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REVIEW

PHOTOSYNTHESIS OF GUARD CELL CHLOROPLAST

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Abstract –Chloroplasts are a central structural feature of stomatal guard cells. Guard cell chloroplasts have both photosystems I and II (PS I and II), carry out O_2 evolution, cyclic and noncyclic photophosphorylation, and possess the Calvin-Benson cycle enzymes involved in CO_2 fixation. These imply that guard cell chloroplasts have a normal photosynthetic carbon reduction pathway just like their mesophyll counterparts, indicating similar functional organization of thylakoid membranes in both types of mesophyll and guard cell chloroplasts. It has been, however, found that guard cell chloroplasts have distinctive and comparative properties in their photosynthetic performance. In this article, I review the intrinsic features on the light reactions of and carbon reduction by guard cell chloroplasts.

INTRODUCTION

Guard cells themselves directly perceive, transduce, and respond to many environmental stimuli, such as light, drought, humidity, temperature, CO_2 and O_2 concentrations, and pollutants. Stomatal movements result from changes in osmotic pressure and turgor of individual guard cells. Stomata open when guard cells swell by releasing protons and thus, hyperpolarizing the plasma membrane. Whereas, stomatal closure is not the reversal process of opening; anion release depolarizes the plasma membrane, and thus guard cells release water and shrink. These characteristic volume changes of guard cells are mediated by transport of K^+ ions and anions as well as by starch-malate interconversion and by energy metabolism.[1,2] The processes are tightly coupled in order to allow an efficient control of stomatal aperture for gas exchange; stomata provide paths for CO_2 intake while minimising the unavoidable efflux of water vapour under continuously changing ambient conditions.

Among a number of studies on the regulation of stomatal movements, much attention has focused on the physiological role of guard cell chloroplasts in the energy relations of stomatal movements. Stomatal opening is well known to be energy-requiring process. The hypothesis that guard cell chloroplasts supply ATP and reducing equivalents for innumerable reactions including ion transport processes at the plasmalemma and tonoplast during stomatal movements is a classic concept in stomatal physiology (Fig. 1).[3,4] On the other hand, for the operation of stomatal opening in the light, guard cell mitochondria could also contribute to the process as an energy source.[5] Stomatal opening in the dark depended on oxidative phosphorylation while opening in the light appeared to require photophosphorylation and the blue light-specific opening could rely on oxidative phosophorylation or a membrane-bound electron transport carrier as an energy source for proton extrusion.[6] ATP synthesis by either guard cell chloroplasts or mitochondria could thus accommodate the requirements of metabolic energy for stomatal opening.

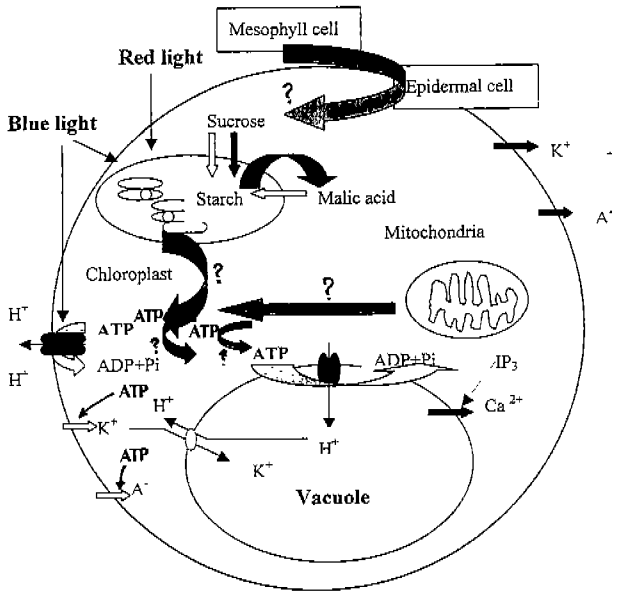

Figure 1. Supposed relationship between ion transport and metabolic energy in guard cell during stomatal opening and closing processes. Full open and closed arrows indicate stomatal opening and closing responses, respectively. Note that proton pump in the plasma membrane which activated by blue light requires metabolic energy. A cytosolic ATP also appears to involve in up-regulation of K^+ and anion channels by phosphorylation events in guard cells. Concerning the energy sources, it is still debated whether it is originated from chloroplast or mitochondria. Malic acid is synthesized from starch and stored in vacuole during stomatal opening. Sucrose has suggested to be originated from mesophyll cell chloroplasts. IP_3 induces the release of Ca_{2+} from vacuole into cytosol, which blocks inward-rectifying K^+ current and activates outward-rectifying A^- current. A^-: anion (Cl^-, malic acid, and etc), IP_3: inositol-1,4,5-triphosphate.

Table 1. Number of chloroplasts per guard cell on the epidermes of *Vicia, Solanum,* and *Arabidopsis* leaves

Plant	No. of chloroplasts	No. of cells counted
Vicia faba L. (cv. Gebag)	19.8 (16-28)	25
Solanum tuberosum L. (cv. Désirée)	20.9 (16-28)	25
Arabidopsis thaliana L. (cv. Colombia)	6.6 (4-8)	25

Values are means with extreme ranges in parentheses by counting the number of chloroplast in a pair of guard cell under microscope (10 × 40). The epidermes were peeled from the abaxial side of mature leaves. In *Arabidopsis* plants, a single of mesophyll cell has about 90 chloroplasts per cell which depends on cell size.[84]

However, no direct evidence have emerged which organelles play the role as an energy source for stomatal opening and closing. The energy relations of guard cells at the physiological and biochemical levels are complex because when one source of energy is inhibited other sources tend to compensate so that the real value is obscured. Above all, the detailed characterization of the intrinsic properties in the photosynthetic performance of guard cell chloroplast must be prerequisite for correct interpreting the physiological role of the organelle in stomatal movements.

Here, I summarize recent efforts for understanding the intrinsic features of the photosynthesis of guard cell chloroplasts which have known to be fairly smaller in size, fewer in number (Table 1), and have fewer granal stacks than their mesophyll counterparts,[7] and explore future research opportunities.

PHOTOBIOLOGICAL PROPERTIES OF STOMATA

The properties of stomatal opening in relation to light are well characterized in elsewhere.[8] About 40% of the radiant energy under natural conditions constitutes blue light of the wavelengths 400-500 nm[9] and thus, the blue light superiority must be an integral component of the stomatal light responses. In general, stomata of most plants open in response to light and close in response to darkness. The light responses of stomatal opening show the hyperbolic shape characteric of photobiological responses.[10] The action mechanisms of light in guard cells, however, are still poorly understood.

Blue light induces stomatal opening in a dose-dependent manner, suggesting that a blue light receptor pigment is involved in the stomatal response to the light.[11] The intracellular location and the chemical identity of the blue-light receptor in guard cells, however, remain unknown. As with most blue light responses of higher plants, it has been debated whether the blue light response in guard cells is mediated by a 'cryptochrome'-like photoreceptor, which is widely believed to be a flavin or a flavoprotein[12] or a carotenoid, zeaxanthin.[13-15] The greater quantum yield efficiency of blue light in stimulating stomatal opening, particularly at low fluence rates (1 μmol $m^{-2}s^{-1}$ or less), is the most definitive of several lines of evidence for a guard cell blue-light receptor. Since chlorophyll in guard cell chloroplasts absorbs both blue and red light, many investigators have determined the blue light responses either on a background of red light to distinguish between the responses of the chlorophyll and a blue light receptor,[16-19] in the absence of chlorophyll response by using wheat seedlings treated with SAN 9789, a compound that inhibits the synthesis of carotenoids,[20] or by using the orchid genus plants with guard cells that lack chlorophyll.[21,22] The blue light response of stomata obeys reciprocity[18,23] and shows an action spectrum with a maximum at 445-450 nm, a large shoulder at 470 nm and a minor shoulder at 420 nm.[18] Blue light in guard cells have known to activate the H^+ pump as the final target[83] and in its signal transduction process, the increase of cytosolic Ca^{2+} level may involve in. Much research is, however, needed to prove the signal transduction cascade of blue light in guard cells. The cellular action mechanisms have been well discussed in detail elsewhere.[24-27]

A single pulse of red light also stimulates stomatal opening.[28] The light-quality response curves of stomatal conductance and photosynthesis between 600 and 700 nm are nearly identical.[29] Red light response of stomatal opening involves chlorophyll; the inhibitor of PS II, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and other PS II inhibitors close stomata in red light.[6,29,30] In addition, red light stimulates proton pump from guard cell protoplasts which are completely inhibited by DCMU[31]. The action spectrum of red light induces stomatal opening strongly correlated with photosynthesis and is thus associated with the chloroplast.

From these photobiological properties of stomata, the response has been understood to be operated by the combined expression of two distinct photoreceptor systems; a blue light-dependent photosystem and the photosynthesis by guard cell chloroplasts.[11,28,29,32] On the other hand, stomatal guard cells possess fairly small chloroplasts, which in contrast to mesophyll cells have poorly-developed grana[7]. In line with those ultrastructural data, previous physiological and biophysical studies could show that guard cells have very different properties from their mesophyll counterparts. These include voltage-dependent K^+ channels and voltage-, calcium- and ATP-dependent anion channels[33-35], red[32] and blue light[24,36]-dependent H^+ pumps. As a result, stomatal physiologists have predicted that energy metabolism of guard cell chloroplasts is pivotal for stomatal opening in light. It should, however, been mentioned that their unique photosynthetic properties with respect to stomatal function are still discussed.[25] The main reason why the current results did not find a broad acceptance is due to the fact that only minor contaminants have strong effects on the metabolite spectrum as well as chlorophyll fluorescence (photosynthetic electron transport) of guard cells. One approach to overcome the contamination problem is to perform single guard cell biochemistry and photobiology.

Table 2. Rates of O_2 uptake and evolution, and noncyclic and cyclic photophosphorylation in guard and mesophyll cells

Guard cell	Mesophyll cell	Plant species	Reference
1. Light O_2 evolution / Dark O_2 uptake (μmolO_2 mg^{-1}Chl h^{-1})			
189/—[a]	87/—	*V. faba*	67
65/	60/	*V. faba*	71
250/200	80/10	*V. faba*	72
150/175	100/6	*V. faba*	44
61/100	73/8.1	*C. communis*	49
162/127	48/6.6	*C. communis*	73
400/300	120/6	*B. napus*	72
—/130	—/7	*P. sativum*	74
—/—	34.4/6.9	*As. sprengi*	73
—/—	30.2/5.4	*N. tabacum*	76
—/—	53.6/10	*N. plumbaginifolia*	77
2. Linear photophosphorylation (μmol ATP mg^{-1}Chl h^{-1})			
103	155	*V. faba*	54
148	160	*V. faba*	71
75.1[b]	100	*V. faba*	55
3. Cyclic photophosphorylation (μmol ATP mg^{-1}Chl h^{-1})			
508	418	*V. faba*	71
193	365	*V. faba*	81

[a]Not specified. [b]It is estimated by electron transport rate, and the value of guard cell was calculated from a single chloroplast and normalized as a percentage when the rate in mesophyll cell was 100.

Table 3. Chlorophyll concentration of guard and mesophyll cells from *Vicia faba* leaves

Guard cell	Mesophyll cell	Reference
1. Chl concentration (Chl ratio/Protein)		
2.86%		43
1.21%	9.01%	41
2. Chl *a/b* ratio		
4.5:1		3
3.8:1	2.2:1	71
2.8:1		43
3.3:1	3.2:1	44
2.7:1	3.1:1	41

It is believed that chlorophyll *a/b* ratios indicate whether chloroplasts are enriched in PS I because of the differing absorption characteristics of the two photosynthetic units; PS I absorbing more strongly in the red light and short-wavelength blue regions of the spectrum (due to its high levels of Chl a), PS II absorbing strongly at wavelengths absorbed by Chl b and carotenoids, particularly xanthophylls. Values approximately between 2 and 3 (typical of C_3 plants) indicate that considerable amounts of both photosystems are present, while values above 3 (as in C_4 plants) indicate that PS I dominates.[8]

LIGHT REACTIONS OF GUARD CELL CHLOROPLASTS

Solar energy arriving at the earth's surface, and available to phototrophs, comprises wavelenghts in the range of 300-1100 nm. The light used to drive syntheses in green plants, photosynthetically active radiation (PAR), is within the range of visible light from 400-700 nm. Light is absorbed in plants by specific pigments of which the chlorophylls are the most important. The othe major classes of light-absorbing pigments are the carotenoids. Guard cell chloroplasts have been known to possess chlorophylls as well as carotenes.[37,38] Like their mesophyll counterparts, the organelles can also operate the xanthophyll cycle,[13,14,39] which may play an important role in the photoinhibition and the photoprotection of the guard cell chloroplasts. In addition, guard cell chloroplasts have UV-absorving flavonoids, which show the maximum at 350 nm.[40,41] Especially, the content of flavonoids is estimated to be two-fold larger in guard cells of adaxial epidermis than abaxial one.[41] In contrast to this, it has been not reported for mesophyll chloroplasts to show such peak at their absorption spectra of UV region.

Several studies conducted over the last few years have made it evident that, qualitatively, guard cell chloroplasts exhibit the typical light reactions known for mesophyll.[27] The studies have shown that, like their mesophyll counterparts, guard cell chloroplasts have functional PS I and PS II[42-46], the ability for photosynthetic electron transport[43,45,47], and photosynthetic O_2 evolution.[44,48,49] The evidences for photophosphorylation in guard cell chloroplasts have been also established by the photochemical studies, chlorophyll a fluorescence transients[44,47,50,51] and the light-induced 518 nm electrochromic shift.[52] Table 2 shows the significant quantitative differences between mesophyll and guard cells in the light reactions of photosynthesis. The rates of photosynthetic O_2 evolution were much higher in guard cell chloroplasts (61-400 μmol O_2 mg^{-1} Chl h^{-1}) than in mesophyll cell ones (30-120 μmol O_2 mg^{-1} Chl h^{-1}). This implies that guard cell chloroplasts have more active PS II complex than that of mesophyll ones. On the other hand, oxygen uptake in the dark also showed higher rates in guard cells (100-300 μmol O_2 mg^{-1} Chl h^{-1}) than in mesophyll ones (5.4-10 μmol O_2 mg^{-1} Chl h^{-1}), indicating a powerful operation of oxidative phosphorylation in guard cells. Dose response curves for noncyclic photophosphorylation shows that half saturation for mesophyll chloroplasts was at 40 μmolm^{-2}s^{-1}, whereas guard cell chloroplasts was at 80 μmolm^{-2}s^{-1} on a chlorophyll basis,[53] indicating 1.5 times lower rate of photosynthetic electron transport in guard cells than their mesophyll counterparts.[54] Most recent studies conducted with a single cell also reported that the photosynthetic electron transport rate of guard cells was distinctly lower than that of mesophyll ones.[55] Electron transport rate in a comparison to a single chloroplast showed that guard cell chloroplast was 3.3 times lower than that of mesophyll counterparts.[56] In general, photosynthetic O_2 evolution closely relates to the rate of photosynthetic electron transport (noncyclic). Photophosophorylation (over 95%) is coupled to linear electron flow from PS II to PS I because the photophosphorylation is completely inhibited by DCMU. Here, we should note why high activity of

photosynthetic O_2 evolution in guard cells does not keep similar or higher rate of electron transport (noncyclic) than did in mesophyll ones. One of the reasons may be resulted from the structural differences of thylakoids of guard cell chloroplasts, which have known to have fewer granal stacks than their mesophyll counterparts.[7] It may lead to a difference in the photosystem stoichiometry (a PS II/PS I ratio) of mesophyll and guard cell chloroplasts, which affects the photosynthetic performance. Table 3 shows the possibility of differential stoichiometries of the two photosynthetic reaction centers, although the possibility of mesophyll contamination in the material preparations can not rule out. Much research is required for understanding PS I and PS II composition or function by measuring chlorophyll fluorescence emission and excitation spectra at 77 K at single guard cells. Current knowledge from these results is that guard cell chloroplasts have both photosystems I and II (PS I and II), carry out O_2 evolution, cyclic and noncyclic photophosphorylation and thus, may produce ATP to accommodate the requirements of metabolic energy for stomatal opening in the light.

CARBON DIOXIDE ASSIMILATION IN GUARD CELL CHLOROPLASTS

Efficient photosynthetic CO_2 fixation is dependent on the light-driven production of NADPH and ATP in appropriate stoichiometric amounts. In guard cell chloroplasts as mentioned in previous chapter, the flow rate of noncyclic electrons showed much lower than that of mesophyll ones. It has kept lower activity of photophosphorylation in guard cell chloroplasts than their mesophyll counterparts. It may thus induce lower activation of enzymes involved in Calvin-Benson cycle in guard cell chloroplasts than that of mesophyll ones.

Since the photosynthetic assimilation of CO_2 is initiated by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), much attention has therefore attempted to detect the enzyme in guard cell chloroplasts. Evidence supporting the presence of Calvin-Benson cycle in guard cells has been now accumulated in the literature (Table 4). In the early reports, a number of studies in guard cells of *Vicia, Pima and Commelina* have indicated the absence of the Calvin-Benson cycle, on the basis of measurement of activities of relevant enzymes[57-59] and incorporation of $^{14}CO_2$.[59-62] Additional studies also indicate that the other enzymes associated Calvin-Benson cycle[8], such as phosphoribulikinase[57,61] and NADP specific glyceraldehyde phosphodehydrogenase[57] have been not found in guard cells. Contrary to these results, some investigators have indicated that Rubisco is present at the low levels (less than 1% relative to mesophyll cells) in guard cells.[1,60,63,64] Another reports show that considerable Rubisco activity (Table 4) and other Calvin cycle enzymes were found in *Vicia* guard cells.[65,66] In addition, guard cell chloroplasts show CO_2-dependent O_2 evolution[67], CO_2 and O_2-sensitive chlorophyll fluorescence,[55,56,66] and DCMU-sensitive

Table 4. Rates of CO_2 uptake and/or Rubisco activity (μmol mg^{-1} Chl h^{-1}) under light by guard cell and mesophyll cell protoplasts determined by different methods

Plant species	guard cell	mesophyll cell	Method	Reference
C. commelina	23.0	74.2	mass spectrometry	48
V. faba	19.6[a]	80[a]	^{14}C	79
	21.3[a]	—[b]	^{14}C	80
	111-345	—[b]	ΔpH	67
	52.5[c]	135.6[c]	enzyme activity	65
	59[d]	100	electrophoresis	81
	40-50[d]	100	immunolabelling	82
	20[d]	100	electrophoresis	55
	120	—b	ΔpH	41

[a]Dark incorporation was omitted. [b]Not specified. [c]Values are the mean of four experiments. [d]The amount of Rubisco in guard cells is normalized to that in mesophyll and represents the percentage value on Chl basis.

sugar production[68] and sucrose accumulation under red light.[69,70] Most recent approach by chlorophyll a fluorescence induction at single cell gave us new insight to illuminate the intrinsic properties of guard cell photosynthesis; the fluorescence kinetics seen under blue light excitation in guard cell protoplasts and guard cell pairs yield distinctive transients that were similar to those obtained with mesophyll cell ones, including the presence of a clear M peak,[55] which indicate the operation of CO_2 fixation. They also found pH gradients across the thylakoid membranes, suggesting ATP consumption for CO_2 fixation in guard cell chloroplasts. The chlorophyll fluorescence from a single cell completely rules out the signal from the other cell, such as mesophyll cells in harvesting the fluorescence intensity.[55] They have thus proposed that guard and mesophyll cells have similar functional organization of thylakoid membranes in chloroplasts. And thus, guard cell chloroplasts possess the Calvin-Benson cycle enzymes involved in CO_2 fixation, implying that guard cell chloroplasts have a normal photosynthetic carbon reduction pathway just like their mesophyll counterparts. The operation of Calvin-Benson cycle in guard cell chloroplast may synthesize starch, which produces malic acid during stomatal opening. The sugar production (sucrose) *via* the Calvin cycle may thus contribute to the osmoticum of guard cells during stomatal opening.

CONCLUDING REMARKS

Guard cells are very attractive model system for elucidating the perception site as well as the transducing mechanisms of environmental signals, ion channels in membrane transport, cell water relations, and many other processes involved in stomatal movements. In this review I have described the intrinsic properties of guard cell chlorolplasts. The most significant advance in this field has been the recent technique being capable of directly quantifying the photosynthetic

activity of guard cell chloroplasts in single stomata and isolated protoplasts by microfluorimetry using an inverted epifluorescence microscope.[55] What is certain is that, like their mesophyll counterparts, guard cell chloroplasts have two functional photosystems I and II, and can fix CO_2 photosynthetically. However, we have raised serious questions about the physiological implications in photosynthetic performance of guard cell chloroplasts during stomatal opening. The firist is that why do guard cell chloroplasts have higher O_2-evolving system than that of mesophyll cell ones? The second is that we should note about small amount of Rubisco for CO_2 fixation in guard cell chloroplasts. Therefore, we must question the physiological implication of the products via CO_2 fixation in guard cell chloroplasts during stomatal opening. Does the sugar production (sucrose) via the Calvin cycle contribute significantly to the osmoticum of guard cells? Further information about the properties of guard cell chloroplasts should also find in a profound study of acclimation of guard cell chloroplasts to changing light environments. For examples, changes of photosystem stoichiometry may affect the photosynthetic performance since light acclimation benefits a chloroplast by maximizing light capture and optimizing light use efficiency. It will of paramount significance for a correct understanding in the functioning of chloroplasts during stomatal movements.

FUTURE PROSPECTS

In stomatal physiology with respect to the energy metabolism of guard cells in light, guard cell chloroplasts have been thus discussed as the potential energy source for the H^+ pumping by plasma membrane ATPase, the import of K^+, and synthesis of the organic counterion (malic acid). Furthermore, it is essential for the maintenance of cytosolic ATP and NADPH levels, carbon reduction and sucrose production, and thus guard cell osmotic potential.[24,25] On the other hand,. Ion channels are integral membrane proteins that mediate the movement of ions down their electrochemical potential gradients in plant cells. Especially, activation of ion channels in guard cells by light may thus occur through the energy metabolism. Concerning the origins and control of the ATP and NAD(P)H pool in the cytosol of guard cells during stomatal movements in response to environmental stimuli, the molecular mechanisms of energy metabolism of guard cells in light and its signal transduction between chloroplasts and plasma membrane ion transport are still unknown. The combination of patch clamp technique and chlorophyll fluorescence imaging system which developed recently may be permitted us to monitor simultaneously both photosynthetic activity and ion channel currents in single guard cell protoplasts. It will be expected to give us valuable information about the steps involved between the chloroplastic factors and the activation of ATP-, pH-, and voltage-dependent plasma membrane ion channels in guard cells in response to environmental stimuli affecting stomatal movements.

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