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REVIEW ARTICLE

The Sustainable Reasons of Controversy over the Mechanisms for the Stomatal Opening

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Abstract Depending on the diversities of the plant species, the mechanisms of the stomatal opening are thought to be different. First, *Paphiopedilum insigna* var. *sanderae* do not have chloroplasts in the guard cells. However, the stomata of this plant function as normal. Plants lacking the chloroplasts in the guard cell imply that the chloroplasts in guard cells are not related to stomatal opening. The guard cells without chloroplasts cannot be the source cells of the photosynthetic products, and the guard cells must be act as the sink cells. Second, the distinctive features of guard cells are specialized cell walls, which are considerably thickened to 5 μm. In contrast, the thickness of mesophyll cell walls is less than 100 nm. In the guard cells, most of the photosynthetic products may be used for the production of cell walls. The low photosynthetic activities of chloroplasts in the guard cell cannot be sufficient to maintain the structures and functions of the guard cell walls. Third, 40~60 chloroplasts in the guard cell of *Lilium longiflorum* which is belong to angiosperm were found in the first time. *Lilium longiflorum* of the relationship with mesophyll cells cannot be completely excluded, but it is assumed that guard cell chloroplasts in *Lilium longiflorum* may play an important role for the stomatal opening.

Keywords: Blue light photorteceptors, Guard cell chloroplasts, *Lilium longiflorum*, Mesophyll cells, Stomata

Introduction

In the early 1980s, Plant Physiology textbook written by Salisbury and Ross was widely used (Salisbury and Ross 1978). Now, most colleges use 'Plant Physiology and Development's 6th editions, co-authored by Taiz and Zeiger, published in 2015, as a teaching material for plant physiology (Taiz and Zeiger 2015). The first edition of the textbook was published in 1991 and lasted 23 years, up to the 6th editions. A new chapter called 'blue light response: stomatal movements and morphogenesis' has been added from the second editions. In the fifth editions of this book, phototropin mediates blue light-dependent phototropism and chloroplast motions. Zeaxanthin is the main blue light receptor for stomatal opening. However, in the 6th editions of the textbook, the functions of the phototropins were changed to the other main blue light receptors for stomatal opening. There are still debates about how stomata open. In the beginning of 2019, it is still unclear the roles of the guard cell chloroplasts and the mesophyll cells for the stomatal opening. Therefore, it was investigated which cause to delay the understanding of the mechanisms for the stomatal opening. In addition to, the stomatal openings by the blue light and the possible mechanisms of stomatal opening were discussed.

The Using of the Isolated Epidermis for the Mechanisms of the Stomatal Opening Can Make Mistakes

It was reported that the use of the isolated epidermis was very different from the stomatal mechanism occurring in intact leaves (Lee and Bowling 1992). The guard cells of the isolated epidermis were more sensitive to incubation solution (including KCl) than the environmental factors. Fig. 1 shows a comparison between the behavior of the stomata in the intact leaf and the isolated epidermis. Grantz and Amnon (1988) first published a distinction between the isolated epidermis and the intact leaf before Lee and Bowling (1992). They were also found similar experiences that in the isolated epidermis, guard cell solute content of open the stomata did not decrease in response to desiccation. In leaf disks, the stomata exhibited clear hydro active stomatal response. Mott clearly suggested that stomata in *Vicia faba* epidermal peels do not respond to changes in light or CO_2 concentration

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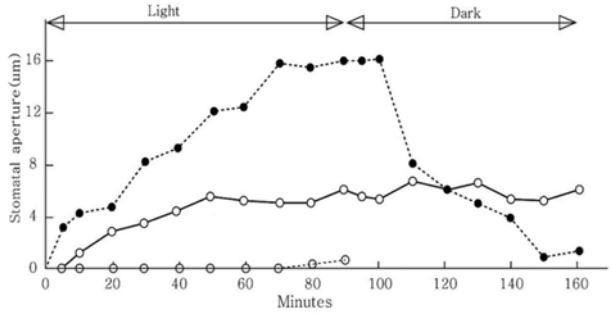

Fig. 1. The different responses between the intact leaves and the isolated epidermis on stomatal opening and closing in *Commelina communis*. Leaves were kept in the dark for 1 h., then exposed to light 90 min. and then returned to the dark. Epidermis was taken from the leaf at the end of the dark period. Each point is the mean of two replicate experiments and 40 stomatal apertures were measured. Bar indicates maximum standard error (±0.89). Closed circles, intact leaves in distilled water; open circles, isolated epidermis in 10 mM MES-KOH buffer (pH 6.15) plus 100 mM KCl, open rectangles, isolated epidermis in buffer plus 10 mM KCl (Lee and Bowling 1992).

(Mott 2009). Sibbernsen and Mott (2010) flooded the intercellular air space with water and used hydrophobic filters to conclude that the signal must be a vapour. Recent study has adopted a unique epidermis–mesophyll transfer experimental approach first recently refined (Mott and Peak 2018). In this experiment, the epidermis was removed from the mesophyll and measured in isolation or replaced back into the mesophyll belonging to the same or different species. This study demonstrated that the stomatal responses to light and CO_2 concentration in the isolated epidermis were not the same as those observed when the epidermis was placed back into the mesophyll. Between the cell walls, there is a middle lamellar which is rich in pectin that binds them tightly. Therefore, the removals of the epidermis of the plant should also peel off the middle lamellar. This is to block the plasmodesmata which are the pathways of photosynthetic products, ions and hormones from the mesophyll cells to the epidermal cells. Therefore, the stomatal responses in the isolated epidermis and the intact leaf may differ for two main reasons. First, the isolated epidermis appears to be damaged during the separation process because it is tightly bound by the middle lamella between the mesophyll cell walls and the epidermis cell walls. Secondly, it is presumed to be due to the blocking of the plasmodesmata between mesophyll cells and the epidermal cells by the removing of the mesophyll cells.

Have Guard Cells Sufficient K^+ Concentration to Open the Stomata?

It is clear that we have studied the mechanisms of the stomatal opening without a deep understanding of the morphology. The stomatal complex is naturally believed to be composed of guard cells and subsidiary cells. And the subsidiary cells will supply the osmotic material needed to open the stomata. However, most dicotyledons and monocotyledons do not have subsidiary cells. Among the monocotyledons, subsidiary cells can be only found in Cyperaceae and Poaceae of Commelinaidae. Also, most dicotyledon and monocotyledon do not have the subsidiary cells. Imamura reported that the influxes of K^+ would occur when the stomata were opened in 1943. Afterwards, starch ↔ sugar hypothesis was discarded, and it was assumed that the stomata were opened by K^+. Early, stomamal reseachers of the UK were skeptical at first that the K^+ ion was the predominant osmotic material of the stomatal opening (Bowling 1976; Travis and Mansfield 1977). In order to balance electrical charge within the guard cells, Cl^-, which is an inflow negative ion, is less than 40% of the K^+ positive charge, and malate has an important role as an osmotic material. What is the real K^+ concentration of the guard cells of intact leaves? Talbott and Zeiger (1996) measured the concentration of K^+ when the stomata were opened and closed using the isolated epidermis that had not been cultured in the KCl culture solution. They reported that the K^+ concentration was increased to 400~800 mM when the stomata were opened. What does it mean that the concentration of K^+ is close to 1 M in guard cell? DeSilva et al. (1996) thought that it could not be possible to 800 mM K^+ increase in single plant cell. They estimate of apoplastic K^+ concentrations in the range of 50~75 mM which are in general agreement with those of Bowling (1976). Recently, many results suggest that the concentration of K^+ in single plant cell including cytoplasm, vacuole and cell wall is generally 100~150 mM (Bowling 1976, 1987; Travis and Mansfield 1977; Desilva et al. 1996). Isolated epidermis, containing 100-150 mM K^+ concentration already in the guard cell, were added with 100 mM KCl pH buffer solution (Fig. 1). Therefore, the maximum concentration of K^+ that the guard cell can contain was 200~250 mM. However, in the intact leaf, the maximum stomatal aperture was 16 μm, but 5 μm in the isolated epidermis although they were incubated in 100 mM KCl. In the isolated epidermis, the size of the stomatal aperture cannot be maximized only by K^+ ion alone. This means that there are other osmotic materials that contributes more to the stomatal opening than K^+ in the natural state. The guard cells should be supplied with sucrose from the mesophyll cells, the source cells of photosynthesis. Therefore, the stomatal opening is presumed to be induced by sucrose together with K^+. It is suggested that sucrose has once again become recognized as one of the most important osmotic substances.

The Chloroplasts in the Guard Cell Do Not Receive Enough Sunlight Because the Abaxial Epidermis is Located in the Back of the Leaf

According to Darwin's natural selection, all living organisms are descendants who are much better adapted to the environment. A better adaptation to the environment does not mean the creatures that are perfectly adapted to the environment. Human beings repeat the same mistakes. That is, humans are incomplete. Plants make mistakes too. A typical example is the case of *Korean forsythia*. If the temperature rises temporarily during the winter and the temperature continues like the spring, then the flowers bloom. *Kerria japonica* generally blooms from April to May, but flowers can be observed in November. From an evolutionary standpoint, stoma is a character to be derived from the process of adapting to the land life of a plant. Numerous stomata on the back of the leaves open and close as the environment changes. Stomata increase resistance to water stress and allows photosynthesis and transpiration, which are the most important parts of plant physiology. The reason that plants can exist as primary producers on the earth is because they synthesize sucrose through photosynthesis. The cell walls of the mesophyll cells in the sponge parenchyma are at the forefront of the process of transpiration. Therefore, the stomatal opening should be affected by the environmental demands of the mesophyll cells. There are stomata in abaxial leaf on the underside of the leaves, and they may be very limited to the light (Fig. 2).

The Chloroplasts in the Guard Cell of CAM Plants

CAM plants have chloroplasts in guard cells. There are about 10 chloroplasts in the guard cell of *Sedum sarmentosum*, a typical CAM plant. The chloroplasts in the guard cell of *Sedum sarmentosum* are about 50% smaller than the chloroplasts in the guard cell of *Commelina communis*. CAM plants such as cacti and *Opuntia ficus–indica* achieve their high water use efficiency by opening their stomata during the cool, desert nights and closing them during the hot, dry days. Signal transduction pathway for stomatal opening by blue light photoreceptors including phototropins and the carotenoid pigment zeaxanthin has been suggested. Blue light regulated signal transduction pathway on stomatal opening could not be applied to CAM plants, but the most possible theory for a nocturnal response of stomata in CAM plants is photoperiodic circadian rhythm. The circadian rhythm will be controlled by the metabolic demand of mesophyll cells, not epidermis cells. Fig. 3 shows the possible hypothesis of stomatal opening in CAM plants. Fundamental sources in the increase in osmotic potential required for stomatal opening might be similar with C_3 and C_4 plants. However, the initial stomatal opening of CAM plants will depend on the circadian rhythms. Various metabolic processes in plants, such as oxygen evolution and respiration, cycle alternately through high-activity and low-activity phases with a regular periodicity of about 24h. Light is a strong modulator of rhythms in plants. Although circadian rhythms that persist under controlled laboratory conditions usually have periods one or more hours longer or shorter than 24h, in nature their periods tend to be uniformly closer to 24h. because of the synchronizing effects of daybreak, referred to as entrainment. The essential mechanism of CAM is the acquisition of inorganic carbon by dark fixation of bicarbonate (HCO_3^-) via phosphoenolpyruvate carboxylase. This leads to an organic acid (mainly malic acid)-concentrating effect in the dark period when organic acid is stored in the

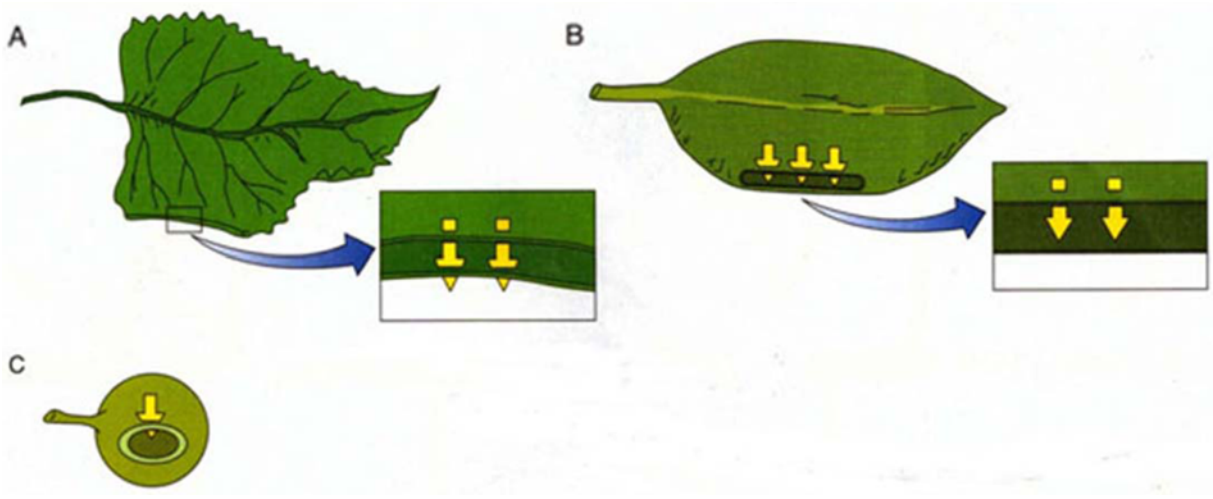

Fig. 2. (A) Leaf blades with thin and flat are common. All cells can be exposed to light, (B) In thick leaves, light cannot penetrate to the bottom of the leaves, so all cells cannot faithfully activate photosynthesis, (C) Because the round leaves do not reach deep inside, only some cells receive light and can act photosynthesis (Lee 2016).

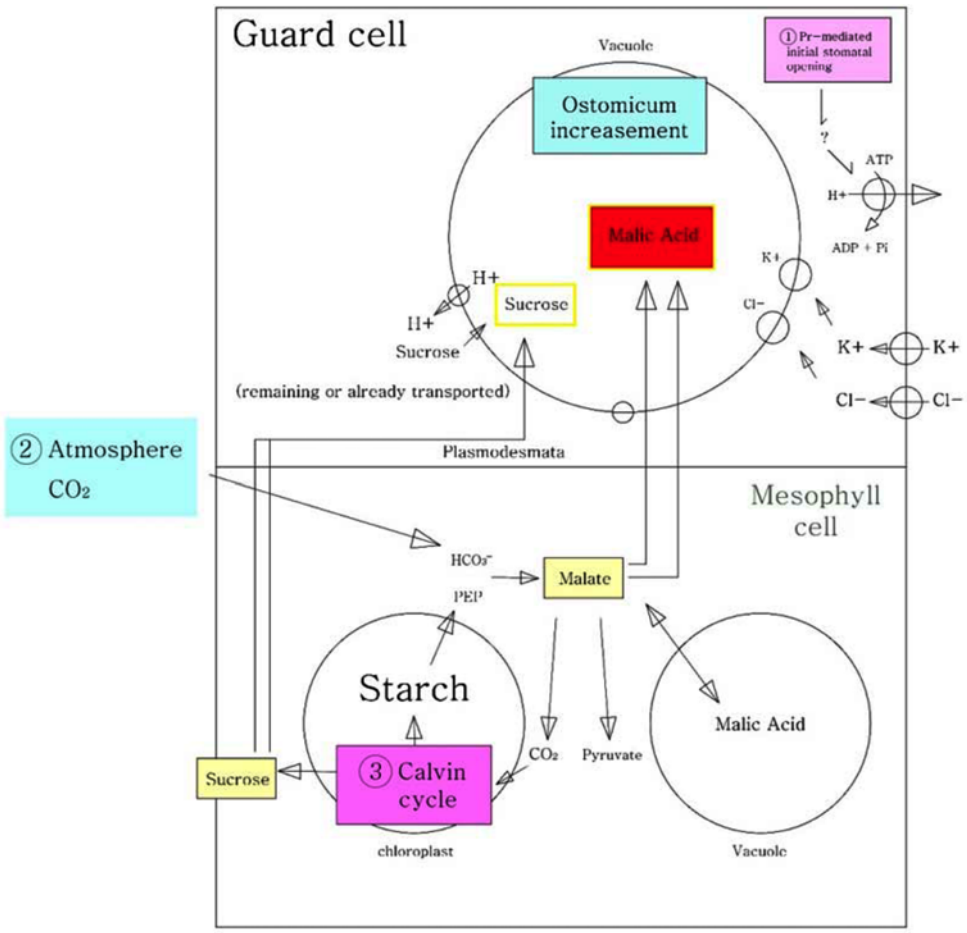

Fig. 3. The possible hypothesis of stomatal opening in CAM plants. A well-described example of a plant circadian rhythm is involved in phytochrome. The initial stomatal opening of CAM plants in darkness could be mediated by phytochrome. ① Phytochrome regulates membrane potentials and ion fluxes. The plasma membrane H^+ pump appears to be activated by phytochrome – mediated signal transduction pathway. K^+ then passively enters the guard cells and some Cl^- is also transported, but a complete charge balance of K^+ is accompanied by the synthesis of malate ② CO_2 is incorporated via carboxylation of phosphoenolpyruvate to oxalacetate, which is then reduced to malate. The malate accumulates and is stored in the large vacuole of mesophyll cells. Substantial amounts of malate among them will transport to guard cell vacuole through plasmodesmata or across cell wall. ③ The carboxylation of CO_2 leads to the net synthesis of glyceraldehyde-3-phosphate (G-3-P) and ribulose 1, 5-bisphosphate that regenerates starting materials of the Calvin cycle. Starch and sucrose are synthesized from G-3-P. The synthesis of starch processes that occur in the chloroplast, but sucrose will be produced in the cytosol. Degradation materials of starch will be transported from the chloroplast to the cytoplasm to synthesize sucrose during the night. The concentration of K^+ in the guard cells is not high enough to open stomata and sucrose can produce in the cytosol of the mesophyll cells in the night. Sucrose transports to vacuole of guard cells through plasmodesmata or across the cell wall. Sucrose can contribute mainly to stomatal opening in CAM plants, but malate can be subsidiary osmoticum.

central cell sap vacuole. In the subsequent light period, organic acid is released from vacuole and again decarboxylated. The CO_2 released is fixed by Rubisco and converted to carbohydrate by the Calvin cycle. Accordingly, sucrose could be the main osmoticum for the increase of guard cell turgor pressure.

Most Guard Cells Do Not Have Subsidiary Cells

Plants have different photosynthetic activities depending on the species and they are C_3, C_4 and CAM plants. The plant with the highest photosynthetic activity on the earth is *Saccharum officinarum*. The photosynthetic activities of guard cells chloroplasts may be also different. Park and Lee (2016) examined stomata as a single character and the possibility of angiosperm' taxonomy. They had difficulties separating the epidermis. It was found that plant taxonomy according to the character of stomata was not possible. In the initial taxonomy it is understood that the stomata show two types in terms of morphological characterizations. The first type is only found in a few monocots including Poaceae and Cyperaceae. In rice and corn, guard cells have the morphological characteristics of dumbbell shape. The morphological

characteristics of dumbbell shape always have subsidiary cells. The other type is found in every dicots and many monocots. They are kidney-shaped guard cells. The plants of kidney-shaped guard cells rarely have subsidiary cells except *Commelina communis* L. Therefore, it could be concluded that two types of the morphological characteristics of guard cells cannot divide according to monocots or dicots (Kim and Lee 2017). The number of chloroplasts in the guard cell varies depending on the species. Guard cells usually contain fewer chloroplasts than the adjacent mesophyll cells typically 10 to 15, depending on species, compared with 30 to 70 in palisade cells (Humble and Raschke 1971). Zuzana et al. (2014) applied, for the first time, the stereological method of an optical disector based on counting chloroplasts in stacks of spruce needle optical cross-sections acquired by confocal laser-scanning microscopy. This estimate was compared with counting chloroplast profiles in 2D sections from the same stacks of sections. Comparing practical measurements of mesophyll cells, calculations performed in a 3D model of a cell with chloroplasts as well as a theoretical analysis showed that the 2D approach yielded biased results, while the underestimation could be up to 10-fold. Many species frequently fall outside the 10-15 range of guard cell chloroplast numbers. *Selaginella* spp. typically has only 2 chloroplasts per guard cell (Brown and Lemmon 1985). In case of *Selaginella*, the number of guard cell chloroplast had 3-6. *Erigeron annuus* (L.) PERS. has 9 chloroplasts per guard cell: *Sedum sarmentosum*, 7; *Chamaesyce supina* MOLD, 8; *Trifolium repens*, 7; *Persicaria tinctoria*, 9; *Portulaca oleracea* L., 8; *Zebrina* spp., 30; *Allium cepa*, 20). Suprisingly, *Lilium longiflorum* has 50-100 chloroplasts. These are similar to the number of chloroplasts in mesophyll cells (30-70) (Alloway and Milthorpe 1976). Up to 100 chloroplasts were found in the guard cell of fern, *Polypodium vulgare* (Stevens and Martin 1978). In the early days, plant without chloroplasts in the guard cells was firstly known in *Paphiopedilum insigna* var. *sanderae* since then new plants were started to discover (Nelson and Mayo 1975). It is now known that there are no chloroplasts in the three plant species, and it is believed that new species will be discovered in the future. Slow photosynthetic induction and low photosynthesis in *Paphiopedilum armeniacum* are related to its lack of guard cell chloroplast and peculiar stomatal anatomy (Zhang et al. 2011). The succulent plant, *Pelagonium zonale* cv. Chelsia gem. have no chloroplasts in guard cells (Avrill and Willmer 1984). The numbers of the guard cell chloroplasts in *Commelina communis*, which is most commonly used in the study of stomata, is about 12, and *Vicia faba* L. has about 10 chloroplasts.

The photosynthetic activities of chloroplasts in guard cells were measured and compared according to plant species (Tracy et al. 2003). They measured the high-resolution images of the chlorophyll fluorescence parameter Fq' / Fm'. The differences in photosynthetic activity were measured in six plant species: *Commelina communis*, *Vicia faba*, *Amaranthus caudatus*, *Polypodium vulgare*, *Nicotiana tabacum*, and *Tradescantia albifora*. The results showed that photosynthetic activities of chloroplasts in guard cells were different depending on the species. During the daytime, chloroplasts in mesophyll cells are assimilated by photosynthesis and carbon is used to synthesize chloroplast starch or at the same time cytoplasm synthesize sucrose. Another noticeable feature of most guard cell chloroplasts is that starch accumulates in the dark and disappears in the night (Willmer and Fricker 1996). It was reported that guard cells of *Arabidopsis* accumulate starch during the day, unlike other guard cells (Stadler et al. 2003).

The Photosynthetic Abilities of the Chloroplasts in the Guard Cells

The hot topics of the controversy for the stomatal opening are the roles of the chloroplasts in the guard cells. The structural characteristics of chloroplasts in guard cells in most angiosperms are as follows. The chloroplasts in the guard cells are about 10 times smaller than those of mesophyll cells. The numbers of chlorophyll are 25 to 100 times lower than those of the mesophyll cells. Differences in structure are linked by differences in function. It is clear that the chloroplasts of the guard cell have photosynthetic activity. However, most studies show that guard cell chloroplasts are only 2~5% of CO_2 fixation compared to mesophyll cells (Reckmann et al. 1990; Gautier et al. 1991; Outlaw and De Vleighere 2001; Outlaw 2003; Kang et al. 2007). Amounts of ribulose 1, 5-bisphophate carboxylase/oxygenase (Rubisco) were determined by the [^{14}C]carboxyarabinitol bisphosphate assay. A guard cell contained about 1.2 and a mesophyll cell about 324 picograms of the enzyme; the ratio was 1:270 (Udo et al. 1990). Several lines of evidence suggest a limited photosynthetic capacity in guard cells compared to mesophyll, and smaller numbers and sizes of chloroplasts in guard cells (Vavasseur and Raghavendra 2005). Using chlorophyll fluorescence imaging, Lawson et al. (2002) proposed that electron transport in guard cells was 20% lower than the underlying mesophyll, but that both cells responded in a similar manner to environmental stimuli. Although early reports suggested that there was no (or little) Calvin cycle activity in guard cells (Lawson et al. 2003). It is now generally accepted that all the Calvin cycle enzymes are present and functional in guard cells, but their activities of the chloroplasts definitely low, and they cannot supply all the osmotic materials to guard cells (Tarczynski et al. 1989; Lawson 2009).

Paphiopedilum insigna var. *sanderae* including other 2 species, have no chloroplasts in the guard cells, but the stomata work normally. These three plant species mean that stomata

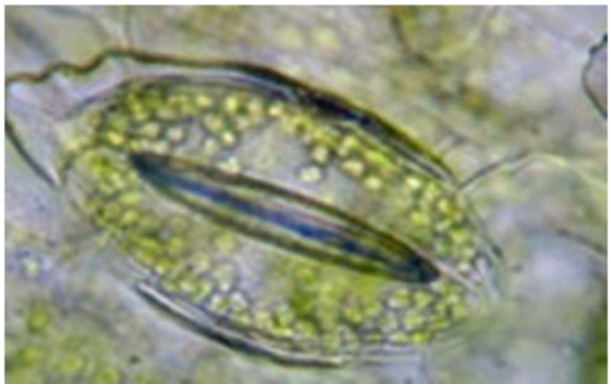

Fig. 4. The chloroplasts in the guard cells of *Lilium longiflorum*. This micrograph was taken by teacher, Park, Sang Hee.

can function normally even if there are no chloroplasts in the guard cells. This clearly means that the three plants may have different mechanisms that do not require chloroplasts. In plants with no chloroplasts in the guard cells, there is no doubt that the guard cells act as sink cells of the photosynthetic products and the mesophyll cells act as source cells. CAM plants open stomata to fix CO_2 and produce sucrose during at night. The phytochrome, known as a circadian rhythm related substance in plants, can be associated with the stomatal opening mechanism of CAM plants (Taiz and Zeiger 2015).

According to Cronquist's taxonomic system, the most evolved plant is Lilyidea of Monocotyledonopsida. The number of guard cell chloroplats in *Lilium longiflorum* was beyond imagination. The number of guard cell chloroplasts in lily was about 40~60 when observed through a two-dimensional microscope (Fig. 4). The numbers of the chloroplasts in the guard cell of *Lilium longiflorum* may be more than 100 in condition of three dimensions. As the shape of the guard cells varies, the structure and the function of the chloroplasts in the guard cell will vary depending on the species. Therefore, the stomatal opening mechanism in *Lilium longiflorum* is hard to imagine, but it is presumed to have a crucial role for the stomatal opening.

The Stomatal Openings by the Blue Light Photoreceptors

The stomatal openings by the blue light receptors have been reported. It is clear that the blue light plays an important role in the stomatal opening (Fig. 5). However, the research papers on the stomatal opening by the blue light have been mainly studied only by the limited teams. In the 6eds textbook, the content of the stomatal opening by the blue light is mainly cited by the results of Zeiger and his student's papers. Therefore, fair discussions of the theory of the stomatal opening by the blue light seem to be necessary. When the red light was alone applied, stomata of about 5 μm were opened after 4 hours, and when the blue light was applied, stomatal apertures of about 6 μm were opened after 2 hours. There is no doubt that stomatal openings are mediated by the blue light photoreceptors. In addition to, the stomatal openings by the red light may be induced by the red light photoreceptors. The opening response of stomata to the red light requires higher irradiance than the blue light and shares characteristics of photosynthesis in its action spectra in the red region (Sharkey and Raschke 1981). Furthermore the red light response can be abolished by 3-(3,4-dichlorophenyl)-1,1-dimethylurea, a PSII inhibitor in whole leaf, epidermal strips, and guard cell protoplasts (Olsen et al. 2002; Messinger et al. 2006). They were also observed the activation of the stomatal opening by the red light and the activation of guard cell plasma membrane (PM) H^+-ATPase in isolated epidermis. The red light also leads to the accumulation of K^+ in guard cells and guard cell PM H^+-ATPase activity in guard cells is inferred to be required for K^+ uptake (Eigo and Toshinori 2018). Daloso et al. (2016) reported that the red light-mediated guard cell responses triggered by mesophyll and guard cell. It was thought that it can be interesting to compare the change of PMPD (plasma membrane potential difference) of guard cells in intact leaves according to different wavelength (Lee 1992). PMPD was hyperpolarized about -5.5 mV when white light saturation was observed, and the change in the red light was -5.2 mV and that in blue light was -2 mV. This means that the red light may have a greater effect on the activity of PM-H^+-ATPase than the blue light in intact leaves. Therefore, it is very likely that the red light is also associated with K^+

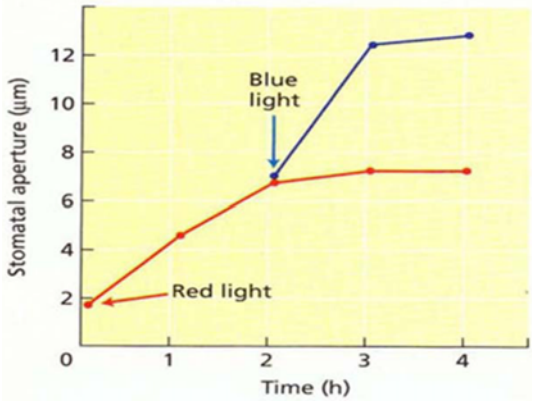

Fig. 5. The response of stomata to blue light under a red-light background. Stomata from detached epidermis of *Commelina communis* (common dayflower) were treated with saturation photon fluxes of red light (red trace). In a parallel treatment, stomata illuminated with red light were also illuminated with blue light, as indicated by arrow (blue trace). The increase in stomatal opening above the level reached in the presence of saturating red light indicates that a difference photoreceptor system, stimulated by blue light, is mediating the additional increases in opening (Schwartz and Zeiger 1984).

absorption facilitation. The blue light has a shorter wavelength and has a higher energy than red light, which can have a greater impact on the general metabolism of the plant.

In the late 1880s, Engelmann used a prism to disperse the sunlight into the rainbow, which then shone onto *the Spirogyra sp.* O_2-seeking bacteria were then added to the culture medium. The bacteria are at the sites where oxygen occurs that the chloroplasts are strongly absorbed in the blue and the red light areas (Fig. 5). In the photosynthetic electron transport, the most efficient wavelengths of the reaction center occur around the reaction center complex, P680 (photosystem II) and P700 (photosystem I). Therefore, it can be considered that the blue light is less important in photosynthesis electron transport. However, when the blue light alone was given to *Spirogyra* sp., photosynthetic electron transport occurs at the same level as when the red light was given.

It was completely activated by the enzyme that regulates the water splitting system of PSII of the algae chloroplast by the blue light. It represents that PSII works perfectly by the blue light. However, the activity of PSII is believed to occur 680 nm. The mechanisms of the stomatal opening are different from the photosynthetic electron transport system. Representative blue light photoreceptors are zeaxanthin, cryptochrome and phototropin. Zeaxanthin is a carotenoid pigment which is terpene compound. Non-photochemical quenching is a major process that regulates excitation energy transfer to the reaction center, which prevents light damage by extinguishing the excited energy delivered to the reaction center of the PSII depending on the light intensities and other conditions. Zeaxanthin is participated in non-photochemical quenching in photosynthetic electron transport and blocks damage to the PSII chlorophyll-antenna complex. β-carotene, the photoreceptor of the chloroplast, exhibits the maximum absorbance between 400 and 500 nm. The main pigments for photosynthesis are chlorophyll a, b and carotenoids.

Algae does not have guard cells. Intercellular space is well developed for gas exchange and supporting action in leaves of aquatic plants, and intercellular space is connected to guard cells.. Leaves submerged in water have no stomata, whereas leaves floating on water have guard cells in the upper epidermis. Evolutionally, guard cells are a new apomorphy from algae. The evolution of the stomata was derived from the process in which algae evolved into terrestrial plants. Guard cells are present in the leaves of bryophytes, fern and almost all vascular plants. The basic role of stomata is to minimize water stress and to maximize the efficiency of photosynthesis activity through photosynthesis and transpiration. Photosynthesis plays a central role in the physiology of plants and the understandings of its response to light are, therefore, critical to any discussion of how plants sense and respond to light. It is likely that many responses exhibited by plants to light are in fact mediated by the response of

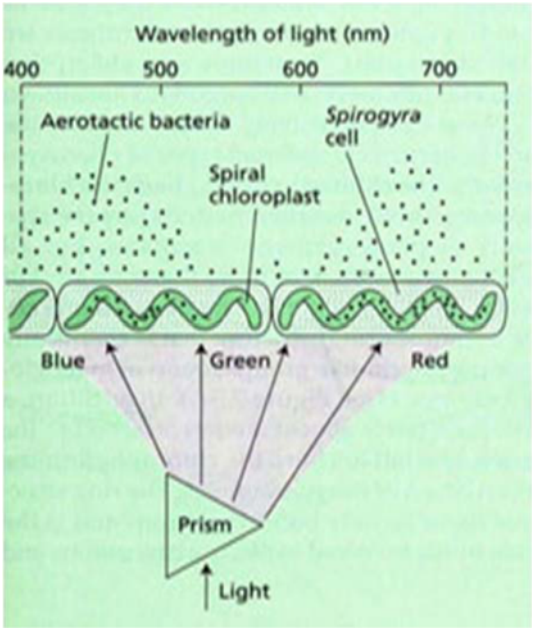

Fig. 6. A schematic diagram of the action spectrum measurements by Engelmann (Taiz and Zeiger 2015).

photosynthesis. The stomatal mechanism could be controlled by the command and the operation. This suggests the idea that the stomata should be controlled according to the demand of the mesophyll cells. In this hypothesis, the command originates in the mesophyll cells and the operation in guard cells (Lee and Bowling 1995). About 95% of total photosynthesis in plants occurs in mesophyll cells of the leaves (Lee 2016). Therefore, it is assumed that photosynthesis products, inorganic ions and hormones between the guard cells and the mesophyll cells through plasmodasmata may be communicated very actively.

Conclusion

The stomatal opening by blue light occurs, but this theory is mainly focused on the blue light. The largest stomata aperture in *Commelina communis* was 16 μm, but in *Vicia faba*, the stomata open up to 18~20 μm. In *Commelina communis*, stomata apertures by the blue light were 6 μm. It is 10 μm less than 16 μm are observed in white light. Therefore, the stomatal apertures by the blue light are much smaller than stomatal apertures under the natural light. It is clear that stomata do not open completely without the help of photosynthetic and signaling systems of mesophyll cells. There is still 'Plant Physiology' textbook that suggests that the guard cell chloroplasts have both osmotic materials and signal transduction pathways required for stomatal opening (Taiz and Zeiger 2015). This textbook did not mention the importance of recent mesophyll cells' impact on the stomatal

opening, but it was consisted of a chapter on stomatal opening with biased the blue light effects. As a result, the students using this textbook may misunderstand that the stomatal opening is only induced by the blue light. As a scholar who studied stomata for 30 years, I found that the content of the chapter was filled with by Zeiger's papers, so it was not reliable. There are chloroplasts in the guard cell of the CAM plant, but the stomata opens at night. This means that the stomatal opening is not induced by the blue light. In most plants, the blue light promotes the stomatal opening, but the stomatal apertures were more than double when the red light was given together. Mesophyll cells have hundreds of millions of evolved chloroplasts optimized for photosynthesis. However, chloroplasts in guard cells have evolved to be less optimized for photosynthesis. This may be seen as a reduction in the role of chloroplasts in the guard cell or a decrease in photosynthetic activity except for *Lilium longiflorum* during evolution.

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Author's Contributions

JSL collected information and wrote the manuscript.

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