Stomata

Secondary article

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Stomata are specialized cells which respond to environmental and endogenous signals and change shape to allow gas exchange. The cells are structurally adapted for movement that occurs as the result of increasing osmotic potential and turgor pressure.

Article Contents

Introduction

Stomata are ancient structures, known from fossils 400 million years old (Edwards *et al*., 1998). They first appear on naked axes and sporangial walls, and only later on leaves or leaf-like structures. Their appearance in land plants of early strata, their conserved structure over time, and their persistence throughout evolutionary history indicate their significance for land plant success. Except for the cuticle and vascular tissue, no other vegetative structure is as ubiquitous in land plants. The cuticle is a waterproof barrier that plays a passive role in water retention, but stomata are active regulators of gas exchange, including water vapour. They balance the plant's need for carbon dioxide with the unavoidable water loss that occurs upon stomatal opening. The guard cells of stomata respond to many environmental and endogenous signals and expand to create an open pore between them to allow gas exchange. Signals are transduced to affect ion channels, ion pumps, and guard cell metabolism. Stomatal movements are driven by turgor pressure changes resulting from increasing osmotic potential in guard cells.

Stomatal Development

Stomata arise late in leaf development, even after other specialized epidermal cell types, such as trichomes, are present. Stomata arise according to the mode of organ growth, that is, in polarized fashion in leaves of lower vascular plants, gymnosperms and monocots, and in mosaic fashion in leaves of dicots. Stomata consist only of paired guard cells, whereas stomatal complexes include epidermal cells associated with guard cell pairs (**Figure 1**). The epidermal cells associated with the complex are termed subsidiary or accessory cells. They differ from other epidermal cells in size, shape and orderly placement around the stoma. Subsidiary cells share a mechanical linkage with guard cells in that when either cell type is damaged, the other cell type bulges into the available space. Such linkage does not occur between epidermal cells or epidermal and subsidiary cells. Subsidiary cell function is not known. Since some stomata have no subsidiary cells, the accessory cells may serve no role or only a secondary role in guard cell movements of stomatal complexes.

Mature guard cells are distinctive in shape (kidney or dumb-bell) and in differential wall thickenings. The cells are structurally engineered to expand in particular locations and thereby form the stomatal pore between them to allow gas exchange. Guard cells arise from a division of a guard mother cell, which is the smaller cell of an unequal division. The guard mother cell sometimes participates in divisions that generate subsidiary cells and in other instances does not. In *Allium*, when the guard mother cell

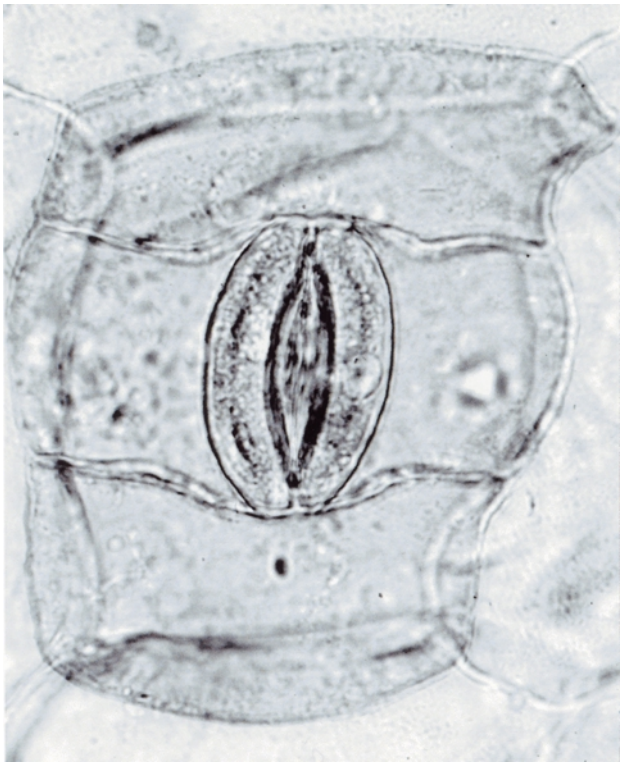

Figure 1 A *Tradescantia* stomatal complex with the guard cells in the centre and the pore oriented longitudinally between them. The ventral walls of the guard cells have prominent ledges. Two pairs of subsidiary cells are associated with this complex; one pair lies on either side (east and west aspects) of the guard cells and the second pair is positioned above and below (north and south aspects) the guard cells.

divides equally, the spindle is canted obliquely across the cell and the poles lie in the cell corners. After anaphase, the entire spindle reorients and the nascent wall is positioned longitudinally. The long common wall between the guard cell pair is the ventral wall. During development there is dissolution of some material in the ventral wall so a pore can form between the paired cells. Parallel to the ventral wall is the dorsal wall, which is adjacent to subsidiary or epidermal cells. The smaller end wall of kidney- and dumb-bell-shaped guard cells contact one another; in dumb-bell guard cells, the end walls are perforate and the cytoplasm is continuous between the pair. At maturity, guard cells are symplasmically isolated, although early in development, open plasmodesmata were present between them and adjacent epidermal cells (Palevitz and Hepler, 1985).

Differential wall thickening

Critical to the cell shape changes that take place during stomatal movements is the placement of wall thickenings. Kidney-shaped guard cells have wall microfibrils (MF) and microtubules (MT) arranged in a fan-like pattern centred on the pore (**Figure 2**). The ventral and outer periclinal (at the surface) walls are much thicker than the dorsal, inner periclinal and end walls. When the guard cells swell, their overall length in face view remains the same, but the dorsal and to some extent the ventral walls bulge outward and increase in length. The ventral wall and a planar microtubule-organizing centre (MTOC) are critical to the correct placement of the MT and cellulosic MF. If the wall does not form or is asymmetric, or if the MTOC or MTs are disrupted at particular developmental times, MFs do not occur in appropriate places. Dumb-bell-shaped guard cells pass through a developmental stage in which the cells are kidney-shaped and have a fan-like pattern of MTs. Subsequently, the middle region of the cell narrows and the MTs cross over one another so that MFs, which are extensive in number, have an overall axial arrangement. The ends of the cells have a flare of MTs and MFs, but the walls in this region are thin in contrast to the walls in the midregion. Ledges commonly are found on the upper edge of the ventral walls where they form an antechamber just above the pore. At maturity, ornamentation on the external walls of guard cells and subsidiary cells may be simple or elaborate, sometimes consisting of interlocking ridges on cells of the complex.

More than 14 configurations and additional subtypes of stomata and stomatal complexes are known (Baranova, 1992). However, the ontogeny of these stomatal types is variable and different sequences of division and division of different cells will produce the same mature produce. Therefore, conclusions about stomatal development cannot be based on mature stomatal appearances. Since as many as five different configurations of stomata or stomatal complexes occur on a single organ, the modal differences also are unlikely to have evolutionary significance.

Stomatal Spacing

Since carbon dioxide is a requisite reactant of photosynthesis, it is not surprising that stomata are found in ordered arrays on leaves and stems for the availability of carbon dioxide near photosynthetic cells must be assured. Stomata are not present in random or clustered distributions, but, according to all research to date, only in ordered arrays. Possible associations between the pathway of carbon

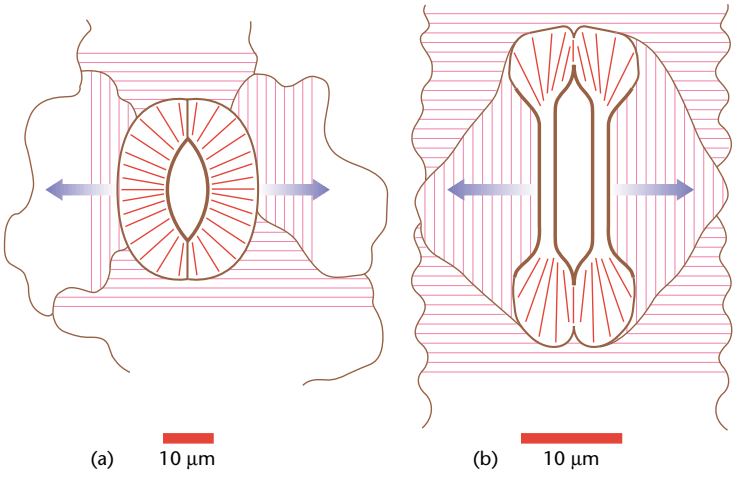

Figure 2 Diagrams of kidney-shaped (a) and dumb-bell-shaped (b) guard cells with dashed lines indicating the orientation of prominent microfibrils. Thickened walls are depicted with heavy black lines; arrows indicate the direction of guard cell expansion during movement. The dorsal wall of the elliptical guard cell expands more than the thickened ventral wall (a). The bulbous ends of the dumb-bell-shaped guard cells expand and push apart the thick walls in the midregion to form the pore (b). Redrawn from Willmer CM (1983) *Stomata*. London: Longman.

fixation (C3, C4, CAM), internal organ design, and stomatal arrays have not been examined. A broad survey examining these associations to assess whether fundamental relationships exist between or among them is crucial to our understanding of this important cell complex.

Interestingly, the orderliness of arrays increases as the organ matures and developing stomata arrest (Croxdale, 1998). The stomatal precursors that are halted in development are selected based on their position, for if arrest were random, the pattern would not change in orderliness. Endogenous inhibitors or competition for a required differentiation factor are possible means of selection. The presence of arrest or aborted stomata is common, although these cells are seldom studied in their own right. Arrest of stomatal initials also indicates that determination of cell fate is progressive, for not all stomatal initials become stomata; some initials switch pathways. In *Tradescantia*, the arrested initials ultimately display characters of epidermal cells in terms of expansion and accumulation of secondary metabolites.

Atmospheric gases and stomatal numbers

Changes in atmospheric gases may result in changes in stomatal numbers. Stomatal numbers are commonly measured by two different means: stomatal frequency on an area basis and stomatal index. To calculate stomatal index all cells within the sample field must be counted, epidermal cells as well as stomata. The labour involved in acquiring index values allows comparison of samples at different stages of development and of samples grown under different conditions. Some species seem especially sensitive to carbon dioxide and show a decrease in stomatal frequency as well as stomatal index from the pre-Industrial Revolution period to current times (Woodward, 1987). However, not all species exhibit such adjustments and there is no absolute correlation between stomatal numbers and concentrations of this atmospheric gas.

In *Tradescantia*, elevated carbon dioxide results in stomatal frequency remaining the same, but more subsidiary cells are associated with each stomatal complex. Stomatal conductance was lower in the enriched carbon dioxide environment than in ambient carbon dioxide. When *Arabidopsis* is grown in low-oxygen conditions, a massive increase in stomatal numbers occurs over those grown in ambient conditions.

Genetics of spacing

Since stomata of some species are sensitive to the environment and respond with a change in their number, it is difficult to separate genetic from physiological effects on spacing. Mutants of stomatal spacing in *Arabidopsis* have clusters of stomata (*too many mouths* and *four lips*) where a single stoma would be found in wild type (Yang and Sack, 1995). Stomata within a cluster in *tmm* are often present in an arc and in wild-type *Arabidopsis* grown under particular conditions, each stoma within a cluster is clonally related (Serna and Fenoll, 1997). Other possible mutants such as those with a random pattern or with few stomata are not yet known.

Patchy stomata

Stomatal movements are not synchronous across a leaf. The lack of uniformity in stomatal function is termed patchiness, patchy stomata (Daley *et al*., 1989), stomatal heterogeneity or nonuniform leaf photosynthesis (Terashima, 1992). These terms mean that stomatal apertures vary across the leaf at any given point in time (Weyers and Lawson, 1997). In selected cases, the lack of uniformity relates to leaf structure. For example, in heterobaric leaves the mesophyll is divided into compartments with structural barriers, usually bundle sheath extensions, that prevent the diffusion of carbon dioxide between compartments (Terashima, 1992). In sunflower leaves fumigated with sulfur dioxide, stomata near the veins close while those further away remain open (Omasa *et al*., 1985). Stomatal movements within an areole (the smallest area of mesophyll served by a vein) tend to be similar (Mott and Buckley, 1998). Stomatal patchiness may be driven by hydraulic considerations. An advantage of such a model is that there is no need for signalling molecules to regulate movements (Mott and Buckley, 1998). The mechanism for patchy function remains elusive.

Stomata as Sensors

Stomata sense a myriad of factors. Stomatal opening signals photosynthetic demand and closing is associated with water stress and decreased photosynthetic demand. Pore changes occur in a few minutes of stimulus application and guard cell volume doubles. Stomata respond to environmental signals such as light, carbon dioxide, temperature, humidity and pollutants and to endogenous signals such as the hormones abscisic acid (ABA) and auxin. Turgor changes in guard cells provide the driving force for stomatal movements. Early on, researchers thought that starch–sugar interconversions provided a large enough change in osmotic potential to drive opening. Today we know that such interconversions do not occur quickly enough or yield enough osmoticum to result in the turgor pressure changes needed for guard cell swelling. Currently, we are aware that the following processes generate osmotic potential involved in stomatal opening (**Figure 3**). A proton pump establishes a proton-motive force that allows for ion uptake. K^+ increases four- to eightfold during opening. The rise in K^+ is balanced by the

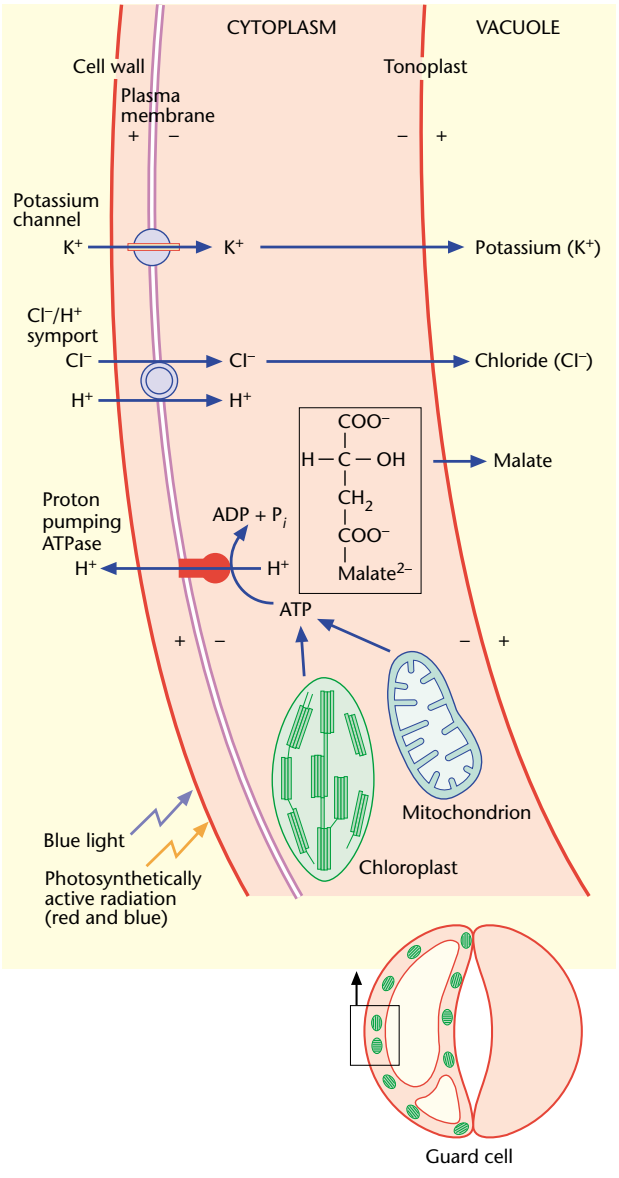

Figure 3 Schematic view of guard cell events involved in stomatal movements. A light-activated H^+-pumping ATPase is stimulated by photosynthetically active radiation (red and blue light) absorbed by chlorophyll. A second blue light signal also stimulates the pump. ATP needed for pump operation comes from the mitochondria or chloroplasts. The action of the ATPase results in a proton gradient that is used to take up K^+ via a specific ion channel. Chloride might be taken up by Cl^-/H^+ symport. Malate is synthesized in the cytoplasm from carbon skeletons formed during starch hydrolysis in the chloroplasts. Redrawn from Taiz L and Zeiger E (1991) *Plant Physiology*. Redwood City, CA: Benjamin/Cummings.

uptake of Cl^-, anions and malate^{2-}. As malate^{2-} is generated, replacement of H^+ lost via the proton pump occurs. Closure is not simply the reverse of the above process, although some ions may be extruded and carbon compounds may be respired or converted during closure.

Signals

Stomata perceive and respond to red and blue light via chlorophyll and a blue light receptor, zeaxanthin (Zeiger and Zhu, 1998). Phytochrome, which also senses red light, is involved in the circadian rhythm of stomatal activity, but not stomatal movements *per se*. Stomatal conductance is correlated to photosynthetically active radiation (PAR) and assimilation from photosynthesis. Chlorophyll within the guard cells absorbs red and blue light, but additional opening can occur via a blue light receptor. Effects of the two receptors are separable from one another. Light activates a proton pump in the plasma membrane and a cascade of ion movement begins. Medium surrounding guard cell protoplasts becomes more acidic when pulsed with blue light. Patch clamp experiments have confirmed that blue light stimulates electrical currents in the guard cell plasma membrane, and the rate of proton pumping depends on the fluence rate of blue light. Red light also stimulates proton pumping, which is blocked by inhibitors of photosynthesis, indicating that the chloroplast mediates this response. Except for chlorophyll and zeaxanthin, signal receptors in guard cells are largely unknown. Establishing receptor identity will help to link signals with their response pathways, including second messenger pathways involving G proteins, calmodulin, kinases/phosphatase, internal calcium levels, and internal pH. The latter two are closely linked with ABA, which is produced in response to stress, especially drought.

Stomata also sense carbon dioxide by closing when levels are elevated and opening when levels are low. The internal concentration of carbon dioxide (c_i) is more important than the ambient carbon dioxide (c_a) level, although c_i is influenced by c_a as well as photosynthesis (Mott and Buckley, 1998). Stomata also respond to air pollutants, and depending on dose, may at first be impaired in movements, but later recover (Omasa *et al.*, 1985). Accumulation of sucrose in the guard cell wall may also be a means of regulating stomatal aperture (Lu *et al.*, 1997).

There has been some debate as to whether stomata sense humidity directly or whether they lose water and the decrease in turgor results in closure. It is now known that guard cells respond metabolically to humidity by losing K^+. ABA, which is carried in the transpirational stream may directly affect closure.

ABA regulation involves both calcium-dependent and calcium-independent pathways, for the hormone activates a calcium-independent protein kinase (Li and Assmann, 1996) and stimulates outward K^+ current, which is correlated with a rise in cytoplasmic pH. A calcium-dependent protein kinase with calmodulin-like domain has

been identified in guard cells and is probably an important component of calcium signalling in stomata (Li *et al.*, 1998). ABA promotes closure by directly or indirectly regulating K^+, anion and calcium channels. Auxins stimulate opening by activating the H pump and shifting the voltage sensitivity of particular anion channels.

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