress may be the best characterized example concerning the long-distance signaling under environmental stresses. ABA as a central signal in the root-to-shoot chemical signaling has been demonstrated to play predominant roles in this process.

Besides the massage delivery process in the long-distance signaling, modulation of the stomatal sensitivity should also be a quite important aspect since it is closely associated with the capability of root signals in the regulation of stomatal movements. Under normal condition, long-distance delivery of some biologically active substances may play potential roles in keeping stomata in a normal state, and under a stressed condition, it may contribute a great deal to the modulation of stomatal sensitivity to the long-distance delivered ABA signal. Stomatal movements can not only respond to a persistent stress stimulus, such as drought, salt, hypoxia and so on, but also respond to a shock stimulus, for example, a heat shock. In case of a shock-stimulus, the stomatal movement displays distinct behaviors not ever seen in case of a persistent stress. Unfortunately, much less is known about the nature of the signals involved in this signaling process. Adaptive responses and stress tolerance, as mediated by long-distance signaling, should play crucial roles in helping plants to cope with various environmental stresses. The regulation of stomatal movements through the long-distance signaling is just one example in this emerging field of plant science. In future, more attentions should be paid to the mechanisms for the stress tolerance associated with long-distance signaling, because it can not only contribute a great deal to our understanding of the mechanisms for plant stress tolerance and adaptive plant behavior, but also to our understanding integrated plant signaling and communication, with implications of the recently emerging "plant neurobiology".[45-47]

Acknowledgements

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