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Review

Calvin cycle and guard cell metabolism impact stomatal function

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ABSTRACT

Stomatal conductance (g_s) determines CO_2 uptake for photosynthesis (A) and water loss through transpiration, which is essential for evaporative cooling and maintenance of optimal leaf temperature as well as nutrient uptake. Stomata adjust their aperture to maintain an appropriate balance between CO_2 uptake and water loss and are therefore critical to overall plant water status and productivity. Although there is considerable knowledge regarding guard cell (GC) osmoregulation (which drives differences in GC volume and therefore stomatal opening and closing), as well as the various signal transduction pathways that enable GCs to sense and respond to different environmental stimuli, little is known about the signals that coordinate mesophyll demands for CO_2. Furthermore, chloroplasts are a key feature in GCs of many species, however, their role in stomatal function is unclear and a subject of debate. In this review we explore the current evidence regarding the role of these organelles in stomatal behaviour, including GC electron transport and Calvin-Benson-Bassham (CBB) cycle activity as well as their possible involvement correlating g_s and A along with other potential mesophyll signals. We also examine the roles of other GC metabolic processes in stomatal function.

1. Introduction

Stomata are the gateway for gas exchange between the plant and the atmosphere. They are present on the surfaces of leaves as well as other organs [1] and are composed of two guard cells (GCs) forming a central pore on the epidermis. The state of turgor and the anatomy of these guard cells determine the size of the pore (stomatal aperture) and therefore the amount of CO_2 entering and H_2O leaving the leaf. The maximum rate of water flux from the leaf is used to determine stomatal conductance (g_s) and used as a measure of stomatal function. At the whole leaf level, stomatal conductance also depends on the abundance of stomata (stomatal density; SD) as well as pore depth and aperture [2,3]. Variation in GC turgor pressure controls GC movements and relies on a connection between ion balance and metabolism in GCs (reviewed in [4,5], Fig. 1). Stomatal opening is associated with an influx of K^+ through the opening of voltage-gated inward-rectifying K^+ channels and through the activity of H^+/K^+ HAK-type symporters [4,6], and the balance of the positive electrical charge by the uptake of inorganic anions, such as Cl^- and $NO3^-$ or organic solutes, mainly malate and sucrose, which are either imported or synthesised within the GC (see below, Fig. 1.3, 1.10, 1.11). These osmolytes are then stored in the vacuole and raise the osmotic potential leading to an influx of water, an increase in turgor pressure and ultimately the opening of the stomatal pore (Fig. 1.4 and 1.5). Stomatal closing mechanisms represent a reverse process where GC turgor pressure is lowered by converting malate into non-osmotic starch and exporting K^+, Cl^- and malate via channels such as outward-rectifying K^+ channels (GORK), slow anion channels (SLAC) and quick anion channels (QUAC) [4].

Stomata respond to numerous external and internal cues to regulate gaseous exchange and balance CO_2 uptake with water loss, to ensure sufficient substrate for photosynthesis, whilst maintaining overall plant water status [7] and evaporative leaf cooling [8]. Regulation of stomatal conductance is complex and usually the result of the integration of many simultaneous environmental cues [3] including light intensity and spectral quality, CO_2 concentration ($[CO_2]$), temperature and vapour pressure deficit (VPD). Light quality or spectra waveband greatly influences stomatal behaviour via two distinct responses known as: 1) the specific blue-light response, and 2) the red-light response (reviewed in [9], Fig. 1). The blue-light response is more effective in promoting stomatal opening, saturates at low fluence rates and is independent of photosynthesis, while the red-light response occurs at high fluence rates and is believed to be dependent on photosynthesis. Blue light is perceived by phototropins resulting in a signalling cascade that invokes the plasma membrane (PM) H^+-ATPase and results in rapid stomatal opening via uptake of K^+ and anions, including malate and sucrose, possibly as a result of starch breakdown [10] (Fig. 1.1–.3). More

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recently, Flütsch et al. [11] have suggested that glucose rather than malate is the main result of starch breakdown and is required for rapid stomatal opening in blue light (Fig. 1.6). The red-light response is considered the primary stomatal response that links and co-ordinates stomatal behaviour with mesophyll demands for CO_2, although the full signal transduction pathway has still not been elucidated. Interestingly recent studies have reported red light induced stomatal opening also via activation of the PM H^+-ATPase linked to photosynthetic electron transport [12]. Although, the location of the red photoreceptor or signalling cascade for the red-light response is under debate (and discussed in more detail below), there are several lines of evidence that suggest that red light is sensed in the chloroplast with no consensus on whether this is in mesophyll or GC chloroplasts [13,14].

Stomatal function impacts on CO_2 supply for assimilation (A) in the Calvin-Benson-Bassham (CBB) cycle and usually there is a close relationship between the rate of photosynthetic CO_2 fixation and stomatal

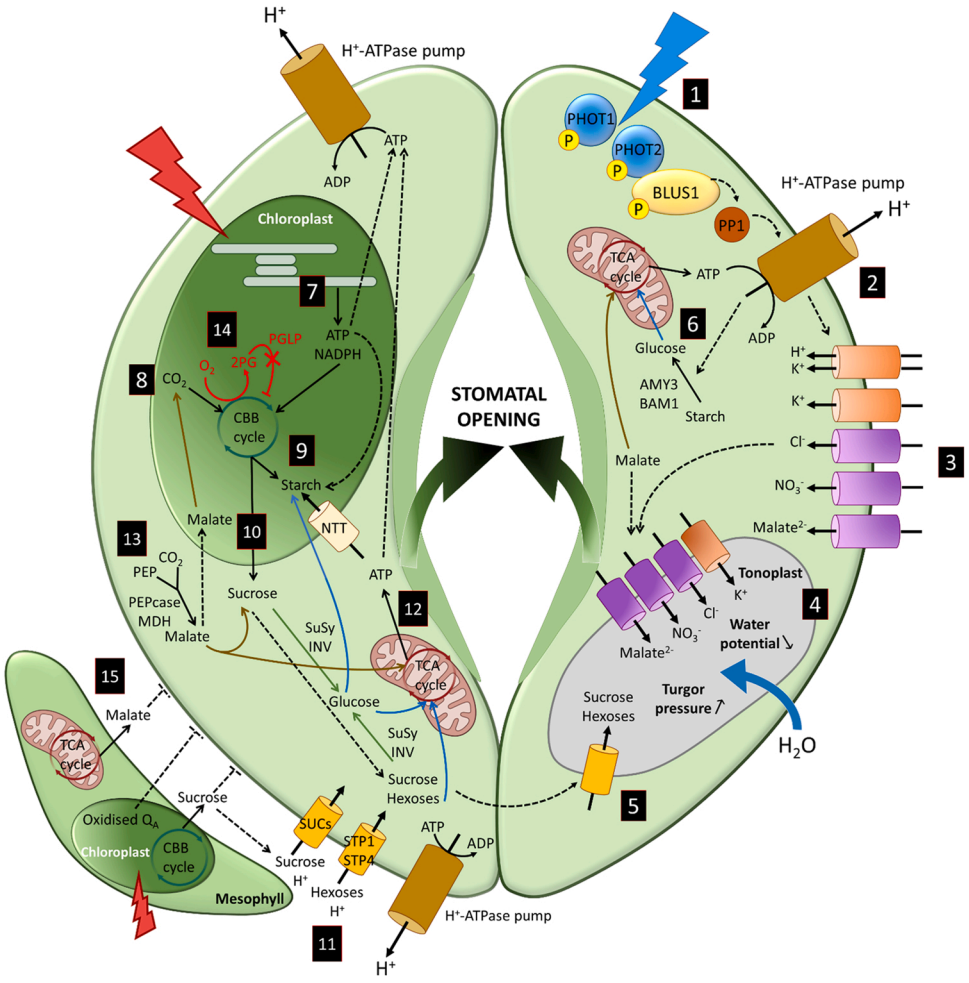

Fig. 1. Impact of mesophyll and guard cell metabolism on stomatal opening. 1. Blue light triggers stomatal opening after perception by the photoreceptors PHOT1 and PHOT2. 2. The signal is transduced via kinases and phosphatases such as BLUS1 and PP1 and ultimately activates the plasma membrane (PM) H^+-ATPase pump leading to membrane hyperpolarisation. 3. This enables the influx of K^+, Cl^-, NO_3^- and malate. Malate can also be synthesised in the guard cell (GC). 4. These osmolytes can be stored in the tonoplast decreasing the water potential leading to an influx of water which in turn increases GC turgor pressure and promotes stomatal opening. 5. In the afternoon, sucrose is believed to be the main osmolyte maintaining turgor pressure [113]. 6. Blue light also triggers starch degradation catalysed by AMY3 and BAM1 and dependent on PM H^+-ATPase pump activity. It yields glucose and helps maintain the cytoplasmic sugar pool for energetic purposes [10,11]. 7. Red light activates photosynthetic processes in GC chloroplasts. ATP and NADPH produced through the chloroplastic electron transport chain can be used for starch synthesis and in the CBB cycle [47,92]. Potentially, this source of ATP could also be used by the PM H^+-ATPase pump. 8. CO_2 is fixed by ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) in the Calvin-Benson-Bassham (CBB) cycle [82,92,95]. The triose-phosphates produced in the CBB cycle are usually used for starch synthesis in the stroma or exported in the cytosol to be converted into sucrose. 9. Starch accumulation is associated with red light-triggered stomatal opening and is also dependent on PM H^+-ATPase pump activity which could energise the uptake of mesophyll-derived sucrose to contribute to starch accumulation [11]. GC chloroplasts do not produce enough ATP to sustain starch synthesis and can import ATP from the cytosol through nucleotide transporters [92,115]. 10. Sucrose derived from GC CBB activity can be stored in the tonoplast or hydrolysed by sucrose synthases and invertases during light-induced stomatal opening to produce glucose [81,82,114,115]. 11. GC can import mesophyll-derived sucrose from the apoplast via the activity of H^+/sucrose symporters SUCs [116]. This exogenous sucrose could also be stored in the tonoplast or hydrolysed to generate glucose. GC can import apoplastic hexoses as well via the activity of H^+/hexose symporters STPs especially early in the morning while sucrose import would be predominant later in the day [111]. 12. The glucose generated from sucrose degradation or imported from the apoplast can be synthesised into starch or go through glycolysis to feed the TCA cycle in the mitochondria which generates most of the ATP supply necessary to sustain starch synthesis and PM H^+-ATPase pump activity in GCs during stomatal opening [69,81,82,92,111,115]. 13. The production of malate via anaplerotic CO_2 fixation involving PEPcase and MDH activities in GCs also plays an important role in stomatal opening. This malate can be involved in sucrose synthesis via gluconeogenesis and can help replenish the intermediates of the TCA cycle [92,95]. Moreover, most of the CO_2 fixed by RuBisCO seems to originate from the decarboxylation of malate imported from the cytosol into the chloroplast [95]. 14. Functional photorespiratory pathways maintain the breakdown via PGLP activity of 2PG which can inhibit enzymes from the CBB cycle [118,119]. Avoiding these inhibitory effects can help maintain CO_2 fixation rates in the mesophyll and/or GCs (only shown in GCs on the figure for simplification), and likely as a consequence, maintain starch accumulation and stomatal opening under high photorespiratory conditions [17,117,118]. 15. Signal(s) derived from the mesophyll such as malate, sucrose and the oxidised Q_A pool could help coordinate stomatal opening with photosynthetic demand from the mesophyll [68,77,81,90]. For an easier representation, organelles and processes in GCs are distributed between the two cells forming a pore. Blue, green and brown arrows represent glucose, sucrose and malate degradation/conversion respectively. Solid arrows represent entry in metabolic pathways while dashed arrows represent regulatory interactions or movements. PHOT: phototropin, BLUS: blue light signalling kinase, PP: protein phosphatase, AMY: alpha-amylase, BAM: beta-amylase, NTT: nucleotide transporter, SuSy: sucrose synthase, INV: invertase, SUC: sucrose transporter, STP: sugar/hexose transporter, TCA: tricarboxylic acid, PEP: phosphoenolpyruvate, PEPcase: phosphoenolpyruvate carboxylase, MDH: malate dehydrogenase, 2PG: 2-phosphoglycolate, PGLP: 2-phosphoglycolate phosphatase, Q_A: quinone A. Created with BioRender.com.

conductance in steady-state [15] and although conserved, this relationship is not always constant [3,16]. The relationship between g_s and A is curvilinear with greater stomatal control of A at low g_s, due to restricted CO_2 diffusion, lowering intercellular [CO_2] (C_i). Photosynthesis can therefore be limited by stomatal conductance in some circumstances. For example, by promoting stomatal closure, drought can lead to a higher oxygenation/carboxylation ratio of ribulose-1,5-biphosphate (RUPB) by RUBP-carboxylase/oxygenase (RubisCO), reducing A [17,18]. Furthermore, under dynamic conditions (e.g. fluctuating light), stomatal responses are an order of magnitude slower to respond than photosynthetic responses and this can lead to a disconnect between A and g_s [16,19–22]. Photosynthesis is initially stomata-limited following a rapid increase in light intensity due to slow stomatal opening, whilst slow closure can lead to unnecessary water loss for no carbon gain and reduced water use efficiency (Fig. 2, [7,20,23]). The foregone carbon assimilation due to slower stomatal opening responses in such dynamic environments can reduce yield potential and strategies to speed up stomatal kinetics represent a promising route to improve crop performance and harvest [22,24–26]. The nature of the signal(s) responsible for this coordination between stomatal behaviour and photosynthesis remains unclear but seems to be mesophyll-driven and photosynthesis-dependent [27,28].

In this review, we focus mainly on how a better understanding of mesophyll and GC metabolism can provide a route to accelerate stomatal kinetics. In the next sections, we review: the potential signal(s) coordinating stomatal function with CO_2 demand from the CBB cycle; the current knowledge on photosynthesis and CBB cycle in GCs; their potential role in stomatal function and kinetics as well as the fate of photoassimilation-derived carbohydrates within GCs. Finally, we explore the importance of other GC metabolic pathways in stomatal function and kinetics. Although it is well established that ion movements in GCs are linked closely to GC metabolism and play an important role in stomatal function and kinetics, this will not be covered in depth here and we refer readers to several excellent reviews [4,29–38].

2. Coordination of stomatal function with CO_2 demand from the Calvin-Benson-Bassham cycle

2.1. Intercellular CO_2 concentration may not be the only signal coordinating photosynthetic activity with stomatal behaviour

The concentration of CO_2 in the intercellular airspaces (C_i) has long been understood to be the signal that links g_s with mesophyll A, and responsible for the mesophyll or red-light response, also commonly referred to as the PAR (photosynthetically active radiation) response [14]. It is hypothesised that variations in mesophyll consumption of CO_2 due to changes in light and other variables are sensed by the guard cells, resulting in changes in aperture to maintain a ratio of C_i to ambient CO_2 concentration (Ca) (C_i:C_a) [39–41]. In C_3 species, A responds to increasing C_i in a well-established and intensively studied hyperbolic response, with photosynthetic rates saturating at a particular C_i value. However, stomatal responses to C_i have been less well studied, most likely due to slower stomata responses and the time required to reach steady-state [3]. Although, it is generally accepted that stomatal aperture or g_s decreases with increasing C_i (usually achieved by changing external [CO_2] (C_a)), this relationship is also not linear [42], and stomatal sensitivities to C_i depend upon species and conditions, such as light intensity [43,44]. However, several more recent studies have highlighted that the relationship between g_s and C_i (as a function of C_i:C_a) can be broken and therefore C_i may not be the only or main signal for coordination of stomatal behaviour and CO_2 requirement of the mesophyll. The characterisation of mutants with impaired photosynthetic (or photorespiratory) processes usually shows an increase in C_i compared to the control plants as a result of decreased A [45]. In most cases, stomata appeared not to respond to the higher C_i as g_s was often maintained at similar levels to the control plants leading to an increase in the C_i:C_a ratio (Table 1). In the majority of these mutants, photosynthetic capacity was reduced via antisense RNA or gene knock-out (KO) technology targeting the electron transport chain, the CBB cycle or the photorespiratory pathway. These mutations included reductions (compared to the control plants) in the subunit of the chloroplastic cytochrome b6f complex, the small subunit of RubisCO, the chloroplastic NADPH-Glyceraldehyde-Phosphate dehydrogenase, sedoheptulose-1,

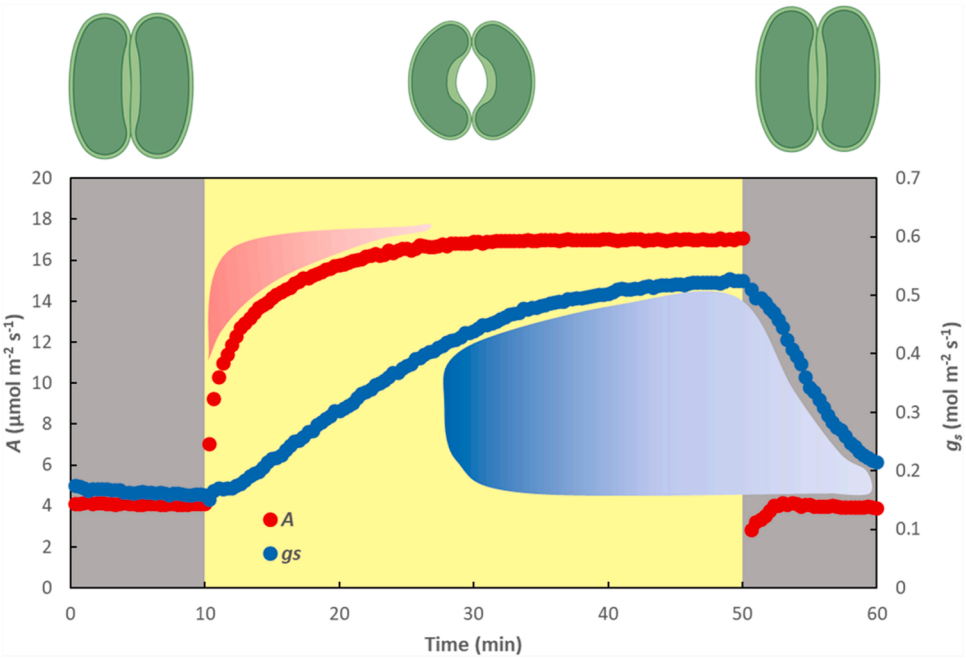

Fig. 2. Dynamics of photosynthetic assimilation and stomatal conductance in a fluctuating light environment. Tobacco leaves were subjected to step changes in light intensity from 100 µmol m^{-2} s^{-1} PPFD to 1500 µmol m^{-2} s^{-1}, then back to 100 µmol m^{-2} s^{-1}. The guard cells above the graph represent the opening/closing status of the stomata. The red shape in the graph represents a loss in carbon assimilation and the blue shapes represent water loss. Created with BioRender.com.

Table 1
Transgenic manipulations affecting photosynthesis and/or guard-cell function. Unless stated otherwise, photosynthesis performance is measured as net CO_2 assimilation at the leaf-level. RbcS: small subunit of Rubisco, RUBP: ribulose-1,5-biphosphate, RubisCO: RUBP carboxylase/oxygenase, SBPase: sedoheptulose 1,7-bisphosphatase, NADP-GAPDH: NADP-glyceraldehyde-3-phosphate dehydrogenase, FBPase: fructose-1,6-bisphosphatase, PRK: phosphoribulokinase, PEPcase: phosphoenolpyruvate carboxylase, PGLP: phosphoglycolate phosphatase,SHM: serine hydroxymethyltransferase, GLYK: glycerate kinase, GDC-H: H-protein of the glycine decarboxylase complex, 2PG: 2-phosphoglycolate, SDH: succinate-dehydrogenase, PsbS: Photosystem II subunit S, AGPase: ADP-Glucose-pyrophosphorylase, pPGI: plastidial phosphoglucose isomerase, AMY: alpha-amylase, BAM: beta-amylase, KO: knock-out, ETR: electron transport rate, NPQ: non-photochemical quenching.

Metabolic process (es)	Altered gene(s)	Function	Manipulation	Promoter/ tissue location	Species	Photosynthesis	Stomatal behaviour	Refs.
CBB cycle	RbcS	Carboxylation of RuBP	Antisense	CaMV 35S	*N. tabacum*	Reduced	No change except decrease when RubisCO content was very low	[45,46, 127–129]
					F. bidentis	Reduced	Higher Ci/Ca suggests g_s not reduced to same extent as A	[130]
CBB cycle	NADP-GAPDH	Conversion of PGA to triose-P	Antisense	CaMV 35S	*N. tabacum*	Reduced	No significant change	[131]
CBB cycle	FBPase	Conversion of fructose-1,6-P to fructose-6-P	Antisense	CaMV 35S	*S. tuberosum*	Reduced	No change or increase depending on relative humidity and CO_2 conditions	[132]
CBB cycle	SBPase	Conversion of sedoheptulose-1,7-P to sedoheptulose-7-P	Antisense	NtRbcS	*N. tabacum*	Reduced	Higher g_s and lower closing rate in mixed light and red light, higher opening rate in red light	[47]
CBB cycle	PRK	Conversion of Ru5P to RuBP	Antisense	NtRbcS	*N. tabacum*	Reduced	No significant change	[133]
Anaplerotic CO_2 fixation	PEPcase	Carboxylation of PEP to form oxaloacetate	Mutants with reduced PEPcase activities	-	*A. edulis*	Reduced	Lower opening rate and g_s in mutants with 3% PEPcase activity compared to WT	[48]
Chloroplastic electron transport chain	PsbS	Promotes NPQ	Overexpression	CaMV 35S	*N. tabacum*	No change	Lower g_s when increasing light intensity	[90]
Chloroplastic electron transport chain	Rieske FeS	Subunit of cytochrome b6f complex	Antisense	CaMV 35S	*N. tabacum*	Reduced	No change except decrease when Rieske FeS content < 10 % of WT	[46,134]
Chloroplastic electron transport chain	Truncated chlorophyllase	Chlorophyll degradation	Overexpression	GC-targeted	*A. thaliana*	Slightly reduced at ambient and elevated [CO_2]	Lower steady-state g_s at ambient [CO_2] but similar responses to [CO_2] shifts	[103]
Photorespiration	PGLP1	Breakdown of 2PG	KO, antisense	CaMV 35S	*A. thaliana*	Reduced	Higher transpiration at high [CO_2] and lower transpiration and gs at ambient [CO_2]	[117, 118]
			Overexpression	ST-LS1		Increased	Higher g_s	[17,118]
Photorespiration	SHM1	Conversion of serine to glycine	KO	-	*A. thaliana*	Reduced ETR	Higher transpiration at high [CO_2] and lower transpiration at ambient [CO_2]	[117]
	GLYK1	Conversion of glycerate to 3-P-glycerate						
Photorespiration	GDC-H	Conversion of glycine to serine	Overexpression	ST-LS1	*N. tabacum*	Increased in high light intensities	Higher g_s in greenhouse conditions	[121]
Photorespiration	PMDH	Reduction of NAD^+ to NADH via malate oxidation	KO	-	*A. thaliana*	Slightly reduced	No change	[135]
Respiration	Fumarate hydratase	Reversible hydration of fumarate to malate	Antisense	CaMV 35S	*S. lycopersicum*	Reduced	Lower g_s, opening and closing rates upon dark to light transition and vice versa	[69]
Respiration	SDH2	Oxidation of succinate in fumarate	Antisense	CaMV 35S	*S. lycopersicum*	Increased	Higher g_s but no change in kinetics	[68]
				AtMYB60 (GC-specific)		No change	No change	

(*continued on next page*)

Table 1 (*continued*)

Metabolic process (es)	Altered gene(s)	Function	Manipulation	Promoter/ tissue location	Species	Photosynthesis	Stomatal behaviour	Refs.
Carbohydrate metabolism	SuSy3 (preferentially expressed in GCs)	Reversible hydrolysis of sucrose	Antisense	CaMV 35S	*S. tuberosum*	Reduced in high light intensities	Lower g_s in high light intensities (morning and midday)	[114]
			Overexpression	StKST1 (GC-specific)	*N. tabacum*	Increased	Higher g_s following dark to light transition	[115]
Carbohydrate metabolism	Acidic invertase	Irreversible hydrolysis of sucrose	Overexpression	Fragment of StAGPase (GC-specific)	*S. tuberosum*	Increased (morning)	Higher g_s (morning)	[114]
Carbohydrate metabolism	Sucrose transporter1	Sucrose import from the apoplast	Antisense	StKST1 (GC-specific)	*N. tabacum*	Reduced (after midday)	Lower g_s	[116]
Carbohydrate metabolism	AGPase	Starch synthesis	KO	Starch deficient in all tissues	*A. thaliana*	Reduced at high $[CO_2]$	Impaired stomatal closure at high $[CO_2]$ and lower stomatal opening rate at low $[CO_2]$	[66]
	pPGI			Starch deficient only in mesophyll		Reduced at high $[CO_2]$	No change in stomatal closure at high $[CO_2]$ and higher g_s upon shift to low $[CO_2]$	
Carbohydrate metabolism	BAM1 (highly expressed in GCs)	Starch degradation	KO, miRNA	CYP86A2 (GC-specific)	*A. thaliana*	Initial slight reduction in double mutant following white light illumination	Lower stomatal opening rate and g_s in double mutant following white light illumination. Lower g_s in double mutant under red light. No change in g_s in double mutant under saturating white light intensities	[10,11]
	AMY3 (highly expressed in GCs)		KO			No change in double mutant under red light or saturating white light intensities		
ATP supply	Nucleotide transporter 1 (preferentially expressed in GCs)	Plastidial ATP/ ADP translocator	KO	-	*A. thaliana*	No significant change	Lower g_s and stomatal opening rate upon dark to light transition, lower stomatal closing rate upon light to dark transition	[92]
Sugar sensing	Hexokinase	Hexose sensor	Overexpression	CaMV 35S StKST1 (GC-specific)	*A. thaliana* *S. lycopersicum* *N. tabacum* *Citrus* (only StKST1)	Reduced in *A. thaliana* in constitutive overexpression. No reduction in other conditions	Lower g_s	[75–80]

7-biphosphatase (SPBase), fructose-1,6-biphosphatase (FBPase), phosphoribulokinase, phosphoenolpyruvate carboxylase (PEPcase) or the peroxisomal NAD-dependent malate dehydrogenase (NAD-MDH) and focused on *Nicotiana tabacum*, *A*rabidopsis *thaliana*, *Solanum tuberosum* and a couple of C_4 species, *Flaveria bidentis* and *Amaranthus edulis* (Table 1). Although most of these studies examined steady-state stomatal behaviour, some included investigations on dynamic responses of stomata to a step increase in light intensity [45–48]. Transgenic tobacco plants with reduced amounts of RubisCO had similar stomatal opening rates and g_s after an increase in mixed red/blue light intensity compared to control plants in a range of $[CO_2]$. Likewise, there was no significant difference in maximal g_s following a step increase in red light intensity in these mutants compared to control plants when grown in low or medium light intensities [45,46]. In transgenic tobacco plants with reduced amounts of Rieske FeS protein, stomatal opening rates and g_s after an increase in red light intensity remained comparable to the control plants except in the mutants with photosynthetic rate reaching only 0.8 % of the control level, which exhibited a 50 % reduction in maximum g_s [46]. These findings left the authors to conclude that the correlation between A and g_s is caused by a signal independent of photosynthesis. However, transgenic tobacco plants with reduced amounts of SBPase showed faster stomatal opening following an increase in red light alone, whilst similar rates to the controls were observed when mixed red/blue light was used, although g_s was higher than WT in both cases [47]. As the reductions in SBPase expression were driven by a RubisCO promoter [49], it is possible that SBPase levels (and CBB activity) were also reduced in the GCs, which in turn would decrease ATP consumption in the CBB cycle, resulting in greater amounts of GC ATP that could be used to drive the PM H^+-ATPase and increase aperture [13,47]. An *A. edulis* PEPcase-deficient homozygous mutant, with a 90 % reduction in A compared to the WT, actually showed a 60 %

reduction in steady-state g_s and 3 times lower stomatal opening rate after a shift from dark to high light [48]. However, C_i:C_a was increased in this mutant and the levels of stomatal response to changes in light intensity and [CO_2] were not correlated with photosynthetic responses. Although more studies with mutants impaired in photosynthesis may be needed to fully understand the dynamics and signalling pathways controlling stomatal behaviour, all those mentioned above suggest that C_i has limited impact on both the steady-state and dynamic stomatal responses. Further evidence disproving C_i as the main coordinating signal between A and g_s comes from studies which have demonstrated a stomatal response to red light even when C_i was held constant [43,47,50]. Alternatively, von Caemmerer et al. [45] suggested that guard cells sense C_a rather than C_i, or mesophyll-driven signal(s) independent of C_i. However, it is important to note that reported C_i values are estimates and the relationship between C_i and g_s is not linear (e.g. [42]) which can make these results difficult to interpret and could lead to underestimating the importance of C_i variations in linking stomatal response to mesophyll demand for CO_2. A recent study revealed a decay in stomatal responsiveness to changes in C_i when *A. thaliana* was subjected to repeated steps in [CO_2] or light intensity [42]. CO_2-induced stomatal closing has been linked to increased GC cytosolic free [Ca^{2+}] [51]. Jezek et al. [42] discovered a connection between endomembrane Ca^{2+} stores and the "carbon memory" or latency in the recovery of stomatal responsiveness to changes in C_i observed following previous closure events. Repeated increases in C_i led to declines in GC endomembrane Ca^{2+} stores and the capacity for Ca^{2+} release to increase cytosolic concentration which in turn slowed stomatal closing, adding further evidence to the complexity and non-linearity of the connection between C_i and g_s. These findings highlight the importance of examining dynamics responses, and to some extent can provide some explanation for the variation reported in stomatal responses between species [20,23] and at different times of the day [52]. Alterations in these dynamic responses can have significant impacts on whole plant carbon assimilation and water use efficiency [19].

2.2. Mesophyll photosynthesis as a coordinating signal

Several studies have tried to elucidate the mesophyll signal that coordinates stomatal responses with mesophyll demands for CO_2. Lee and Bowling [53–55] showed that stomatal aperture in isolated epidermis of *Commelina communis* floated on a liquid buffer did not respond to changes in light intensity or [CO_2]. Similar results were obtained in *Tradescantia pallida* and *Pisum sativum* where stomata from isolated epidermis regained responsiveness to light and [CO_2] when placed on exposed mesophyll cells [27]. The same authors also showed that stomata only responded to incubation with mesophyll cell culture if the mesophyll cells had previously been incubated in the light but not when they were kept in the dark, implying a metabolic signal. Altogether, these results suggest the production of a soluble compound by the mesophyll is responsible for light and CO_2-induced stomatal opening. More recent experiments performed on *C. communis* epidermal strips placed on buffer-containing gel rather than floated on liquid buffer (to avoid a potential effect of filling substomatal cavities with liquid when they should be air-filled as in intact leaves) and using different spacers suggested an aqueous phase signal that moves in the apoplast [56], whilst previous studies had suggested a vapour-phase signal [57]. However, other studies have reported that stomata from a range of species do respond to various stimuli in epidermal peels, in which no mesophyll is present [42,58,59], suggesting that part of the sensory mechanism must be located in the epidermis [60,61]. Nevertheless, responses in peels have also been shown to be significantly slower than in intact leaves [62,63].

Whether this potential mesophyll-driven signal is dependent on photosynthesis or not also remains controversial. As mentioned previously, several transgenic plants impaired in their photosynthetic rate showed unchanged stomatal behaviour (Table 1). Decreased photosynthetic rates due to reduced amounts of enzymes involved in the CBB cycle or components from the chloroplastic electron transport chain suggest that stomatal behaviour is not strongly reliant on either of these processes. Conversely, stomatal opening under red light was abolished in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), an inhibitor of the photosystem II, in *C. communis and Helianthus annuus* [56,64]. DCMU also induced stomatal closure under white light and elevated [CO_2] [50]. Moreover, albino leaf patches in *Vicia faba* and *Chlorophytum comosum* variegated leaves revealed no stomatal opening under red light although guard cells contained functional chloroplasts in the albino patches of *C. comosum* [65]. These findings support a requirement for photosynthetically active mesophyll cells for stomatal opening in response to red light. Conversely, Azoulay-Shemer et al. [66] showed that Arabidopsis mutants with whole-leaf reduced photosynthesis had unaltered high [CO_2]-induced stomatal closing suggesting mesophyll photosynthesis is not the major mediator of CO_2 control of stomatal closing.

2.3. Possible mesophyll signals

Malate plays an essential role in stomatal movements as an osmoregulator and a counter-ion for K^+. When accumulated in the vacuole of GCs, malate lowers the water potential and promotes stomatal opening. It can be synthesised in the cytosol from PEP through the catalytic activities of PEPcase and NAD-MDH (Fig. 1.13), or imported from the apoplast (Fig. 1.3) [5,67]. During stomatal closure, malate can be dissipated via decarboxylation through malic enzyme or PEP carboxykinase activities or released into the apoplast through the rapid-type anion channel [5,67]. Malate provided by adjacent mesophyll cells could be (one of) the link(s) between mesophyll activity and stomatal behaviour (Fig. 1.15). Transgenic tomato plants with antisense inhibition of two enzymes involved in the mitochondrial tricarboxylic acid (TCA) cycle led to modifications in malate content at the leaf level and changes in A and g_s [68,69]. Plants with lower fumarase content showed higher malate leaf content, higher malate and fumarate apoplastic concentrations, lower A, slower stomatal kinetics and lower g_s [68,69] suggesting a role in either stomatal opening or the coordination between A and g_s. Plants with decreased content in the iron sulphur subunit of the succinate dehydrogenase complex (SDH) showed lower fumarate and malate leaf contents and apoplastic concentrations alongside enhanced A and g_s in leaves as well as increased O_2 evolution rates in both GC and mesophyll cell protoplasts [68]. Although the rates of stomatal opening and closing were unchanged in these mutants following a transition into light or dark respectively, their maximal stomatal aperture was larger than the control plants. Additionally, exogenous application of malate and to a lesser extent fumarate on leaves reduced stomatal aperture in control plants in a concentration-dependent manner and independently of osmotic effects. When this antisense inhibition of SDH was GC specific, no difference was observed either in apoplastic malate and fumarate concentrations or A and g_s. These findings support a role for malate from the mesophyll and not the GCs as a signal to coordinate mesophyll photosynthesis and stomatal behaviour. Arabidopsis KO plants missing a tonoplast dicarboxylate transporter responsible for malate accumulation in the vacuole of mesophyll and GCs (although more expressed in the former than the latter) showed reduced malate content in their leaves but no difference in A and stomatal behaviour [70]. However, the apoplastic concentrations of malate were unchanged compared to the control plants which could be an argument in favour of a role for malate as a mesophyll-driven signal diffusing to the epidermis through the apoplast. Moreover, a rise in extracellular malate concentration has been shown to increase the activity of GC rapid-type anion channels responsible for malate efflux into the apoplast possibly leading to a feedforward mechanism during stomatal closing and supporting a signalling role of mesophyll-derived malate in stomatal response [4,71,72]. Yet, cytosolic oxaloacetic acid could have a larger control on the activity of these channels as suggested by the work of Wang et al. [73]. Several studies report a role for PEPcase in stomatal behaviour by providing

malate to the GCs. PEPcase constitutive overexpressing and antisense potato plants showed respectively higher and lower leaf malate contents and stomatal opening rates compared to the control plants [74]. Similarly and as mentioned earlier, Cousins et al. [48] showed a decrease in g_s and slower stomatal opening in a PEPcase-deficient homozygous mutant where PEPcase contents were decreased to a comparable extent in mesophyll and GCs. However, these studies do not report apoplastic malate concentrations and highlight the effect of constitutive genetic transformations. It would be interesting to compare these results with experiments on GC specific overexpressing or antisense plants to clarify the role of mesophyll PEPcase in producing malate as a signal to coordinate mesophyll photosynthesis and stomatal behaviour. The malate produced could also be used directly as a counter ion or osmolyte for stomatal movements.

Sugar-sensing has also been proposed to play a role in the coordination of stomatal function and mesophyll demand. In this hypothesis, sucrose produced in the mesophyll acts as a signal. Glucose, as a product of sucrose hydrolysis, can be sensed by hexokinases whose overexpression either constitutive or in a GC specific manner has been shown to reduce g_s in Arabidopsis, tomato, tobacco and citrus plants [75–80]. Moreover, the addition of exogenous sucrose or glucose exacerbates the reduction in stomatal aperture in tomato plants [77] (Fig. 1.15). In these studies, the authors suggest that, as a feedback inhibition mechanism, excess sucrose from mesophyll photosynthesis is transported in the transpiration stream to the GCs where it can be sensed via hexokinases to trigger stomatal closure and prevent water loss. Additionally, Medeiros et al. [81] reported that high concentrations of exogenous sucrose promoted stomatal closure in detached leaves and GC-enriched epidermal peels in Arabidopsis (Fig. 1.15). Conversely, Daloso et al. [82] did not observe a repressive effect of an exogenous sucrose treatment at a lower concentration during light-induced stomatal opening in tobacco GC-enriched epidermal fragments, suggesting a concentration-dependent effect. Although, the hypothesis that sucrose and/or its hydrolysis products could act as a signal, coordinating A and g_s, this could only really explain longer-term diurnal feedback in which photosynthesis often reduces towards the end of the light period, rather than a short-term dynamic signal, as rates of high photosynthesis (and therefore high apoplastic sugar concentration) [83,84] are not normally correlated with low g_s [85].

In addition to malate and sugars, other signals including chloroplastic ATP, NADPH, RUBP and zeaxanthin have been proposed [15,54,86–89]. More recently, a role for chloroplastic quinone A (Q_A) redox state in light-induced stomatal opening has been suggested [14,90] (Fig. 1.15). Q_A is the primary electron acceptor downstream of photosystem II (PSII) in the photosynthetic electron transport chain. Its redox state reflects the balance between the excitation energy at PSII and downstream processes that act as sinks for electrons including CBB cycle activity. Głowacka et al. [90] observed that higher levels of Photosystem II subunit S (PsbS) in transgenic tobacco were correlated with more oxidised Q_A pool and reduced stomatal opening when light intensity increased without impacting steady-state photosynthesis. PsbS is involved in non-photochemical quenching (NPQ) decreasing the excitation pressure at PSII by heat dissipation [91] resulting in a less reduced Q_A pool which could be a signal that coordinates stomatal response to light with mesophyll demand [14]. Since the manipulation in PsbS-overexpressing tobacco plants is constitutive [90], we cannot rule out a potential importance of this signal directly within the GCs.

Using photosynthetic mutants as outlined above that have been generated with constitutive promoters to drive expression changes in key photosynthetic and metabolic gene targets makes it extremely difficult to determine if changes in mesophyll or GC metabolism or both are responsible for any impact on stomatal behaviour and/or the coordination with mesophyll A. The importance of CBB cycle activity as well as other photosynthetic processes in GCs for stomatal function and/or as a signalling coordinating stomata with mesophyll is still not fully understood and debated.

3. Stomatal function and guard-cell photosynthesis

The role of GC chloroplasts and whether GCs perform photosynthetic carbon reduction has been the subject of much debate over the last several decades. There appears to now be a consensus that both electron transport and the enzymes of the CBB cycle are present and functional (see reviews by [13,85]). But there is on-going discussion about the quantity of any end production from these processes and how useful they may be for GC osmoregulation and turgor changes as well as signal perception and transduction [4,5], with several more recent studies suggesting GCs behave more like a sink than a source tissue [60,92]. Although features are variable across species, GC chloroplasts tend to be less abundant and have a smaller size and less granal stacking compared to mesophyll cells [93,94]. Moreover, Lawson et al. [47,63] observed a reduction in quantum efficiency of PSII electron transport in GCs compared to mesophyll cells in tobacco (5–25 %), *C. communis* and *T. pallida* (20–30 %) using high resolution chlorophyll fluorescence microscopy. However, changes in CO_2 and O_2 concentrations indicated that RubisCO was a major sink for the end products of GC electron transport [63] (Fig. 1.7). More recently Lim et al. [92] imaged compartment-specific fluorescent ATP and NADPH sensor proteins in Arabidopsis and could not detect ATP and NADPH production in illuminated GC chloroplasts. They suggested that photosynthetic electron transport rate must be very low in GCs and that mitochondria are the main source of ATP. However, in the same study starch levels decreased when GC PSII was inhibited with DCMU, confirming that GC photosynthesis is active and required for proper starch accumulation. Furthermore, other studies have shown that CBB cycle activity in GCs is a major sink for ATP and NADPH produced through photosynthetic electron transport as suggested by the reduction in PSII quantum efficiency observed in GCs in tobacco plants with reduced SBPase activity [47]. Accordingly, isotope labelling experiments have also demonstrated CO_2 fixation by RubisCO in GCs [82,95] (Fig. 1.8). Robaina-Estévez et al. [95] also report that the CBB cycle is a driver of sucrose and starch syntheses in GCs (Fig. 1.8–.10). However, the amount of photosynthates GCs can produce is still unclear. Different amounts and activities of the photosynthetic electron transport chain and CBB cycle enzymes measured in GCs and mesophyll cells have been reported, including lower amounts of PSII subunits, chloroplastic FPBase, RubisCO and ATP synthase in GCs [92,96–98]. Some studies report that sucrose production by GC photosynthesis would represent as low as 2 % of the osmoticum necessary for stomatal movement while others suggest amounts up to 40 % [98–100]. It therefore appears that photosynthetic electron transport and CBB activity does occur in GCs. However, the extent of each appears to be species specific and the role they both play in stomatal function unclear.

3.1. The role of guard-cell photosynthesis in stomatal function and responses to the environment

Experiments from Goh et al. [101,102] revealed similar photosynthetic capacities in adaxial and abaxial GC protoplasts from *V. faba* although abaxial stomatal aperture is greater than on the adaxial surface under the same light intensity. These observations sustain the idea that GC photosynthesis is not essential for light-induced stomatal opening. Still, the contribution of GC photosynthesis in stomatal function remains controversial and some studies report evidence in favour of an important role. As mentioned earlier, transgenic tobacco plants with reduced SBPase or RubisCO content and activity showed reduced quantum efficiency of PSII electron transport in the GCs but greater stomatal opening rate in response to red light [47] and no difference in response to mixed red/blue light compared to the control plants [45,47] suggesting that GC photosynthesis is not essential for stomatal opening, but could play a role in providing additional ATP. On the other hand, Azoulay-Shemer et al. [103] showed GC-targeted overexpression of a chlorophyllase in Arabidopsis yielded stomata with reduced chlorophyll content, and

although stomatal conductance and assimilation rate were reduced in the transgenic plants, stomata still responded to changes in [CO_2]. This suggests GC photosynthesis does not directly modulate CO_2 signal transduction. However, a proportion of these stomata exhibited a deflated phenotype highlighting the importance of GC photosynthesis for turgor production. The debate around the role of GC chloroplasts and photosynthetic activity could be due to species specific differences, with significant variation in the number of chloroplasts in GCs of different species [104]. For example, the GC of species of the orchid genus *Phaphiopedilum* can be devoid of chloroplasts or their chloroplasts lack thylakoids and contain low amounts of chlorophyll, and interestingly high-fluence red light does not induce stomatal opening in these species [105–107], however they do respond to blue light [105]. Stomatal responses to low fluence red light have been reported in these plants and suggested to be driven by a phytochrome signal [107]. The stomatal response to [CO_2] is also weaker in *Phaphiopedilum* compared to closely related orchids with chlorophyllous GCs suggesting an involvement of GC photosynthesis in this response [108]. Further support for a role for GC chloroplasts in stomatal function has been demonstrated in the Arabidopsis crumpled leaf mutant that lack GC chloroplasts and display 40–50 % reduced stomatal aperture compared to the control [109]. Suetsugu et al. [110] also showed in Arabidopsis GCs that red light enhanced blue light-dependent proton-pumping, necessary for light-induced stomatal opening, and that the use of the PSII inhibitor DCMU eliminated this effect highlighting an important role for GC photosynthesis in stomatal response to light. Additionally, Olsen et al. [59] observed stomatal opening in response to decreased [CO_2] in epidermal peels of *V. faba* which was abolished with DCMU suggesting the response is dependent on GC photosynthetic electron transport. However, several studies have suggested both autotrophic and heterotrophic behaviour in guard cells [11,82,92,95,111] that may explain the conflicting reports regarding the importance of various aspects of GC metabolism for stomatal function. But the contribution of the different processes to stomatal responses is not clear yet. Recently Flütsch et al. [112] revealed that GCs synthesise starch using carbon substrates derived from photosynthesis in both mesophyll and guard cells and that the amount from each cell type was dependent on the time of the day. This work supports research in Arabidopsis that showed the enzymes for GC photosynthetic CO_2 fixation are present in small amounts and contribute to starch synthesis required for stomatal opening [92]. The same study also revealed different pathways for starch metabolism in the two cell types, further highlighting the complexity of GC metabolism.

3.2. Photoassimilation-derived carbohydrate metabolism in stomatal function

Triose-phosphates produced through the CBB cycle are usually used for starch biosynthesis in the stroma or exported in the cytosol to be converted into UDP-glucose and then sucrose (Fig. 1.8–.10). These different carbohydrates derived from photoassimilation play crucial roles in stomatal opening and closing. However, their relative contribution to stomatal function remains to be fully elucidated and could depend on species, light conditions and time of the day among other parameters.

Sucrose accumulation in the guard cells is thought to be particularly important for maintaining stomatal opening in the afternoon as sucrose would then be the main osmolyte, rather than K^+ in the morning [113] (Fig. 1.4 and 1.5). However, several studies with Solanaceae have also shown the role of sucrose is not solely osmotic and its degradation plays a determinant role in stomatal opening [82,114,115] (Fig. 1.10). Transgenic potato [114] and tobacco plants [115] with altered GC activity of a sucrose synthase (SuSy) or an acid invertase catalysing sucrose degradation showed a positive correlation between sucrolytic activity and g_s. Accordingly, Daloso et al. [82] showed a decrease in sucrose and glucose levels but no change in starch levels in tobacco GC enriched epidermal fragments during light-induced stomatal opening as well as a higher SuSy activity in the GCs compared to the whole leaf. Medeiros et al. [81] confirmed degradation of sucrose in Arabidopsis GCs during light-induced stomatal opening by using ^{13}C-labelling. In these studies, they observed increased levels and ^{13}C-labelling in metabolites associated with the TCA cycle suggesting glucose generated from sucrose degradation is used in glycolysis to feed the TCA cycle and produce ATP necessary for stomatal opening [81,82,115] (Fig. 1.12). It is unclear whether GCs need exogenous sucrose to produce sufficient energy for stomatal opening. Daloso et al. [82] showed light-induced stomatal opening in epidermal fragments isolated from the mesophyll suggesting tobacco GCs are not dependent on sucrose from the mesophyll. Similarly, adding exogenous sucrose to Arabidopsis epidermal fragments did not improve light-induced stomatal opening [81]. However, Antunes et al. [116] reported a decreased g_s in tobacco plants with reduced sucrose transporter expression and sucrose content in GCs suggesting they needed access to apoplastic sucrose to maintain this function (Fig. 1.11).

Several studies in Arabidopsis focused on the role of starch in stomatal opening depending on the light conditions [10,11,111] (Fig. 1.6 and 1.9). They compared starch and sucrose levels, and stomatal behaviour in protoplasts isolated from the mesophyll versus GCs, and also in GCs from mutants with altered PM H^+-ATPase, genes involved in starch degradation or genes involved in blue-light signalling including phototropins (PHOT1, PHOT2), blue light signalling kinase (BLUS1) and protein phosphatase (PP1). They showed photosynthesis-driven stomatal opening, also known as the red-light response, involves starch accumulation in GCs and was dependent on PM H^+-ATPase activity [11]. Their results suggest uptake of mesophyll-derived sucrose energised by PM H^+-ATPase in GCs contributes to starch accumulation under red light [11] (Fig. 1.9). Further studies shed light on the importance of mesophyll-derived glucose uptake from the apoplast for starch biosynthesis in GCs since Arabidopsis *stp1stp4* mutants with knocked-out plasma membrane hexose transporters have GCs devoid of starch at dawn, accumulate substantially less starch in response to light and had reduced g_s [92,111] (Fig. 1.11). Flütsch et al. [111] proposed a model with major import of glucose in the GCs in the early morning while sucrose uptake would become predominant later in the day. Although these results strongly indicate the need for GCs to access mesophyll-derived sugars to sustain sufficient starch biosynthesis for light-induced stomatal opening, the addition of a photosystem II inhibitor to isolated *stp1stp4* GCs led to a complete lack of starch. This confirms photosynthetic CO_2 fixation in GCs directly contributes to starch synthesis under red light (Fig. 1.8 and 1.9). The importance of starch synthesis in GCs is also suggested by the presence of larger amounts of AGPase than RubisCO [92]. Conversely, the blue-light response triggers starch degradation specifically in GCs to promote stomatal opening at the start of the day (Fig. 1.6). Mutants with reduced starch degradation in GCs show slower stomatal opening under non-saturating (150 μmol m^{-2} s^{-1}) white light conditions compared to the control [11] indicating starch metabolism is a factor to consider in the rapidity of stomatal kinetics. Blue-light induced starch degradation is dependent on proton extrusion from PM H^+-ATPase activity (Fig. 1.2), which occurs at a fast rate during the first hour of light [10] and yields glucose rather than malate [11]. Starch degradation products do not directly affect the capacity to transport H^+, K^+ and Cl^- across the GC plasma membrane, but they seem to help maintain the cytoplasmic sugar pool for energetic purposes [11]. The signal linking blue-light induced starch degradation and PM H^+-ATPase activity during stomatal opening remains to be elucidated [10,11], and therefore the interaction between guard cell metabolism and membrane transport requires further research. Guard-cell starch metabolism also plays a role in CO_2-induced stomatal closing [66]. Arabidopsis mutants with impaired starch biosynthesis in all leaf tissues have reduced stomatal closing at high [CO_2] while it is unaltered in mutants where starch synthesis is only affected in mesophyll cells. Azoulay-Shemer et al. [66] suggest the mechanism behind impacted stomatal closing could be a shift of

triose-phosphate use towards sucrose biosynthesis which could then lead to accumulation of sucrose breakdown products and organic acids. Reduced levels of starch could also lead to decreased amounts of malate whose export into the apoplast is involved in efficient stomatal closing.

4. Stomatal function and other guard-cell metabolic pathways

4.1. Photorespiratory pathway

Within the CBB cycle, RubisCO can either catalyse the carboxylation of RUBP which generates two molecules of 3-phosphoglycerate (3PGA) or the oxygenation of RUBP which produces one molecule of 3PGA and one molecule of 2-phosphoglycolate (2PG) instead. In the event of RUBP oxygenation, the photorespiratory pathway then scavenges this 2PG and releases CO_2 following reactions that occur in chloroplasts, mitochondria, peroxisomes and cytosol. Therefore, photorespiratory and photosynthetic metabolisms are intimately linked. Although it can be described as a wasteful process, a functional photorespiratory pathway is essential to maintain electron transport rate and CO_2 fixation under conditions prone to increase RUBP oxygenation and 2PG production [117,118]. The recycling of 2PG is crucial as it is an inhibitor of the activity of two enzymes from the CBB cycle, SBPase and triose-phosphate isomerase (TPI) which is also involved in starch synthesis [118,119].

Recently, studies have shown altered g_s and transpiration rate in transgenic plants affected in the photorespiratory pathway which suggests a role for photorespiration in stomatal function [17,117,118,120,121] (Fig. 1.4). When subjected to high photorespiratory conditions such as high [O_2] or low [CO_2] levels, water shortage, elevated temperatures or light intensities, Arabidopsis plants with reduced activity of phosphoglycolate phosphatase (PGLP) (in photosynthetic tissues), which catalyses 2PG breakdown, exhibited lower g_s and transpiration rates compared to the control while the overexpressing lines showed higher g_s, transpiration and A [17,117,118]. Similarly, Arabidopsis KO for serine hydroxymethyltransferase or glycerate kinase, (which are involved in the photorespiratory pathway downstream of PLGP), showed decreased g_s as well as lower electron transport rates compared to the control plants upon a shift to lower [CO_2] [117]. Additionally, Arabidopsis plants with a KO thioredoxin involved in the redox regulation of mitochondrial photorespiratory metabolism showed reduced amounts of serine hydroxymethyltransferase and glycine decarboxylase (GDC) H and L subunits and had impaired g_s [120]. Accordingly, Lopez et al. [121] observed higher g_s along with higher A in greenhouse-grown tobacco plants overexpressing GDCH in their photosynthetic tissues.

Although the underlying mechanisms are yet to be fully understood, it seems that optimising fluxes through the photorespiratory pathway could help maintain or improve stomatal movements via higher carbon fixation and starch production. Indeed, several Arabidopsis mutants with KO photorespiratory genes show downregulated expression of starch metabolism genes, and impaired stomatal function [117]. Moreover, expression levels of PGLP correlate with leaf starch content at the end of the day [17,118]. Faster synthesis rates rather than slower degradation rates seem responsible for higher starch accumulation, at least in the lines overexpressing PGLP [17,118]. This increased starch biosynthesis is probably the consequence of increased A, as observed in transgenic plants overexpressing some components of the photorespiratory pathway [17,118,121]. Likely, higher starch accumulation helps with maintaining stomatal opening under high photorespiratory conditions. Conversely, steady-state levels of sucrose are not affected by differential PGLP expression levels [17,118]. Whether mesophyll or GC photosynthesis and starch accumulation are responsible for maintaining stomatal function under high photorespiratory conditions is yet to be determined. GC-specific manipulations would be necessary to identify which of these cell types have a determinant role in this process. Interestingly, the expression of PHOT1 and PHOT2 involved in the blue light-dependent stomatal opening, was reduced in Arabidopsis mutants with KO photorespiratory genes suggesting a direct or indirect role of functional photorespiration in this stomatal response as well [117].

4.2. Respiration, anaplerotic CO_2 fixation and oxidative pentose phosphate pathway

As mentioned previously, several studies argue GC photosynthesis only plays a minor role in providing energy, reducing power and osmolytes necessary for stomatal function and other metabolic pathways have a much higher contribution to this process. A recent study in Arabidopsis reported that mitochondria are the primary source of ATP for GCs and PM H^+-ATPase activity upon illumination is mainly fuelled by ATP produced via mitochondrial respiratory metabolism (TCA cycle and oxidative phosphorylation, [92]) (Fig. 1.12). Using plastid-targeted FRET sensors, they could not detect ATP and NADPH production in GC chloroplasts upon illumination. Furthermore, they determined a GC-specific role of the plastidial ATP/ADP translocator NTT1 (nucleotide transporter), responsible for importing cytosolic ATP into chloroplasts to sustain starch synthesis (Fig. 1.9). *ntt1* mutants were nearly devoid of starch in GCs and had impaired stomatal kinetics. Additionally, respiratory inhibitors depleted GC cytosolic ATP levels. Altogether, these observations point toward a major role of mitochondrial respiratory pathways to provide sufficient energy for stomatal function. This is supported by previous studies where increased fluxes through the TCA cycle in GCs were reported during light-induced stomatal opening [81,82,115]. Nunes-Nesi et al. [69] also highlighted the importance of functional respiratory pathways and the TCA cycle in tomato plants with reduced fumarate hydratase expression which had slower stomatal kinetics. These transgenic plants were constitutively impaired so the relative importance of GC versus mesophyll respiration in this context remains to be established. Furthermore, GCs also seem to have high glycolytic activity in the light as suggested by their low amounts of cytosolic FBPase catalysing the reverse reaction of phosphofructokinase in glycolysis [92]. Flütsch et al. [111] used the Arabidopsis *stp1stp4* mutants with knocked-out plasma membrane hexose transporters to show that the import of glucose at dawn was an important carbon source for GC metabolism, via glycolysis to produce pyruvate that is then fed into the TCA cycle to provide energy for stomatal movement (Fig. 1.11 and 1.12), as well as being important for starch accumulation. The importance of a mesophyll photosynthetic metabolite for GC respiratory processes that facilitates rapid and early morning stomatal opening is supported by a model developed by Vialet-Chabrand et al. [122].

There is supporting evidence for a role in anaplerotic CO_2 fixation in GCs to provide energy and osmotica for stomatal movements [82,92,95]. Anaplerotic CO_2 fixation helps replenish intermediates of the TCA cycle and involves carboxylation of PEP by PEPcase, producing oxaloacetate which can be converted by MDH into malate to feed the TCA cycle. Additionally, PEPcase-mediated CO_2 fixation has been shown to provide the carbons for gluconeogenesis and sucrose synthesis in GCs [95] (Fig. 1.13). Both RubisCO and PEPcase have been shown to fix CO_2 simultaneously in GCs [82] but anaplerotic CO_2 fixation has been reported to be higher in GCs compared to mesophyll cells. This is supported by larger amounts of PEPcase and MDH present in GCs than mesophyll cells [92]. Moreover, the main source of CO_2 fixed by RuBisCO has been argued to originate from the decarboxylation of malate imported from the cytosol into the chloroplast suggesting a C_4-like metabolism in GCs, even in C_3 plants [95] (Fig. 1.13). However, it should be borne in mind that C_4 metabolism requires spatial separation and compartmentalisation of RubisCO from PEPcase which is not the case in GCs and therefore such analogies may not be the best way to suggest alternative mechanisms involving C_4 enzymes.

5. Future directions

Chloroplasts are a key feature in guard cells (GCs) in the majority of species, however their role to date is unclear. There is substantial

evidence that both electron transport and CBB cycle activity take place in these organelles, however the extent to which these processes contribute to stomatal movements is still unclear. It is also possible that these plastids could provide the site of perception or the signal that coordinates stomatal conductance with mesophyll demands for CO_2. To date the majority of studies that have investigated GC photosynthesis have done so using plants that have altered expression of key enzymes in these metabolic processes driven by constitutive and/or photosynthetic promoters rather than specific changes to GC expression, making it difficult to assess the contribution or role of GC chloroplasts in stomatal behaviour [7,85]. This review (and others) has highlighted that there are many osmoregulatory pathways that possibly exist in GC and that the time of day influences the relative contribution of each to stomatal opening and closing [112,113], and this could provide an explanation for conflicting reports from different groups. These processes are likely to be complex and there is functional redundancy, in that when one process is manipulated another compensates. Future research in this area should take advantage of modern advances in molecular biology and use GC specific promoters to drive altered expression of key enzymes in electron transport and the CBB cycle to fully understand the importance of these processes in stomatal function. Single cell transcriptomics and metabolomics will facilitate a full understanding of the different fluxes of metabolites and their origins over the diel period to fully appreciate the complexity of GC metabolism and osmoregulation. Altering GC metabolism directly could provide an unexploited mechanism to speed up stomatal responses and more closely align these responses with mesophyll demands for CO_2, providing a target for improving both photosynthetic CO_2 uptake as well as water-use efficiency. Although not reviewed here, ion movements in the GCs have been shown to play an important role to stomatal function and kinetics and manipulating these fluxes has been a successful approach to improve carbon assimilation and/or reduce water use in dynamic light conditions in several studies [25,26]. The GC-specific expression of the synthetic, blue-light induced K^+ channel BLINK1 in *A. thaliana* promoted K^+ fluxes across the GC plasma membrane leading to a reduction in stomatal opening and closing halftimes in response to an increase or decrease in blue light intensity respectively. Under fluctuating light, this manipulation increased dry biomass and long-term water use efficiency [25]. Similar improvements under fluctuating light were observed in *A. thaliana* when the clustering of GORK channels which are responsible for K^+ efflux during stomatal closure, was suppressed in a GC-specific manner highlighting the importance of native ion channel regulation in stomatal kinetics [26]. Ion movements and metabolism in GCs are connected in a complex manner and how these links impact stomatal function and kinetics needs to be further investigated. Additionally, further research is needed to fully understand the signalling mechanisms that regulate stomatal movements, as well as potential differences in stomatal sensitivities between leaf surfaces and how they may impact on mesophyll assimilation rate [123–125]. Takahashi et al. [126] recently discovered the long-sought primary CO_2/bicarbonate sensor in *Arabidopsis* GCs that will aid in our understanding of these complex processes. Another consideration for future research not covered here, is the importance and contribution of subsidiary cells to stomatal movements, especially in grasses, and the energetics required for movement of solutes between these two cells. It is only with a full appreciation of these processes that we can begin to understand their potential for exploitation to improve CO_2 uptake and plant water status.

Conflict of Interest

No conflicts of interest.

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References

[1] T. Lawson, A. Milliken, Photosynthesis – beyond the leaf, New Phytol., n.d.
[2] J.D.B. Weyers, T. Lawson, Heterogeneity in stomatal characteristics, Adv. Bot. Res. (1997) 317–352, https://doi.org/10.1016/s0065-2296(08)60124-x.
[3] T. Lawson, J.I.L. Morison, Stomatal function and physiology, The Evolution of Plant Physiology, 2004, pp. 217–42. ⟨https://doi.org/10.1016/b978-012339552-8/50013-5⟩.
[4] M. Jezek, M.R. Blatt, The membrane transport system of the guard cell and its integration for stomatal dynamics, Plant Physiol. 174 (2017) 487–519.
[5] D. Santelia, T. Lawson, Rethinking guard cell metabolism, Plant Physiol. 172 (2016) 1371–1392.
[6] M.R. Blatt, G.M. Clint, Mechanisms of fusicoccin action: kinetic modification and inactivation of K^+ channels in guard cells, Planta 178 (1989) 509–523.
[7] T. Lawson, M.R. Blatt, Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency, Plant Physiol. 164 (2014) 1556–1570.
[8] C.E. Moore, K. Meacham-Hensold, P. Lemonnier, R.A. Slattery, C. Benjamin, C. J. Bernacchi, T. Lawson, A.P. Cavanagh, The effect of increasing temperature on crop photosynthesis: from enzymes to ecosystems, J. Exp. Bot. 72 (2021) 2822–2844.
[9] T. Lawson, J. Matthews, Guard cell metabolism and stomatal function, Annu. Rev. Plant Biol. 71 (2020) 273–302.
[10] D. Horrer, S. Flütsch, D. Pazmino, J.S.A. Matthews, M. Thalmann, A. Nigro, N. Leonhardt, T. Lawson, D. Santelia, Blue light induces a distinct starch degradation pathway in guard cells for stomatal opening, Curr. Biol. 26 (2016) 362–370.
[11] S. Flütsch, Y. Wang, A. Takemiya, S.R.M. Vialet-Chabrand, M. Klejchová, A. Nigro, A. Hills, T. Lawson, M.R. Blatt, D. Santelia, Guard cell starch degradation yields glucose for rapid stomatal opening in Arabidopsis, Plant Cell 32 (2020) 2325–2344.
[12] E. Ando, T. Kinoshita, Red light-induced phosphorylation of plasma membrane H-ATPase in stomatal guard cells, Plant Physiol. 178 (2018) 838–849.
[13] T. Lawson, Guard cell photosynthesis and stomatal function, New Phytol. 181 (2009) 13–34.
[14] F.A. Busch, Opinion: the red-light response of stomatal movement is sensed by the redox state of the photosynthetic electron transport chain, Photosynth. Res. 119 (2014) 131–140, https://doi.org/10.1007/s11120-013-9805-6.
[15] S.C. Wong, I.R. Cowan, G.D. Farquhar, Stomatal conductance correlates with photosynthetic capacity, Nature 282 (1979) 424–426, https://doi.org/10.1038/282424a0.
[16] T. Lawson, D.M. Kramer, C.A. Raines, Improving yield by exploiting mechanisms underlying natural variation of photosynthesis, Curr. Opin. Biotechnol. 23 (2012) 215–220.
[17] S. Timm, F. Woitschach, C. Heise, M. Hagemann, H. Bauwe, Faster removal of 2-phosphoglycolate through photorespiration improves abiotic stress tolerance of Arabidopsis, Plants 8 (2019), https://doi.org/10.3390/plants8120563.
[18] T.N. Buckley, How do stomata respond to water status? New Phytol. 224 (2019) 21–36.
[19] T. Lawson, S. von Caemmerer, I. Baroli, Photosynthesis and stomatal behaviour, in: Progress in Botany 72, Springer, Berlin Heidelberg, Berlin, Heidelberg, 2010, pp. 265–304.
[20] L. McAusland, S. Vialet-Chabrand, P. Davey, N.R. Baker, O. Brendel, T. Lawson, Effects of kinetics of light-induced stomatal responses on photosynthesis and water-use efficiency, New Phytol. 211 (2016) 1209–1220.
[21] R.M. Deans, T.J. Brodribb, F.A. Busch, G.D. Farquhar, Plant water-use strategy mediates stomatal effects on the light induction of photosynthesis, New Phytol. 222 (2019) 382–395.
[22] H. Kimura, M. Hashimoto-Sugimoto, K. Iba, I. Terashima, W. Yamori, Improved stomatal opening enhances photosynthetic rate and biomass production in fluctuating light, J. Exp. Bot. 71 (2020) 2339–2350.
[23] M. Faralli, J. Matthews, T. Lawson, Exploiting natural variation and genetic manipulation of stomatal conductance for crop improvement, Curr. Opin. Plant Biol. 49 (2019) 1–7.
[24] S.P. Long, S.H. Taylor, S.J. Burgess, E. Carmo-Silva, T. Lawson, A.P. De Souza, L. Leonelli, Y. Wang, Into the shadows and back into sunlight: photosynthesis in fluctuating light, Annu. Rev. Plant Biol. 73 (2022) 617–648.
[25] M. Papanatsiou, J. Petersen, L. Henderson, Y. Wang, J.M. Christie, M.R. Blatt, Optogenetic manipulation of stomatal kinetics improves carbon assimilation, water use, and growth, Science 363 (2019) 1456–1459.
[26] W. Horaruang, M. Klejchová, W. Carroll, F.A.L. Silva-Alvim, S. Waghmare, M. Papanatsiou, A. Amtmann, A. Hills, J.C. Alvim, M.R. Blatt, B. Zhang, Engineering a K^+ channel "sensory antenna" enhances stomatal kinetics, water use efficiency and photosynthesis, Nat. Plants 8 (2022) 1262–1274.
[27] K.A. Mott, E.D. Sibbernsen, J.C. Shope, The role of the mesophyll in stomatal responses to light and CO_2, Plant Cell Environ. 31 (2008) 1299–1306.
[28] T. Lawson, I. Terashima, T. Fujita, Y. Wang, Coordination between photosynthesis and stomatal behavior, in: The Leaf: A Platform for Performing Photosynthesis, Springer International Publishing, Cham, 2018, pp. 141–161.
[29] M.R. Blatt, Cellular signaling and volume control in stomatal movements in plants, Annu. Rev. Cell Dev. Biol. 16 (2000) 221–241.

[30] M.R. Blatt, C. Garcia-Mata, S. Sokolovski, Membrane transport and Ca2 oscillations in guard cells, Rhythms Plants (2007) 115–133, https://doi.org/10.1007/978-3-540-68071-0_6.
[31] M.R. Blatt, M. Jezek, V.L. Lew, A. Hills, What can mechanistic models tell us about guard cells, photosynthesis, and water use efficiency? Trends Plant Sci. 27 (2022) 166–179.
[32] S. Pandey, W. Zhang, S.M. Assmann, Roles of ion channels and transporters in guard cell signal transduction, FEBS Lett. 581 (2007) 2325–2336.
[33] H. Kollist, M. Nuhkat, M.R.G. Roelfsema, Closing gaps: linking elements that control stomatal movement, New Phytol. 203 (2014) 44–62.
[34] The regulation of ion channels and transporters in the guard cell, in: Advances in Botanical Research, Academic Press, 2018, pp. 171–214.
[35] S. Saito, N. Uozumi, Guard cell membrane anion transport systems and their regulatory components: an elaborate mechanism controlling stress-induced stomatal closure, Plants 8 (2019) 9.
[36] C. Eisenach, A. De Angeli, Ion transport at the vacuole during stomatal movements, Plant Physiol. 174 (2017) 520–530.
[37] M.R.G. Roelfsema, R. Hedrich, In the light of stomatal opening: new insights into "the Watergate", New Phytol. 167 (2005) 665–691.
[38] M.R.G. Roelfsema, R. Hedrich, Making sense out of Ca^{2+} signals: their role in regulating stomatal movements, Plant Cell Environ. 33 (2010) 305–321.
[39] K.A. Mott, Do stomata respond to CO_2 concentrations other than intercellular? Plant Physiol. 86 (1988) 200–203.
[40] M.R.G. Roelfsema, S. Hanstein, H.H. Felle, R. Hedrich, CO_2 provides an intermediate link in the red light response of guard cells, Plant J. 32 (2002) 65–75.
[41] J.T. Ball, I.E. Woodrow, J.A. Berry, A model predicting stomatal conductance and its contribution to the control of photosynthesis under different environmental conditions, in: Progress in Photosynthesis Research, Springer, Netherlands, Dordrecht, 1987, pp. 221–224.
[42] M. Jezek, F.A.L. Silva-Alvim, A. Hills, N. Donald, M.R. Ishka, J. Shadbolt, B. He, T. Lawson, J.F. Harper, Y. Wang, V.L. Lew, M.R. Blatt, Guard cell endomembrane Ca-ATPases underpin a "carbon memory" of photosynthetic assimilation that impacts on water-use efficiency, Nat. Plants 7 (2021) 1301–1313.
[43] J.I.L. Morison, P.G. Jarvis, Direct and indirect effects of light on stomata II. In *Commelina communis* L, Plant Cell Environ. 6 (1983) 103–109, https://doi.org/10.1111/j.1365-3040.1983.tb01882.x.
[44] P.G. Jarvis, J.I.L. Morison, The control of transpiration and photosynthesis by the stomata, in: Stomatal Physiology, Cambridge University Press, 1981, pp. 247–279.
[45] S. von Caemmerer, T. Lawson, K. Oxborough, N.R. Baker, T.J. Andrews, C. A. Raines, Stomatal conductance does not correlate with photosynthetic capacity in transgenic tobacco with reduced amounts of Rubisco, J. Exp. Bot. 55 (2004) 1157–1166.
[46] I. Baroli, G.D. Price, M.R. Badger, S. von Caemmerer, The contribution of photosynthesis to the red light response of stomatal conductance, Plant Physiol. 146 (2008) 737–747.
[47] T. Lawson, S. Lefebvre, N.R. Baker, J.I.L. Morison, C.A. Raines, Reductions in mesophyll and guard cell photosynthesis impact on the control of stomatal responses to light and CO_2, J. Exp. Bot. 59 (2008) 3609–3619.
[48] A.B. Cousins, I. Baroli, M.R. Badger, A. Ivakov, P.J. Lea, R.C. Leegood, S. von Caemmerer, The role of phosphoenolpyruvate carboxylase during C_4 photosynthetic isotope exchange and stomatal conductance, Plant Physiol. 145 (2007) 1006–1017.
[49] T. Lawson, B. Bryant, S. Lefebvre, J.C. Lloyd, C.A. Raines, Decreased SBPase activity alters growth and development in transgenic tobacco plants, Plant Cell Environ. 29 (2006) 48–58.
[50] S.M. Messinger, T.N. Buckley, K.A. Mott, Evidence for involvement of photosynthetic processes in the stomatal response to CO_2, Plant Physiol. 140 (2006) 771–778.
[51] A.A.R. Webb, M.R. McAinsh, T.A. Mansfield, A.M. Hetherington, Carbon dioxide induces increases in guard cell cytosolic free calcium, Plant J. 9 (1996) 297–304, https://doi.org/10.1046/j.1365-313x.1996.09030297.x.
[52] J.S.A. Matthews, S. Vialet-Chabrand, T. Lawson, Acclimation to fluctuating light impacts the rapidity of response and diurnal rhythm of stomatal conductance, Plant Physiol. 176 (2018) 1939–1951.
[53] J. Lee, D.J.F. Bowling, The effect of a mesophyll factor on the swelling of guard cell protoplasts of *Commelina communis* L, J. Plant Physiol. 142 (1993) 203–207.
[54] J. Lee, D.J.F. Bowling, Effect of the mesophyll on stomatal opening in *Commelina communis*, J. Exp. Bot. 43 (1992) 951–957.
[55] J. Lee, D.J.F. Bowling, Influence of the mesophyll on stomatal opening, Aust. J. Plant Physiol. 22 (1995) 357–363.
[56] T. Fujita, K. Noguchi, I. Terashima, Apoplastic mesophyll signals induce rapid stomatal responses to CO_2 in *Commelina communis*, New Phytol. 199 (2013) 395–406.
[57] E. Sibbernsen, K.A. Mott, Stomatal responses to flooding of the intercellular air spaces suggest a vapor-phase signal between the mesophyll and the guard cells, Plant Physiol. 153 (2010) 1435–1442.
[58] G. Tallman, E. Zeiger, Light quality and osmoregulation in *Vicia* guard cells: evidence for involvement of three metabolic pathways, Plant Physiol. 88 (1988) 887–895.
[59] R.L. Olsen, R.B. Pratt, P. Gump, A. Kemper, G. Tallman, Red light activates a chloroplast-dependent ion uptake mechanism for stomatal opening under reduced CO_2 concentrations in *Vicia* spp, New Phytol. 153 (2002) 497–508.
[60] C. Willmer, M. Fricker, Stomata, Springer Science & Business Media, 1996.
[61] S. Frechilla, L.D. Talbott, E. Zeiger, The CO_2 response of *Vicia* guard cells acclimates to growth environment, J. Exp. Bot. 53 (2002) 545–550.
[62] M.G. Santos, P.A. Davey, T.A. Hofmann, A. Borland, J. Hartwell, T. Lawson, Stomatal responses to light, CO_2, and mesophyll tissue in and *Vicia faba* and *Kalanchoë fedtschenkoi*, Front. Plant Sci. 12 (2021), 740534.
[63] T. Lawson, K. Oxborough, J.I.L. Morison, N.R. Baker, Responses of photosynthetic electron transport in stomatal guard cells and mesophyll cells in intact leaves to light, CO_2, and humidity, Plant Physiol. 128 (2002) 52–62.
[64] Y. Wang, K. Noguchi, I. Terashima, Photosynthesis-dependent and -independent responses of stomata to blue, red and green monochromatic light: differences between the normally oriented and inverted leaves of sunflower, Plant Cell Physiol. 52 (2011) 479–489.
[65] M.R.G. Roelfsema, K.R. Konrad, H. Marten, G.K. Psaras, W. Hartung, R. Hedrich, Guard cells in albino leaf patches do not respond to photosynthetically active radiation, but are sensitive to blue light, CO_2 and abscisic acid, Plant Cell Environ. 29 (2006) 1595–1605.
[66] T. Azoulay-Shemer, A. Bagheri, C. Wang, A. Palomares, A.B. Stephan, H.-H. Kunz, J.I. Schroeder, Starch biosynthesis in guard cells but not in mesophyll cells is involved in CO_2-induced stomatal closing, Plant Physiol. 171 (2016) 788–798.
[67] W.L. Araújo, A.R. Fernie, A. Nunes-Nesi, Control of stomatal aperture: a renaissance of the old guard, Plant Signal. Behav. 6 (2011) 1305–1311.
[68] W.L. Araújo, A. Nunes-Nesi, S. Osorio, B. Usadel, D. Fuentes, R. Nagy, I. Balbo, M. Lehmann, C. Studart-Witkowski, T. Tohge, E. Martinoia, X. Jordana, F. M. Damatta, A.R. Fernie, Antisense inhibition of the iron-sulphur subunit of succinate dehydrogenase enhances photosynthesis and growth in tomato via an organic acid-mediated effect on stomatal aperture, Plant Cell 23 (2011) 600–627.
[69] A. Nunes-Nesi, F. Carrari, Y. Gibon, R. Sulpice, A. Lytovchenko, J. Fisahn, J. Graham, R.G. Ratcliffe, L.J. Sweetlove, A.R. Fernie, Deficiency of mitochondrial fumarase activity in tomato plants impairs photosynthesis via an effect on stomatal function, Plant J. 50 (2007) 1093–1106.
[70] D.B. Medeiros, K.A. Barros, J.A.S. Barros, R.P. Omena-Garcia, S. Arrivault, L.M. V. Sanglard, K.C. Detmann, W.B. Silva, D.M. Daloso, F.M. DaMatta, A. Nunes-Nesi, A.R. Fernie, W.L. Araújo, Impaired malate and fumarate accumulation due to the mutation of the tonoplast dicarboxylate transporter has little effects on stomatal behavior, Plant Physiol. 175 (2017) 1068–1081, https://doi.org/10.1104/pp.17.00971.
[71] R. Hedrich, I. Marten, Malate-induced feedback regulation of plasma membrane anion channels could provide a CO_2 sensor to guard cells, EMBO J. 12 (1993) 897–901.
[72] S. Meyer, P. Mumm, D. Imes, A. Endler, B. Weder, K.A.S. Al-Rasheid, D. Geiger, I. Marten, E. Martinoia, R. Hedrich, AtALMT12 represents an R-type anion channel required for stomatal movement in Arabidopsis guard cells, Plant J. 63 (2010) 1054–1062.
[73] Y. Wang, M.R. Blatt, Anion channel sensitivity to cytosolic organic acids implicates a central role for oxaloacetate in integrating ion flux with metabolism in stomatal guard cells, Biochem. J. 439 (2011) 161–170, https://doi.org/10.1042/bj20110845.
[74] J. Gehlen, R. Panstruga, H. Smets, S. Merkelbach, M. Kleines, P. Porsch, M. Fladung, I. Becker, T. Rademacher, R.E. Häusler, H.J. Hirsch, Effects of altered phosphoenolpyruvate carboxylase activities on transgenic C_3 plant *Solanum tuberosum*, Plant Mol. Biol. 32 (1996) 831–848.
[75] G. Kelly, A. Egbaria, B. Khamaisi, N. Lugassi, Z. Attia, M. Moshelion, D. Granot, Guard-cell hexokinase increases water-use efficiency under normal and drought conditions, Front. Plant Sci. 10 (2019) 1499.
[76] G. Kelly, R. David-Schwartz, N. Sade, M. Moshelion, A. Levi, V. Alchanatis, D. Granot, The pitfalls of transgenic selection and new roles of AtHXK1: a high level of AtHXK1 expression uncouples hexokinase1-dependent sugar signaling from exogenous sugar, Plant Physiol. 159 (2012) 47–51.
[77] G. Kelly, M. Moshelion, R. David-Schwartz, O. Halperin, R. Wallach, Z. Attia, E. Belausov, D. Granot, Hexokinase mediates stomatal closure, Plant J. 75 (2013) 977–988.
[78] N. Lugassi, B.S. Yadav, A. Egbaria, D. Wolf, G. Kelly, E. Neuhaus, E. Raveh, N. Carmi, D. Granot, Expression of hexokinase in tobacco guard cells increases water-use efficiency and confers tolerance to drought and salt stress, Plants 8 (2019), https://doi.org/10.3390/plants8120613.
[79] N. Lugassi, G. Kelly, L. Fidel, Y. Yaniv, Z. Attia, A. Levi, V. Alchanatis, M. Moshelion, E. Raveh, N. Carmi, D. Granot, Expression of Arabidopsis hexokinase in citrus guard cells controls stomatal aperture and reduces transpiration, Front. Plant Sci. 6 (2015) 1114.
[80] L.G. Acevedo-Siaca, K. Głowacka, S.M. Driever, C.E. Salesse-Smith, N. Lugassi, D. Granot, S.P. Long, J. Kromdijk, Guard-cell-targeted overexpression of Arabidopsis Hexokinase 1 can improve water use efficiency in field-grown tobacco plants, J. Exp. Bot. 73 (2022) 5745–5757.
[81] D.B. Medeiros, L. Perez Souza, W.C. Antunes, W.L. Araújo, D.M. Daloso, A. R. Fernie, Sucrose breakdown within guard cells provides substrates for glycolysis and glutamine biosynthesis during light-induced stomatal opening, Plant J. 94 (2018) 583–594.
[82] D.M. Daloso, W.C. Antunes, D.P. Pinheiro, J.P. Waquim, W.L. Araújo, M. E. Loureiro, A.R. Fernie, T.C.R. Williams, Tobacco guard cells fix CO_2 by both Rubisco and PEPcase while sucrose acts as a substrate during light-induced stomatal opening, Plant Cell Environ. 38 (2015) 2353–2371.
[83] Y. Kang, W.H. Outlaw Jr, P.C. Andersen, G.B. Fiore, Guard-cell apoplastic sucrose concentration–a link between leaf photosynthesis and stomatal aperture size in the apoplastic phloem loader Vicia faba L, Plant Cell Environ. 30 (2007) 551–558.

[84] W.H. Outlaw Jr, X. De Vlieghere-He, Transpiration rate. An important factor controlling the sucrose content of the guard cell apoplast of broad bean, Plant Physiol. 126 (2001) 1716–1724.
[85] T. Lawson, A.J. Simkin, G. Kelly, D. Granot, Mesophyll photosynthesis and guard cell metabolism impacts on stomatal behaviour, New Phytol. 203 (2014) 1064–1081.
[86] G.D. Farquhar, S.C. Wong, An empirical model of stomatal conductance, Funct. Plant Biol. 11 (1984) 191, https://doi.org/10.1071/pp9840191.
[87] M. Tominaga, T. Kinoshita, K. Shimazaki, Guard-cell chloroplasts provide ATP required for H^+ pumping in the plasma membrane and stomatal opening, Plant Cell Physiol. 42 (2001) 795–802.
[88] T.N. Buckley, K.A. Mott, G.D. Farquhar, A hydromechanical and biochemical model of stomatal conductance, Plant Cell Environ. 26 (2003) 1767–1785.
[89] E. Zeiger, J. Zhu, Role of zeaxanthin in blue light photoreception and the modulation of light-CO_2 interactions in guard cells, J. Exp. Bot. 49 (1998) 433–442.
[90] K. Głowacka, J. Kromdijk, K. Kucera, J. Xie, A.P. Cavanagh, L. Leonelli, A.D. B. Leakey, D.R. Ort, K.K. Niyogi, S.P. Long, Photosystem II Subunit S overexpression increases the efficiency of water use in a field-grown crop, Nat. Commun. 9 (2018) 868.
[91] J. Kromdijk, K. Głowacka, L. Leonelli, S.T. Gabilly, M. Iwai, K.K. Niyogi, S. P. Long, Improving photosynthesis and crop productivity by accelerating recovery from photoprotection, Science 354 (2016) 857–861.
[92] S.-L. Lim, S. Flütsch, J. Liu, L. Distefano, D. Santelia, B.L. Lim, Arabidopsis guard cell chloroplasts import cytosolic ATP for starch turnover and stomatal opening, Nat. Commun. 13 (2022) 652.
[93] G.D. Humble, K. Raschke, Stomatal opening quantitatively related to potassium transport: evidence from electron probe analysis, Plant Physiol. 48 (1971) 447–453.
[94] K.-I. Shimazaki, S. Okayama, Calvin Benson cycle enzymes in guard-cell protoplasts and their role in stomatal movement, Biochem. Physiol. Pflanz. 186 (1990) 327–331.
[95] S. Robaina-Estévez, D.M. Daloso, Y. Zhang, A.R. Fernie, Z. Nikoloski, Resolving the central metabolism of Arabidopsis guard cells, Sci. Rep. 7 (2017) 8307.
[96] K. Shimazaki, J. Terada, K. Tanaka, N. Kondo, Calvin-benson cycle enzymes in guard-cell protoplasts from *Vicia faba* L: implications for the greater utilization of phosphoglycerate/dihydroxyacetone phosphate shuttle between chloroplasts and the cytosol, Plant Physiol. 90 (1989) 1057–1064.
[97] R. Hedrich, K. Raschke, M. Stitt, A role for fructose 2,6-bisphosphate in regulating carbohydrate metabolism in guard cells, Plant Physiol. 79 (1985) 977–982.
[98] U. Reckmann, R. Scheibe, K. Raschke, Rubisco activity in guard cells compared with the solute requirement for stomatal opening, Plant Physiol. 92 (1990) 246–253.
[99] M. Poffenroth, D.B. Green, G. Tallman, Sugar concentrations in guard cells of *Vicia faba* illuminated with red or blue light: analysis by high performance liquid chromatography, Plant Physiol. 98 (1992) 1460–1471.
[100] W. Wu, S.M. Assmann, Photosynthesis by guard cell chloroplasts of *Vicia faba* L.: effects of factors associated with stomatal movement, Plant Cell Physiol. 34 (1993) 1015–1022.
[101] C.-H. Goh, T. Oku, K.-I. Shimazaki, Photosynthetic properties of adaxial guard cells from *Vicia* leaves, Plant Sci. 127 (1997) 149–159.
[102] C.-H. Goh, P. Dietrich, R. Steinmeyer, U. Schreiber, H.-G. Nam, R. Hedrich, Parallel recordings of photosynthetic electron transport and K^+-channel activity in single guard cells, Plant J. 32 (2002) 623–630.
[103] T. Azoulay-Shemer, A. Palomares, A. Bagheri, M. Israelsson-Nordstrom, C. B. Engineer, B.O.R. Bargmann, A.B. Stephan, J.I. Schroeder, Guard cell photosynthesis is critical for stomatal turgor production, yet does not directly mediate CO_2- and ABA-induced stomatal closing, Plant J. 83 (2015) 567–581.
[104] T. Lawson, K. Oxborough, J.I.L. Morison, N.R. Baker, The responses of guard and mesophyll cell photosynthesis to CO_2, O_2, light, and water stress in a range of species are similar, J. Exp. Bot. 54 (2003) 1743–1752.
[105] E. Zeiger, S.M. Assmann, H. Meioner, The photobiology of *Paphiopedilum* stomata: opening under blue light but not red light, Photochem. Photobiol. 38 (1983) 627–630, https://doi.org/10.1111/j.1751-1097.1983.tb03394.x.
[106] E.D. D'Amelio, E. Zeiger, Diversity In guard cell plastids of the Orchidaceae: a structural and functional study, Can. J. Bot. 66 (1988) 257–271, https://doi.org/10.1139/b88-044.
[107] L.D. Talbott, J. Zhu, S.W. Han, E. Zeiger, Phytochrome and blue light-mediated stomatal opening in the orchid, *Paphiopedilum*, Plant Cell Physiol. 43 (2002) 639–646, https://doi.org/10.1093/pcp/pcf075.
[108] S.M. Assmann, E. Zeiger, Stomatal responses to CO_2 in *Paphiopedilum* and *Phragmipedium*: role of the guard cell chloroplast, Plant Physiol. 77 (1985) 461–464.
[109] Y. Wang, A. Hills, M.R. Blatt, Systems analysis of guard cell membrane transport for enhanced stomatal dynamics and water use efficiency, Plant Physiol. 164 (2014) 1593–1599.
[110] N. Suetsugu, T. Takami, Y. Ebisu, H. Watanabe, C. Iiboshi, M. Doi, K.-I. Shimazaki, Guard cell chloroplasts are essential for blue light-dependent stomatal opening in Arabidopsis, PLoS One 9 (2014), e108374, https://doi.org/10.1371/journal.pone.0108374.
[111] S. Flütsch, A. Nigro, F. Conci, J. Fajkus, M. Thalmann, M. Trtílek, K. Panzarová, D. Santelia, Glucose uptake to guard cells via STP transporters provides carbon sources for stomatal opening and plant growth, EMBO Rep. 21 (2020), e49719.
[112] S. Flütsch, D. Horrer, D. Santelia, Starch biosynthesis in guard cells has features of both autotrophic and heterotrophic tissues, Plant Physiol. 189 (2022) 541–556.
[113] L.D. Talbott, E. Zeiger, Central roles for potassium and sucrose in guard-cell osmoregulation, Plant Physiol. 111 (1996) 1051–1057.
[114] W.C. Antunes, N.J. Provart, T.C.R. Williams, M.E. Loureiro, Changes in stomatal function and water use efficiency in potato plants with altered sucrolytic activity, Plant Cell Environ. 35 (2012) 747–759.
[115] D.M. Daloso, T.C.R. Williams, W.C. Antunes, D.P. Pinheiro, C. Müller, M. E. Loureiro, A.R. Fernie, Guard cell-specific upregulation of sucrose synthase 3 reveals that the role of sucrose in stomatal function is primarily energetic, New Phytol. 209 (2016) 1470–1483.
[116] W.C. Antunes, D. de Menezes Daloso, D.P. Pinheiro, T.C.R. Williams, M. E. Loureiro, Guard cell-specific down-regulation of the sucrose transporter SUT1 leads to improved water use efficiency and reveals the interplay between carbohydrate metabolism and K^+ accumulation in the regulation of stomatal opening, Environ. Exp. Bot. 135 (2017) 73–85.
[117] M. Eisenhut, A. Bräutigam, S. Timm, A. Florian, T. Tohge, A.R. Fernie, H. Bauwe, A.P.M. Weber, Photorespiration is crucial for dynamic response of photosynthetic metabolism and stomatal movement to altered CO availability, Mol. Plant. 10 (2017) 47–61.
[118] F. Flügel, S. Timm, S. Arrivault, A. Florian, M. Stitt, A.R. Fernie, H. Bauwe, The photorespiratory metabolite 2-phosphoglycolate regulates photosynthesis and starch accumulation in Arabidopsis, Plant Cell 29 (2017) 2537–2551.
[119] L.E. Anderson, Chloroplast and cytoplasmic enzymes. II. Pea leaf triose phosphate isomerases, Biochim. Biophys. Acta 235 (1971) 237–244.
[120] P. da Fonseca-Pereira, P.V.L. Souza, L.-Y. Hou, S. Schwab, P. Geigenberger, A. Nunes-Nesi, S. Timm, A.R. Fernie, I. Thormählen, W.L. Araújo, D.M. Daloso, Thioredoxin h2 contributes to the redox regulation of mitochondrial photorespiratory metabolism, Plant Cell Environ. 43 (2020) 188–208.
[121] P.E. López-Calcagno, S. Fisk, K.L. Brown, S.E. Bull, P.F. South, C.A. Raines, Overexpressing the H-protein of the glycine cleavage system increases biomass yield in glasshouse and field-grown transgenic tobacco plants, Plant Biotechnol. J. 17 (2019) 141–151.
[122] S. Vialet-Chabrand, J.S.A. Matthews, T. Lawson, Light, power, action! Interaction of respiratory energy- and blue light-induced stomatal movements, New Phytol. 231 (2021) 2231–2246.
[123] K.A. Mott, D. Peak, Effects of the mesophyll on stomatal responses in amphistomatous leaves, Plant Cell Environ. 41 (2018) 2835–2843.
[124] S. Wall, S. Vialet-Chabrand, P. Davey, J. Van Rie, A. Galle, J. Cockram, T. Lawson, Stomata on the abaxial and adaxial leaf surfaces contribute differently to leaf gas exchange and photosynthesis in wheat, New Phytol. 235 (2022) 1743–1756.
[125] Y. Wang, K. Noguchi, I. Terashima, Distinct light responses of the adaxial and abaxial stomata in intact leaves of *Helianthus annuus* L, Plant Cell Environ. 31 (2008) 1307–1316.
[126] Y. Takahashi, K.C. Bosmans, P.-K. Hsu, K. Paul, C. Seitz, C.-Y. Yeh, Y.-S. Wang, D. Yarmolinsky, M. Sierla, T. Vahisalu, J.A. McCammon, J. Kangasjärvi, L. Zhang, H. Kollist, T. Trac, J.I. Schroeder, Stomatal CO_2/bicarbonate sensor consists of two interacting protein kinases, Raf-like HT1 and non-kinase-activity requiring MPK12/MPK4, Sci. Adv. 8 (2022) eabq6161.
[127] G.S. Hudson, J.R. Evans, S. von Caemmerer, Y.B. Arvidsson, T.J. Andrews, Reduction of ribulose-1,5-bisphosphate carboxylase/oxygenase content by antisense RNA reduces photosynthesis in transgenic tobacco plants, Plant Physiol. 98 (1992) 294–302.
[128] M. Lauerer, D. Saftic, W.P. Quick, C. Labate, K. Fichtner, E.-D. Schulze, S. R. Rodermel, L. Bogorad, M. Stitt, Decreased ribulose-1,5-bisphosphate carboxylase-oxygenase in transgenic tobacco transformed with "antisense" rbcS, Planta 190 (1993), https://doi.org/10.1007/bf00196962.
[129] W.P. Quick, U. Schurr, R. Scheibe, E.D. Schulze, S.R. Rodermel, L. Bogorad, M. Stitt, Decreased ribulose-1,5-bisphosphate carboxylase-oxygenase in transgenic tobacco transformed with "antisense" rbcS: I. Impact on photosynthesis in ambient growth conditions, Planta 183 (1991) 542–554.
[130] S. Von Caemmerer, A. Millgate, G.D. Farquhar, R.T. Furbank, Reduction of ribulose-1,5-bisphosphate carboxylase/oxygenase by antisense RNA in the C_4 plant *Flaveria bidentis* leads to reduced assimilation rates and increased carbon isotope discrimination, Plant Physiol. 113 (1997) 469–477.
[131] G.D. Price, J.R. Evans, S. von Caemmerer, J.W. Yu, M.R. Badger, Specific reduction of chloroplast glyceraldehyde-3-phosphate dehydrogenase activity by antisense RNA reduces CO_2 assimilation via a reduction in ribulose bisphosphate regeneration in transgenic tobacco plants, Planta 195 (1995) 369–378.
[132] M. Muschak, L. Willmitzer, J. Fisahn, Gas-exchange analysis of chloroplastic fructose-1,6-bisphosphatase antisense potatoes at different air humidities and at elevated CO_2, Planta 209 (1999) 104–111.
[133] M.J. Paul, J.S. Knight, D. Habash, M.A.J. Parry, D.W. Lawlor, S.A. Barnes, A. Loynes, J.C. Gray, Reduction in phosphoribulokinase activity by antisense RNA in transgenic tobacco: effect on CO_2 assimilation and growth in low irradiance, Plant J. 7 (1995) 535–542.
[134] G.D. Price, S. von Caemmerer, J.R. Evans, K. Siebke, J.M. Anderson, M.R. Badger, Photosynthesis is strongly reduced by antisense suppression of chloroplastic cytochrome bf complex in transgenic tobacco, Funct. Plant Biol. 25 (1998) 445.
[135] A.B. Cousins, I. Pracharoenwattana, W. Zhou, S.M. Smith, M.R. Badger, Peroxisomal malate dehydrogenase is not essential for photorespiration in Arabidopsis but its absence causes an increase in the stoichiometry of photorespiratory CO_2 release, Plant Physiol. 148 (2008) 786–795.