Journal of Plant Biology, October 2007, 50(5) : 523-526 **REVIEW**

Current Theories for Mechanism of Stomatal Opening: Influence of Blue Light, Mesophyll Cells, and Sucrose

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Since the late 1960s, researchers have observed that starch in the chloroplasts of the guard cells breaks down during the day and accumulates in the dark. Based on this, carbohydrates have historically been regarded as the primary osmotica modulating stomatal opening. However, the discovery of an important role for potassium uptake has led to the replacement of that starch-sugar hypothesis. Current research now focuses mainly on how K^+ is transported in and out of cells when the stomata open or close. However, questions remain concerning photoreceptors, and the functioning of guard cell chloroplasts is still disputed. Coincidentally, some recent study results have again suggested that sucrose may play a major role in guard cell osmoregulation, thus supporting the original theory of starch-sugar involvement.

Keywords: guard cell, guard cell chloroplasts, mesophyll cells, stomatal opening

Environmental factors, such as light and CO_2 concentration, trigger events that may result in stomatal opening. Stomatal apertures are controlled by the solute content in the guard cells. The classical theory (starch ⇔ sugar during stomatal opening) invoked an osmotic role for sugars with guard cells sufficient to create the requisite turgor pressure. This theory was based on observations of a reciprocal relationship between guard cell starch content and stomatal aperture size.

Nevertheless, upon the discovery that massive K^+ accumulation in the guard cells accompanies stomatal opening, and that K^+ loss from those cells is associated with stomatal closure, the previous theory was discarded (Hsiao, 1976). It is now widely agreed that potassium ions and malic acid are the major solutes that produce the turgor pressure that drives stomatal opening (Humble and Raschke, 1971; Mansfield, 1986; Serrano et al., 1988). However, there is some doubt among most stomatal researchers about how light might trigger the events that lead to K^+ uptake in the guard cells.

Until the early 1990s, the most popular hypothesis for stomatal activity involved fluxes of inorganic cations and anions across the plasmalemma and tonoplasts of the guard cells, which was associated with the synthesis and degradation of organic anions. When the stomata opened, protons are first pumped out from the guard cell, resulting in hyperpolarization of the plasmalemma potential differences. Edwards et al. (1988) reported that previously darkened leaves exposed to light exhibited quenching of fluorescence in the apoplast surrounding the guard cells up to 20 min before stomatal opening. They showed that proton efflux originating at the guard cells also preceded that event, confirming earlier work that suggested proton efflux was a necessary precursor of stomatal opening (Raschke and Humble, 1973). K^+ then passively entered the guard cells under higher osmotic potential. Some Cl^- also was transported, but a complete charge balance of excess K^+ was accompanied by the synthesis of malate (MacRobbie, 1987).

However, several lines of evidence now suggest that soluble sugars may also be significant as osmotica in the guard cells of open stomata (Poffenroth et al., 1992; Lu et al., 1997; Talbott and Zeiger, 1998; Taiz and Zeiger, 2003). MacRobbie (1987) have noted that when stomata in the detached leaf epidermis of *Commelina communis* are induced to open by light exposure, the rates of K^+ and malate accumulation cannot account for all of the osmotica necessary to support the rate of stomatal opening in the early stages, i.e., when apertures are 10 μm. Outlaw and Manchester (1979) have removed the epidermis from leaves of *Vicia faba* in which the stomata are either open or closed and have shown that opening to 10 μm is accompanied by an increase of 420 to 720 fmol of hexose sugar and 140 fmol of sucrose per guard cell.

Tallman and Zeiger (1988) have reported the involvement of three metabolic pathways for stomatal opening. About 80% of guard cells K^+ have a higher content after 1 h of incubation but that decreases to 10% after 5 h. When the incubation medium lacks KCl, the stomata open slowly in response to 25 μmol m^{-2} s^{-1} of blue light, without any K^+ gain or starch loss. In dual-beam experiments, stomata irradiated with 50 μmol m^{-2} s^{-1} of red light for 3 h also open without detectable starch loss or K^+ gain; in contrast, the addition of 25 μmol m^{-2} s^{-1} of blue light causes a further gain of 4.4 μm in aperture size, which is accompanied by substantial K^+ uptake and starch loss. When one compares the guard cells of opened stomata in epidermal peels with those induced to open in intact discs, the content is much higher in the latter type. Talbot and Zeiger (1998) have demonstrated that both K^+ and sucrose are primary guard cell osmotica, and that the use of these two solutes is separated into two distinct phases in which one or the other constitutes the dominant osmoticum. For an intact leaf where the stomata open at the beginning of a daily cycle, the K^+ content in guard cells decreases dramatically, and sucrose then becomes the dominant osmoticum.

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The signal transduction chain for a blue light photoreceptor in the guard cells has been suggested as the carotenoid pigment zeaxantin (Zeiger et al., 2002, Talbott et al., 2003). Phototropin has also been postulated as a blue light photoreceptor (Kinoshita et al., 2001). However, neither the location nor the nature of the red light responses by stomatal guard cells is very clear. The ability of guard cells to fix carbon photosynthetically also is still in dispute (Outlaw, 1989, 1996; Lee and Bowling, 1995; Asai et al., 2000, Lawson et al., 2002, 2003; von Caemmerer et al., 2004). Nevertheless, it has been thought that light-induced stomatal opening depends mainly on a blue light signal transduction pathway.

In their review, Talbott and Zeiger (1998) have concluded that sucrose plays a major role in guard cell osmoregulation. Experiments run in a growth chamber under constant illumination have shown that guard cell sucrose increases slowly early in the day, but accumulates rapidly in the afternoon. Subsequent research has indicated that guard cell K^+ peaks at mid-morning before declining in the afternoon (Talbott and Zeiger, 1998, 2003).

Studies of the daily course for stomatal movement in intact leaves have proven that potassium content in the guard cells increases in parallel with early-morning opening, but that it decreases in the early afternoon under conditions in which the apertures continue to enlarge (Talbott and Zeiger, 1998; Tallman and Zeiger, 1988). That is, sucrose content in the guard cells rises slowly in the morning but, upon potassium efflux, that sugar becomes the dominant osmotically active solute, and stomatal closing at the end the day is correlated with a decline in the amount of guard cell sucrose.

Interest in guard cell carbon metabolism involving sugars has been stimulated by two sets of observations. First, Gotow et al. (1988) have reported that sugar phosphates are formed by photosynthesis in the guard cells of broad bean (*Vicia faba* L.). Second, Tallman and Zeiger (1988) have determined that red light causes an increase in stomatal size on epidermal peels and a decrease in guard cell ψ_s without either an elevation in the guard cell K^+ concentration or a reduction in the guard cell starch content. Under other conditions, the latter research group has found that the stomata also open without a rise in guard cell K^+ concentration but with a loss of guard cell starch content. As a comparison, Outlaw. (1996) have noted that the stomata induced to open in intact leaves have a substantially higher K^+ content than those of the epidermal peels. In summary, the research presented above argues for two major revisions to the paradigm that existed prior to 1988 (Outlaw, 1996). First, the changes in osmotic potential required for stomatal opening in the morning are mediated by potassium and its counterions, whereas the afternoon changes are mediated by sucrose. Second, a certain level of PCRP (photosynthetic carbon reduction pathway) activity in the guard cells is sufficient to contribute to the osmotic requirements for stomatal movement; this, however, is not consistent with the results of Outlaw (1996).

Lu et al. (1997) have pulse-labeled the leaflets of broad bean with $^{14}CO_2$, then harvested whole leaf pieces and rinsed epidermal peels for subsequent processing in a histochemical analysis. There, sucrose-specific radioactivity shows a peak (111 GBq mol^{-1}) in the palisade cells at 20 min. In contrast, the ^{14}C content and sucrose-specific radioactivity are very low in the guard cells for the first 20 min, implying little CO_2 incorporation. However, both then peak at 40 min.

Lee and Bowling (1992) have reported that, upon transfer to the light, stomata from intact *Commelina* leaves open to a maximum aperture of around 16 μm after 70 min. At 40 min, those stomata open to around 10 μm. Lu et al. (1997) have shown with *Vicia* that the ^{14}C content and sucrose-specific radioactivity peak at 40 min. All these results indicate that sucrose uptake occurs as the stomata open, even though its absolute content may be negligible early on. The aperture is largest, ~16 μm, after 70 min in *Commelina* (Lee and Bowling, 1992). When the stomata are opened wide, sucrose content can be maintained maximally. Poffenroth et al. (1992) have found that when *Vicia* stomata are exposed to red light for 3 h, their apertures are increased by as much as 6.7 μm, with the concentration of total soluble sugar being 289 fmoles per guard cell, thereby indicating that sugar is essential for stomatal opening.

Based on the research summarized above, one can argue for two revisions to the prevalent paradigm. First, sucrose uptake occurs rapidly. Second, these results do not coincide with the theory that K^+ is the initial osmoticum, followed by sugars as the day-cycle osmotica (Tallman and Zeiger, 1988; Talbott and Zeiger, 1998). Lu et al. (1997) have proposed a hypothesis by which sucrose in the guard cell wall is a physiological signal that integrates the rates of transpiration, photosynthesis, and translocation. Considered together, the kinetics of total soluble ^{14}C in guard cells and the kinetics of sucrose-specific radioactivities in the palisade and guard cells clearly imply that the latter import ^{14}C from the mesophyll, the only tissue capable of supporting high rates of $^{14}CO_2$ fixation (Lu et al., 1997).

Ritte et al. (1999) have proposed that sucrose could replace potassium and malate as the osmoticum for stomatal opening on intact leaves. Assmann and Zeiger (1987) have reported that the guard cell chloroplasts themselves could supply all the requirements of ATP to fuel the plasma membrane proton pump coupled to potassium influx, thereby leading to stomatal opening. However, Outlaw (1989) has disputed the notion for the operation of the Calvin cycle in guard cells. Other researchers also have shown that guard cells have low levels of Rubisco activity (Gotow et al., 1988; Reckmann et al., 1990).

The basic role of the stomata is to regulate transpiration and photosynthesis, the latter playing a central role in plant processes. Therefore, an understanding of the response to light is critical to any discussion of how plants sense this stimulus. It is likely that many responses are, in fact, mediated by the reaction of photosynthesis to light (Lee and Bowling, 1995; Lee, 2006).

Lee and Bowling (1995) have determined that stomata in the isolated epidermis from *Commelina* leaves behave differently from those in the intact leaf because the former do not respond to light. However, when segments of isolated epidermis are then transferred onto partially exposed mesophyll cells, their stomatal apertures enlarge to almost half the size of those measured from intact leaves. Therefore, the

presence of those mesophyll cells can restore the sensitivity of the stomata to light by controlling their apertures. Likewise, Shabala (1998) has concluded that a resonant analysis approach may be used to distinguish between the contributions of stomatal and mesophyll cells to the composite bioelectric response measured on the leaf surface. Stomatal cells are the major contributors, in a frequency range of tens of minutes. Furthermore, the contribution of the mesophyll cells is substantial at high frequencies. The effect of light quality on changes in membrane potential difference has also been investigated with the guard cells of intact leaves from *Tradescantia virginiana* (Lee and Bowling, 1993; Lee, 2004). There, under red light, the greatest fluctuation in potential difference is approximately –5 mV compared with –2 mV under blue light, when the saturation points of light intensity are separately given.

These results clearly indicate parallel responses between photosynthesis and the stomatal apertures of guard cells. One concept that the white light effect is mediated through the mesophyll is attractive because it explains how photosynthesis can exert some control over stomatal conductance. This has been suggested by the results of Wong et al. (1978), and it helps us understand how stomata that lack chloroplasts, such as those in the orchid *Paphiopedilum*, can respond to light (Nelson and Mayo, 1975). Jamison and Willmer (1984) also have used fluorescence microscopy to demonstrate that chlorophyll is absent from the epidermal and guard cells that overlie all white and green areas in the variegated leaves of *Pelargonium zonale*, cv. Chelsea Gem. In general, stomata with chlorophyll-free guard cells respond normally to light and CO_2, as gauged by both direct measurements of stomatal aperture and studies of transpirational water loss (Jamieson and Willmer, 1984). In addition to these two examples, onion (*Allium cepa*) guard cells contain no chloroplasts but still respond to light (data not published).

Shabala (1998) has used an extracellular electrophysiological technique to investigate the bioelectric responses of leaves to rhythmic variations in irradiance, and has observed that all those species examined respond in a frequency-dependent manner. Furthermore, based on a resonant analysis approach, Shabala (1998) has concluded that the mesophyll component is substantial at higher frequencies. Doi et al. (2006) also have reported that stomata in the intact leaves of sporophytes open in response to red light, but not to blue light, which suggests that ferns of Leptosporangoipsida lack a blue light-specific stomatal response. Nevertheless, *Adiantum* does possess the functional phototropin band plasma membrane H^+-ATPase.

Although the use of isolated epidermis as material for studying the stomatal mechanism helps us understand stomatal control, examining such tissue alone prevents us from evaluating any contribution by the mesophyll cells. This then can lead to some confusion about the real stomatal mechanism in the intact leaf. That might be one factor when explaining changes in stomatal theories. Therefore, other matters that should be investigated include the existence of a red light photoreceptor. Almost all plant photosynthesis occurs in the mesophyll cells, and it is difficult to accept the theory that light-induced stomatal opening is mainly controlled by blue light. Focus should also be directed toward determining the real role of the guard cell chloroplasts, how low concentrations of CO_2 might trigger stomatal opening, and the validity of the theory that K^+ is the initial osmoticum, followed by sugars as the day-cycle osmotica (Tallman and Zeiger, 1988; Talbott and Zeiger, 1998).

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