Research News

The long and the short of stomatal density signals

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Recent studies have shown that environmental factors such as light and CO_2 are sensed by the mature leaves, which relay this information to developing leaves via long-distance systemic signals. The developing leaves respond by adjusting their stomatal density or stomatal index. These long-distance signals might interact with short-range signals that determine the density and patterning of stomata on the developing leaves. Clues to the nature of these interactions and the molecules involved, are bringing fresh understanding of the factors that regulate the density and patterning of stomata in response to environmental stimuli.

Stomata allow the exchange of CO_2, O_2 and water vapour between the leaf and the atmosphere. It has long been known that short-term regulation of gas exchange across the leaf surface is effected by controlling the stomatal aperture, which, in turn, is regulated by turgor-induced shape changes of the stomatal guard cells. Thus, for example, the stomatal aperture is reduced during drought stress. Under extreme stress, the stomata can close completely. Gas exchange can also be influenced by the abundance of stomata on the leaf surface, measured as the number of stomata per unit area of leaf surface (stomatal density) or the proportion of stomata relative to the total epidermal cell number (stomatal index). Regulation of stomatal density can be considered as a means of setting limits on the rate of gas exchange for a given set of conditions. Recently, some remarkable observations have shed light on the factors controlling stomatal density[1–3]. These studies are also beginning to reveal important clues about the mechanisms underlying stomatal patterning and epidermal cell fate. In most plant species, it appears that stomatal density is lower in plants that are grown in high levels of CO_2 (Ref. 4). Thus, plants grown in reduced CO_2 levels have higher numbers of stomata than those grown in modern-day CO_2 concentrations. Further increases of CO_2 can result in further small decreases in stomatal density. These observations are consistent with comparisons of stomatal density of modern day and preserved specimens collected before post-industrial atmospheric CO_2 elevation[5]. There are also good correlations between paleaoclimate model-derived estimates of atmospheric CO_2 levels and the stomatal density and index of fossil plants[6,7], and are therefore useful indicators of past atmospheric CO_2 levels[8].

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Other environmental factors, notably light, might also exert a control over stomatal density. It has been known for several years that leaves of plants developing under high levels of light have increased stomatal densities[9].

Signalling between old and new leaves

Ian Woodward and co-workers have shown that the density of stomata in developing leaves is regulated by a mechanism that senses CO_2 levels around mature leaves rather than by developing leaves themselves[1]. These workers showed that selectively exposing mature leaves of *Arabidopsis* to elevated CO_2 resulted in developing leaves with a reduced stomatal density and index that were exposed only to ambient CO_2. Conversely, exposing mature leaves to low CO_2 and developing leaves to elevated CO_2 resulted in a higher stomatal density and index in the developing leaves. A similar communication from old to new leaves was observed with light treatments. Reduced incident light on fully expanded leaves leads to decreased stomatal density and index of developing leaves. However, in a manner similar to the CO_2 response, this effect was only observed in developing leaves in response to shading of mature leaves. It makes sense that mature leaves exposed directly to prevailing conditions should act as sensors so that the developing leaves, which are tightly enclosed by developing leaf layers can regulate their stomatal density in anticipation of the conditions that they are likely to face. This work indicates the involvement of a long-distance systemic signal in the regulation of stomatal density and index. Systemic signals, where a stimulus is sensed by one organ and the response occurs in another, have long been known in plants. In photoperiodic control of flowering, the leaves sense daylength and the response is shown as the regulation of floral meristem initiation (e.g. Ref. 10). Adaptation to excess light excitation energy involves systemic signalling[11], and the production of systemin in response to wounding serves as a systemic signal which, along with ethylene and jasmonate, leads to changes in gene expression in systemic acquired resistance[12,13].

Localized signals regulating stomatal density and patterning

The above observations lead to two fundamental questions. First, what is the nature of the long-distance signal(s) involved in the stomatal density response to elevated CO_2? Second, how do these signals interact with the developmental mechanisms controlling stomatal density in the leaf surface? Stomata are not randomly distributed in normally developing leaves. They are rarely, if ever, found directly adjacent to one another. It is also becoming clear that existing stomata might suppress the differentiation of adjacent cells by a lateral inhibition process. One line of evidence to support this comes from studies of stomatal density mutants of *Arabidopsis* that are deficient in the stomatal-density response to high levels of CO_2 (*high carbon dioxide*, *hic1*)[2]. Indeed, these plants show increased rather than decreased stomatal index when grown in elevated CO_2. Cloning of the *HIC* gene showed that it probably encodes for a guard cell-specific 3-keto acyl coenzyme A synthase. Co-A synthases are involved in the production of very-long-chain fatty acids, constituents of the waxy cuticle on the outer surface of the epidermis[14]. Under normal growth conditions, plants defective in the *HIC* gene retain the wild-type stomatal patterning phenotype. It is thought[2] that the component of the extracellular matrix encoded by *HIC* might

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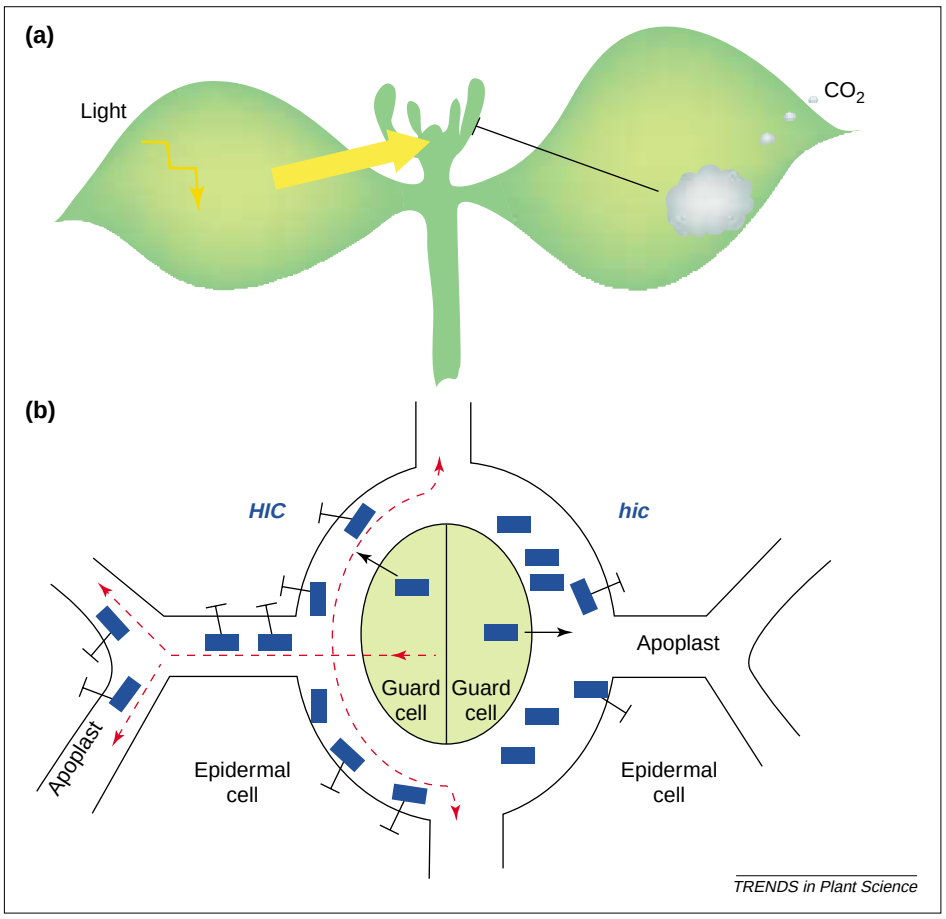

Fig. 1. Hypothetical model for the roles of long-and short-distance signals in the regulation of stomatal density of developing leaves. (a) CO_2 and light can be sensed by mature leaves, which signal systemically to developing leaves. (b) Putative inhibitory factors are secreted into the apoplast where they suppress differentiation of adjacent epidermal cells into guard cells and are involved in controlling the stomatal density response to elevated CO_2. The diffusion of these signals is dependent on the correct composition of wax esters in the cell wall or cuticle encoded, for example by the *HIC* gene product (left). *hic* mutants (right) are defective in these extracellular components, effectively reducing the range over which signals can act. The *HIC* gene product is represented by red broken arrows, the putative inhibitory factor is represented by dark-blue blocks.

allow the controlled movement of a signal in the apoplast that represses guard cell differentiation (Fig. 1). In the absence of the *HIC* gene product, diffusion of this signal is restricted so that it can operate only over shorter distances. This model implies that the pattern of guard cell differentiation is regulated by a factor or factors that operate over short-to-medium distances to prevent epidermal cell differentiation into guard cells during leaf development.

Although we can only speculate about the identity of the short-range factors involved in suppressing guard cell differentiation in response to CO_2 or light, studies of *sdd1-1* (*stomatal density and distribution*) mutants of *Arabidopsis* have indicated the nature of at least one type of signal involved in stomatal development[3]. *sdd1-1* plants have a higher than normal stomatal density and relatively high numbers of stomata that are in direct contact with their neighbours. The *SDD1* gene product has homology with a subtilisin-like protease. These proteins are thought to have roles in protein processing in signal transduction pathways, for example by converting inactive peptide signals into active forms. Examples in plants include the pro-systemin binding protein SBP50, which converts pro-systemin to systemin[14]. However, there is currently no evidence that *SDD1* is involved in the regulation of stomatal density in response to environmental factors.

Conclusions

The observations discussed here raise the intriguing possibility that inhibitory factors in developing leaves, themselves regulated by long-distance signals that initiate in mature leaves, act as negative regulators of guard cell differentiation over short distances. It appears that the composition of the cuticle is fundamental to the movement of signals in the apoplast of leaf epidermis. This hypothesis implies that the cell wall plays a pivotal role as a channel for fate-determining signals. Understanding the nature of these signals in the guard cell density response to CO_2 must be a key goal of plant biology research. The short-range signals identified to date appear to act as repressors of guard cell differentiation. This begs the question of what factors determine the initiation of guard cells from protodermal precursors in the first place?

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