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Surrounded by luxury: The necessities of subsidiary cells

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Abstract

The evolution of stomata marks one of the key advances that enabled plants to colonise dry land while allowing gas exchange for photosynthesis. In large measure, stomata retain a common design across species that incorporates paired guard cells with little variation in structure. By contrast, the cells of the stomatal complex immediately surrounding the guard cells vary widely in shape, size and count. Their origins in development are similarly diverse. Thus, the surrounding cells are likely a luxury that the necessity of stomatal control cannot do without (with apologies to Oscar Wilde). Surrounding cells are thought to support stomatal movements as solute reservoirs and to shape stomatal kinetics through backpressure on the guard cells. Their variety may also reflect a substantial diversity in function. Certainly modelling, kinetic analysis and the few electrophysiological studies to date give hints of much more complex contributions in stomatal physiology. Even so, our knowledge of the cells surrounding the guard cells in the stomatal complex is far from complete.

KEYWORDS

CO_2, development, humidity, physiological functions, stomata, stomatal kinetics, surrounding (subsidiary) cell

1 | INTRODUCTION

From an evolutionary point of view, the guard cell is a revolutionary design that enabled the dominance of plants on dry land and facilitated their colonisation across a wide range of habitats. The vast majority of plant species share a common, kidney-shaped design that can be traced back to pores on sporangia of fossil moss-like plants at the end of the Ordovician period throughout their evolution (Clark et al., 2022; Edwards et al., 1986; Salamon et al., 2018). Guard cells appear as pairs of cells that surround a pore through the epidermal cell layer. The swelling and shrinking of the guard cells opens and closes the pore, thereby regulating water loss via transpiration from the plant and CO_2 diffusion from the atmosphere for photosynthesis. The only major innovation in design, with dumbbell-shaped guard cells, appeared more recently in the evolution of the grasses (Poaceae) some 100 Myr ago (Cai et al., 2017; Chen et al., 2017). By contrast, a very active diversification is evident across multicellular streptophytes in the epidermal cells adjacent to the guard cells, sometimes referred to as subsidiary cells and what we identify broadly as surrounding cells (Clark et al., 2022; Sack, 1987). In large measure, it is the surrounding cell organisation that defines the variety in structures between stomatal complexes; this diversity in surrounding cell shape, size and organisation most likely also contributes to differences in stomatal function without altering the vital design of the stoma.

Although their definition within the stomatal complex is simple, identifying the surrounding cells of the complex remains challenging. Often, surrounding cells are distinguishable by their smaller size when compared with other epidermal pavement cells, their morphology and organisation around the guard cells (Figure 1). Paracytic stomata, for

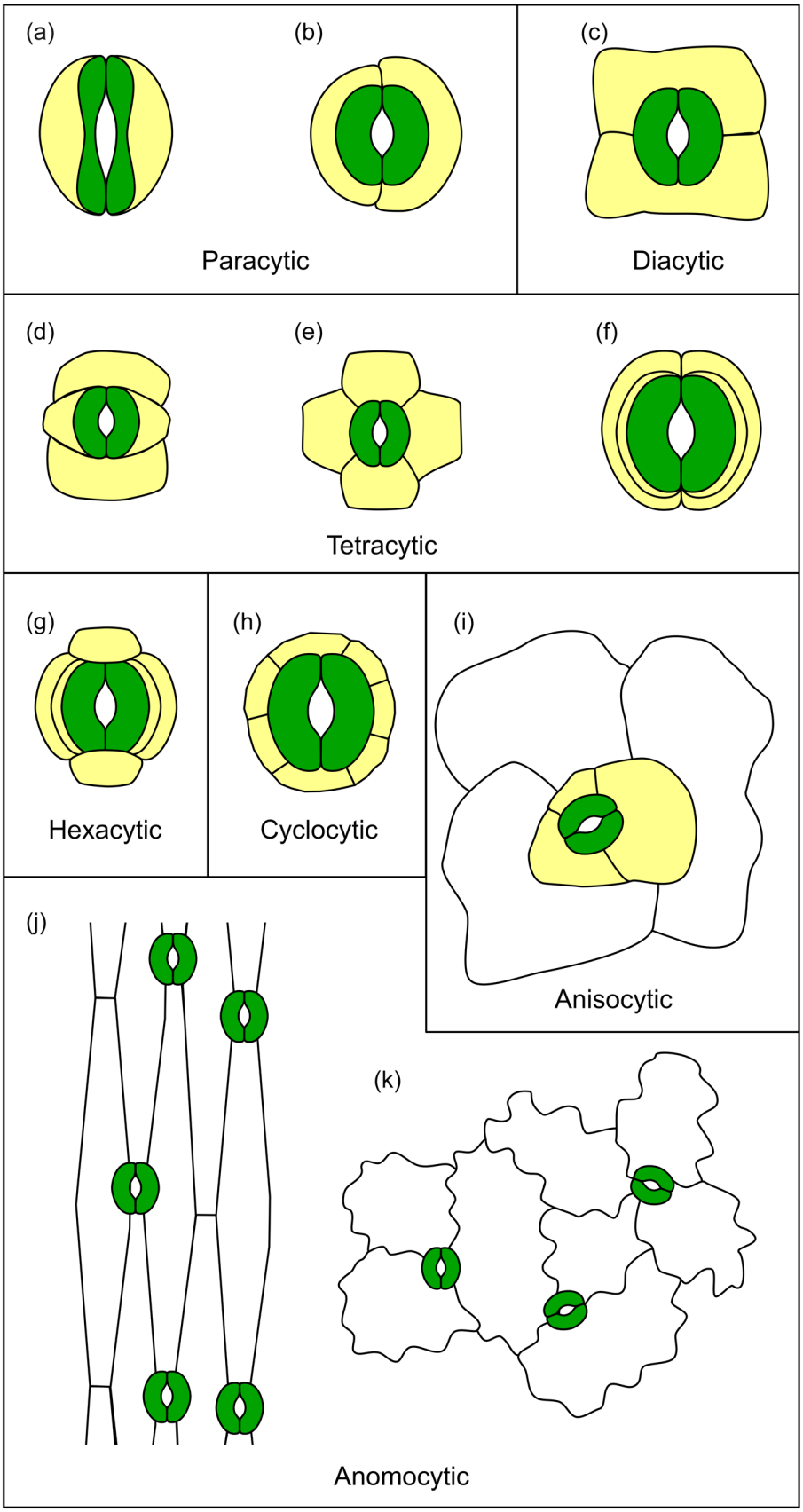

FIGURE 1 Typical stomatal complex structures. (a, b) Paracytic stomata. (a) Grass-type stomata (paracytic) in members of Poaceae. (b) Paracytic stomata in horsetail (*Equisetum*) and coffee (*Coffea*). (c) Diacytic stomata of *Dianthus*. (d–f) Tetracytic stomata of *Tradescantia* (d), *Agave* (e) and *Anacampseros rufescens* (f). (g) Hexacytic stomata of *Commelina communis*. (h) Cyclocytic stomata of *Ginkgo bibola*, *Kanenchoë* and cycads. (i) Anisocytic stomata of the Brassicaceae and Arabidopsis. (j, k) Anomocytic stomata in onion (*Allium cepa*) (j) and watermelon (*Citrullus lanatus*) (k). colour code: guard cell—green, surrounding cell—light yellow, pavement cell—white. [Color figure can be viewed at wileyonlinelibrary.com]

example, occur with pairs of surrounding cells aligned parallel to guard cells. Such complexes are found among horsetails (*Equisetum*) (Cullen & Rudall, 2016; Hauke, 1957), coffee (*Coffea*) (Pompelli et al., 2010) and grasses. Diacytic stomata form complexes with a pair of surrounding cells at right angles above and below the guard cells, such as in *Dianthus* species (Ramayya & Rajagopal, 1980). Stomatal complexes with four and six surrounding cells are termed tetracytic and hexacytic, respectively. These stomata often appear with a pair of terminal (or polar) subsidiary cells at both ends of the guard cells (Rudall et al., 2017) and are common to *Tradescantia* (Kappen & Haeger, 1991), *Agave* (Gray et al., 2020; Monja-Mio et al., 2015) and *Commelina* species (Schwartz et al., 1994). The tetracytic stomatal complex of *Anacampseros rufescens*, however consists of four lateral surrounding cells (Gray et al., 2020). In *Ginkgo biloba* (Gray et al., 2020; Kausik, 1974) and cycads (Coiro et al., 2021; Pant & Mehra, 1964), the guard cells are wholly encircled by a narrow ring of four or more surrounding cells, forming a cyclocytic stomatal complex (Van Cotthem, 1970a).

However, many plants exhibit so-called anomocytic stomata, those lacking morphologically distinct surrounding cells, including onion (*Allium cepa*) (Schnabl & Ziegler, 1977), watermelon (*Citrullus lanatus*) (Duan et al., 2014) and members of the Ranunculaceae (Van Cotthem, 1970b). The model plant Arabidopsis has both anomocytic and anisocytic stomata, with three unequally sized surrounding cells, a pattern which is shared across the cruciferous plants (Pant & Kidwai, 1967). In these instances, identifying the surrounding cells is more challenging and, even with gene expression signatures, may differ quantitatively rather than qualitatively from other pavement cells.

To date, the gene *PATROL1* is the only molecular marker that identifies the surrounding cells in Arabidopsis (Gray et al., 2020; Higaki et al., 2014). The *PATROL1* promoter was found to be active in guard cells and one or two of the (usually smaller) adjacent cells. However, the lack of uniformity in expression does raise questions about the utility of *PATROL1* as a surrounding cell marker. PATROL1 was first identified in association with H^+-ATPase traffic to the plasma membrane (Hashimoto-Sugimoto et al., 2013). As guard cell function depends heavily on H^+-ATPase activity, it is no surprise that *PATROL1* is always expressed in guard cells (Higaki et al., 2014) and may also explain why it is more prevalent in the surrounding cells. Of course, H^+-ATPase activity contributes also to cell expansion and apoplastic acidification for relaxing of the cell wall (Hager et al., 1991; Rayle & Cleland, 1970; Xia et al., 2019). This association, too, might also have connected *PATROL1* expression to the smaller surrounding cells, if they were still expanding. In other words, caution must be taken when interpreting data using *PATROL1* as a surrounding cell marker.

Much the same issue applies to expression of several of the genes encoding K^+ channels in subsidiary cells (Büchsenschütz et al., 2005; Nguyen et al., 2017). These channels are found also in guard cells and the mesophyll and, hence, cannot be used as subsidiary cell markers. To date, only Closed Stomata1 (CST1), a protein belonging to SWEET-family of sugar transporters, appears to mark maize subsidiary cells but not the guard cells or any other plant tissues (Wang, Yan, et al., 2019), suggesting that this gene could be a potential molecular marker of utility. Whether the CST1 orthologues in other grass species also show a similar pattern in expression and whether its homologue in non-grass species may also mark all surrounding cells remains to be seen. Certainly, establishing a set of molecular markers for the cells surrounding the guard cells, in general, will facilitate future research.

2 | THE ORIGINS OF SURROUNDING CELLS

The stomatal complex forms through a series of asymmetric cell divisions (Facette & Smith, 2012; Gray et al., 2020). In Arabidopsis, after differentiating from a protodermal cell, the meristemoid mother cell (MMC) divides asymmetrically to generate a small meristemoid cell and a stomatal lineage ground cell. The meristemoid cell then differentiates into a guard mother cell (GMC) which later gives rise to a pair of guard cells by symmetric cell division. The associated surrounding cell arises before the symmetric division of GMC, but need not derive from the same lineage. This difference in cell lineages therefore gives rise to three different classifications based on the surrounding cells (Figure 2) (Rudall et al., 2013). Surrounding cells arising from the asymmetric division of meristemoid cells, so from the same cell lineage with guard cells, are mesogenous. Arabidopsis is a typical example of a mesogenous lineage in which the surrounding cells originate through amplification divisions of meristemoid cells (Lau & Bergmann, 2012). By contrast, perigenous stomata arise with the surrounding cells recruited from protodermal cells adjacent to the GMC. Perigenous surrounding cells are typical of the grass stomatal complex (Cheng & Raissig, 2023; Wu et al., 2019). Finally, some species exhibit mesoperigenous surrounding cells, with one or more mesogenous and perigenous surrounding cells. The stomatal complexes of several *Amborella* and *Austrobaileya* species follow this pattern (Rudall & Knowles, 2013).

Extensive studies, primarily of Arabidopsis, have uncovered many regulators, notably three transcription factors, SPEECHLESS (SPCH), MUTE and FAMA that act in stomatal initiation, proliferation and differentiation (Herrmann & Torii, 2021; Raissig et al., 2016). The underlying developmental processes are widely reviewed and we point readers also to those of Spiegelhalder and Raissig (2021) and Wu et al. (2019). Orthologues of the Arabidopsis transcription factors in grass species similarly play central roles in stomatal developments (Liu et al., 2009). While the activity of MUTE in Arabidopsis defines only the fate of GMC, its orthologues in grasses also recruit and facilitate development in surrounding cells (Raissig et al., 2017; Serna, 2020; Wang, Guo, et al., 2019). Mobile MUTE orthologs appear specific to grasses—species with perigenous surrounding cells—and, to date, have been studied in *Brachypodium*, maize and rice; these orthologues diffuse from GMCs to neighbouring epidermal cells where they trigger asymmetrical cell divisions to generate surrounding cells (Raissig et al., 2017; Wang, Guo, et al., 2019). To

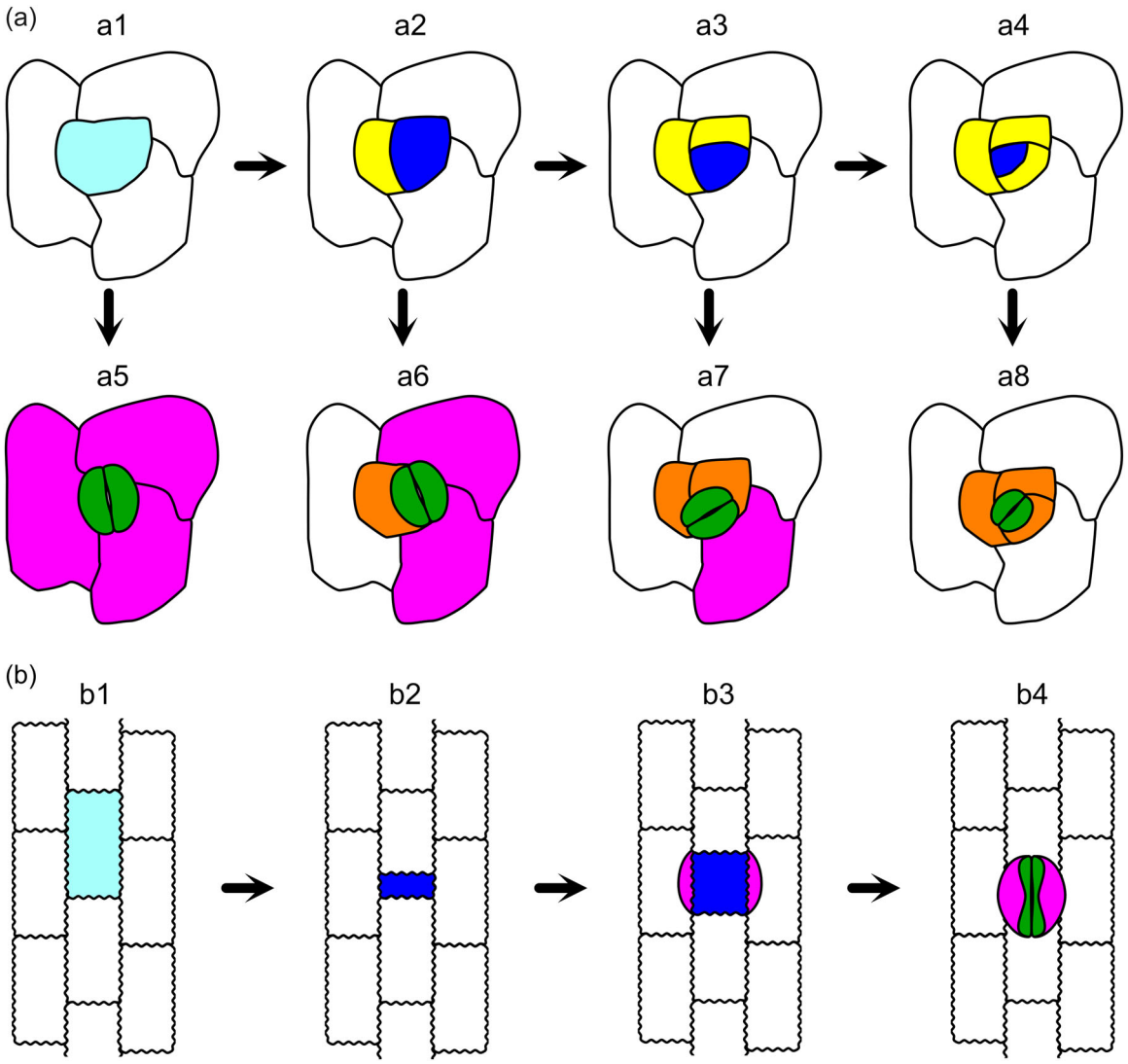

FIGURE 2 Origins of surrounding cells. (a) The formation of perigenous (a1→a5), mesoperigenous (a1→a2→a6 and a1→a2→a3→a7) and mesogenous (a1→a2→a3→a4→a8) stomata from a protodermal cell (a1—light blue). Surrounding cells that do not share the same cell lineage with the guard cells (green) are perigenous (pink). In contrast, the successive division of the meristemoid cell (blue) gives rise to the stomatal lineage ground cells (a2, a3 and a4, yellow) which later develop into mesogenous surrounding cells (a6, a7 and a8, orange). The mesoperigenous stomata have both mesogenous and perigenous surrounding cells (a6 and a7). (b) The development of the grass-type stomata (perigenous). An asymmetric division of the protodermal cell (light blue) (b1) gives rise to the guard mother cell (GMC) (blue) (b2). The perigenous surrounding cells (pink) are recruited from the two lateral pavement cells in direct contact with the GMC (b3) by asymmetric cell division before the formation of the pair of guard cells (b4, green). Figure adapted from Rudall et al. (2013). [Color figure can be viewed at wileyonlinelibrary.com]

prove the mobile property of MUTE in *Brachypodium*, Raissig et al. (2017) expressed the gene fused with YFP in wild-type Arabidopsis under the control of the GMC-specific *MUTE* promoter of Arabidopsis and observed YFP fluorescence also in cells adjacent to GMC (Pillitteri et al., 2007; Raissig et al., 2017). In the reciprocal experiment where the corresponding Arabidopsis *MUTE* construct was expressed in *Brachypodium* under its *MUTE* promoter, the fluorescence was only observed in GMC. These results suggest that (1) the Arabidopsis MUTE protein is immobile, (2) *Brachypodium MUTE* expression is specific to the GMC and (3) the *Brachypodium* MUTE is able to move from the GMC to adjacent cells. This intrinsic mobility seems to be common in grass species as the YFP fluorescence of constructs with *Brachypodium*, maize and rice *MUTE* genes were always observed in the GMC and the cells adjacent to the GMC (Raissig et al., 2017; Wang, Guo, et al., 2019). Overexpressing *Brachypodium MUTE* using a constitutive ubiquitin promoter resulted in polarised cell divisions in the majority of pavement cells, finally forming multiple rings of surrounding (subsidiary) cell layers around the guard cells that resembled the structure of stomatal complexes of *Commelina communis* (Raissig et al., 2017). This result is consistent with the idea that *Brachypodium* MUTE alone is sufficient to define surrounding cell fate.

The MUTE transcription factor is not the sole player controlling surrounding cell fate. Downstream controls are also mediated by

POLAR in Arabidopsis and its homologue in *Brachypodium* (Han et al., 2018; Zhang et al., 2022). Abnormal surrounding cells were observed in the *pangloss1* (*pan1)* and *pangloss2* (*pan2)* mutants, and in the double mutant *pan1/pan2* in maize (Cartwright et al., 2009; Humphries et al., 2011; Zhang et al., 2012). The *PAN* gene products are leucine-rich repeat receptor-like proteins localised at the site of contact with GMC, suggesting the importance of signal transduction from GMC in surrounding cell formation of maize. Interacting with PAN1, the two Plant Rho family GTPases (ROPs), ROP2 and ROP9, were also found involved in maize surrounding cell development as their partial loss of function enhance the *pan1* phenotype (Humphries et al., 2011). Additionally, in maize, the *LINC KASH AtSINE-like2* (*MLKS2*) gene, encoding a member of the linker of nucleoskeleton and cytoskeleton (LINC), was shown to be responsible for the correct division of maize surrounding cells (Gumber et al., 2019). The *mlks2* mutants displayed abnormal surrounding cell phenotype, which was later proven to be associated with incorrect division plane due to a defect in premitotic nuclear migration and nuclear position stabilisation (Ashraf et al., 2023). Recently, a new gene affecting surrounding cell formation was found in maize (Cui et al., 2023): the mutation of *LOST SUBSIDIARY CELL*, encoding a large subunit of ribonucleotide reductase, led to the abortion of 50% polarised cell divisions generating the SMC. This result suggested the sensitivity of surrounding cell development to dNTP deficiency.

Finally, it is worth noting that the position of stomata within the leaf epidermis is often not at the same height relative to other cells on the leaf surface (Gray et al., 2020). Stomata may be positioned either above or below the epidermal surface, depending on the plant species, and this characteristic results in elevated and sunken stomata. Although the real role of stomatal position in plant adaptation to the environment remains controversial, the origins of stomatal positioning may depend, in part, on surrounding cell development (Gray et al., 2020).

2.1 | Surrounding cells in stomatal function

Almost nine decades ago, Heath observed how surrounding cells could alter the stomatal aperture (Heath, 1938). By alternately puncturing guard cells and surrounding cells, he could show that pressure from the surrounding cells on the dorsal wall of the guard cells opposed stomatal opening. Glinka (1971) extended these studies to show the mechanical advantage of the surrounding cells by manipulating the epidermal cell osmotic potential. Later modelling work again confirmed the mechanical advantage of surrounding cells (DeMichele & Sharpe, 1973). This mechanical advantage is of particularly importance in stomatal closing: Itai et al. (1978) noted that full closure of *Commelina* guard cells in abscisic acid (ABA) was only observed in stomata surrounded by living surrounding cells. In short, it has long been recognised that changes in the turgor pressure of the surrounding cells imposes a substantial and opposing pressure on the guard cells that affects steady-state aperture. Furthermore, by surrounding the guard cells, these larger neighbouring cells gain considerable physical—or mechanical—advantage to dominate the final pore size.

The mechanical advantage of surrounding cells also creates problems when the guard cells are displaced laterally toward the space occupied by surrounding cells during stomatal opening (Franks & Farquhar, 2007). Pressure probe measurements of stomatal aperture versus guard cell turgor highlighted the problem with the opening of paracytic stomata (stomata with parallel-oriented surrounding cells) when epidermal cell turgor is maximal. A cryo-section of these stomata in the open-state showed that the surrounding cells deformed allowing lateral displacement of guard cells, suggesting a decrease in turgor pressure against the guard cells (Franks & Farquhar, 2007). In this work, the authors also pointed out the discrepancy between experimental data and the model in the stomatal conductance (g_s) in response to different value of leaf-to-air vapour pressure differences (VPD). Mechanical advantage alone predicts a proportional change of g_s with the change of VPD which cannot explain how a plant can attain full aperture under high humidity when the turgor pressure of the surrounding cell is high. To solve this discrepancy, the authors proposed an exchange in turgor pressure in inverse proportion with that of the guard cells. A similar explanation has been invoked for the fast and large dynamic range of barley stomata (Durney et al., 2023) and was modelled successfully as part of the OnGuard systems platform to explain the discrepancies between guard cell transport and the kinetics of stomatal movements (Jezek et al., 2019). Durney et al. reported that the simulations captured the steady-state aperture of open and closed stomata, while Jezek et al. emphasised the importance of a turgor exchange in the kinetics of stomatal movements.

It is worth noting that turgor exchange as a means to control stomatal aperture requires active mechanisms to adjust and reverse the turgor pressure of the surrounding cells. These processes pose some interesting mechanical challenges. Guard cells lack functional plasmodesmata (Palevitz & Hepler, 1985; Wille & Lucas, 1984) and, therefore, can adjust their turgor pressure efficiently. Such is not obviously the case for surrounding cells, which retain plasmodesmatal connections with their adjacent epidermal cells. Does the presence of functional plasmodesmata mean that changes in turgor pressure in the surrounding cells are temporary? Could these plasmodesmatal connections be regulated during stomatal movements to alter or buffer turgor changes in the surrounding cells?

Certainly, comparisons between simulations and experimental data suggest a transient and reversible element to the turgor exchange between guard and surrounding cells. Jezek et al. (2019) used the OnGuard platform, which incorporates the quantitative characteristics of guard cell solute transport and metabolism, to describe the kinetics of stomatal movements and define the differential between simulation and experiment. Their analysis uncovered a dynamic and reversible process of constraint relaxation and recovery by the surrounding cells that acts on the guard cells. In this analysis, during stomatal opening the surrounding cells lose solute and reduce in turgor as the guard cells take up solute; the

surrounding cells then recover slowly to regain their turgor. This 'constraint-relaxation-recovery' (CRR) model is supported by experimental data obtained from maize subsidiary cells (Mumm et al., 2011; Raschke & Fellows, 1971). Raschke and Fellows showed a shuttle of K^+ between the guard cells and surrounding cells suggesting a cooperatively reciprocal change in turgor pressure of these cell types during stomatal movements (Raschke & Fellows, 1971). Mumm et al. (2011) observed that the free-running voltage of the surrounding cells followed a transient depolarisation upon the transition from dark to light with a slow recovery in voltage analogous to the changes in solute flux predicted by Jezek et al. (2019; Mumm et al., 2011). While there remains no substantive proof, it is to be anticipated that the transient depolarisation could reflect a rapid solute loss and decrease in turgor pressure in these cells while the slower recovery associates with solute and turgor recovery by the surrounding cells. Thus, the CRR model of Jezek et al. (2019) explains how a transient change in the turgor pressure of surrounding cells contributes to a fast kinetic reversibility in stomatal movements (Figure 3).

Finally, it is worth noting that the CRR concept finds support also in the characteristics of the *mute* mutant of *Brachypodium* noted above that lack surrounding cells. The mutant plants showed significantly slower stomatal movements in response to light changes when compared to wild-type plants (Raissig et al., 2017). These mutants also had lower stomatal conductance and significantly lower fresh weight compared to wild type. This result strongly suggests that subsidiary cells are important and actively contribute to the control of stomatal kinetics.

2.2 | Solute transport in surrounding cells

Since water can only flow across the cell membrane following the water potential, cellular turgor depends on the regulation of the solute content. Potassium has long been recognised in determining guard cell turgor (Raschke & Humble, 1973), as it is in virtually all plant cells. The charge on the K^+ ion is balanced principally by Cl^- and the malate anion, much of the latter synthesised in the guard cells (Travis & Mansfield, 1977). Surrounding cells are also known to accumulate K^+ (Squire & Mansfield, 1972). The first strong evidence of K^+ and Cl^- shuttling between guard cells and surrounding cells was reported in maize using histochemical staining (Raschke & Fellows, 1971). Later, similar staining methods were used to confirm the phenomenon in 22 different plant species (Dayanandan & Kaufman, 1975). However, such studies place K^+ within a constrained environment since, after peeling, only guard cells and surrounding cells survive and plasmodesmatal connections to the pavement cells are broken. So a question remains whether pavement cells of the epidermis may serve as an additional ion reservoir. Certainly, the surrounding cells of the stomatal complex are known to adjust their content of K^+ during stomatal movements: a reduction in the K^+ content of these cells was often observed accompanied by K^+ accumulation in the guard cells during stomatal opening (Sawhney & Zelitch, 1969; Squire & Mansfield, 1974).

Pallaghy (1971) first described the different kinetics of K^+ content changes of maize surrounding cells in response to light and CO_2 that opposed those of the guard cells. Similarly, on treating epidermal peels of *Commelina* with ABA, which triggers stomatal closure, K^+ was found to accumulate in the surrounding cells with a corresponding increase in turgor (Itai & Meidner, 1978; Itai et al., 1982). These data are consistent with the role of ion reservoir and sink for guard cell solute exchange during stomatal movements. Indeed, measurements of *Commelina* stomata showed an apparent exchange of K^+ and Cl^- in the surrounding cells with the guard cells that was directed to the guard cells during stomatal opening and reversed during stomatal closure (Figure 4) (Bowling, 1987; Penny & Bowling, 1974; Penny et al., 1976). These seeming directional movements of ion suggest the presence of ion transport systems in the surrounding cells that facilitate an ion shuttle with the guard cells.

What transport systems in the surrounding cells might contribute to ion shuttling? Much of our knowledge to date comes from the grass models of maize and rice (Table 1). Not surprisingly, histochemical assays revealed the expression of a subset of K^+ channel genes, among these one encoding an outward- and two encoding inward-rectifying channels; all of these channels were found in both guard cells and subsidiary cells of rice (Hwang et al., 2013; Nguyen et al., 2017). In maize, the expression of *ZMK1* and *ZORK* encoding inward- and outward-rectifying K^+ channels, respectively, were identified in surrounding cells (Büchsenschütz et al., 2005). In many respects, the characteristics of these channels mirror those found in Arabidopsis guard cells. In Arabidopsis, *KC1* and *AKT2* were also found expressed in all the epidermis including guard cells, surrounding cells, pavement cells and trichomes (Lacombe et al., 2000; Nieves-Cordones et al., 2022; Pilot et al., 2003). KC1 is thought to be a so-called 'silent' channel subunit that assembles with other inward-rectifying channel subunits but does not yield a current by itself; AKT2 is a weakly rectifying channel that facilitates net K^+ flux both inward and outward, depending on the prevailing electrochemical driving force. Expression of *SLAH3*, a gene encoding a slow-activating anion channel, was also reported in Arabidopsis pavement cells (Negi et al., 2008) although in another study, this gene appeared to be present only in the guard cells (Zheng et al., 2015). Note, however, that all of these genes are also expressed in guard cells with the sole exception of ZMK1.

In rice, mutation of *OsK5.2*, normally encoding an outward-rectifying K^+ channel, slowed down both stomatal opening and closing kinetics (Nguyen et al., 2017). While the slow closing of *osk5.2* mutants is linked to the function of this gene in guard cells, the slow stomatal opening kinetics is more readily explained by its activity in the surrounding cells if its absence were to slow the release of K^+ from these cells and thereby favour a higher turgor in the surrounding cells. These findings echo those of the *kc1* mutant in Arabidopsis that appears to enhance stomatal conductance (Nieves-Cordones et al., 2022). The KC1 subunit normally suppresses channel activity when assembled with other inward-rectifying subunits, and may suggest the importance of a lower K^+ conductance in the pavement cells for stomatal function. To date, the glucose

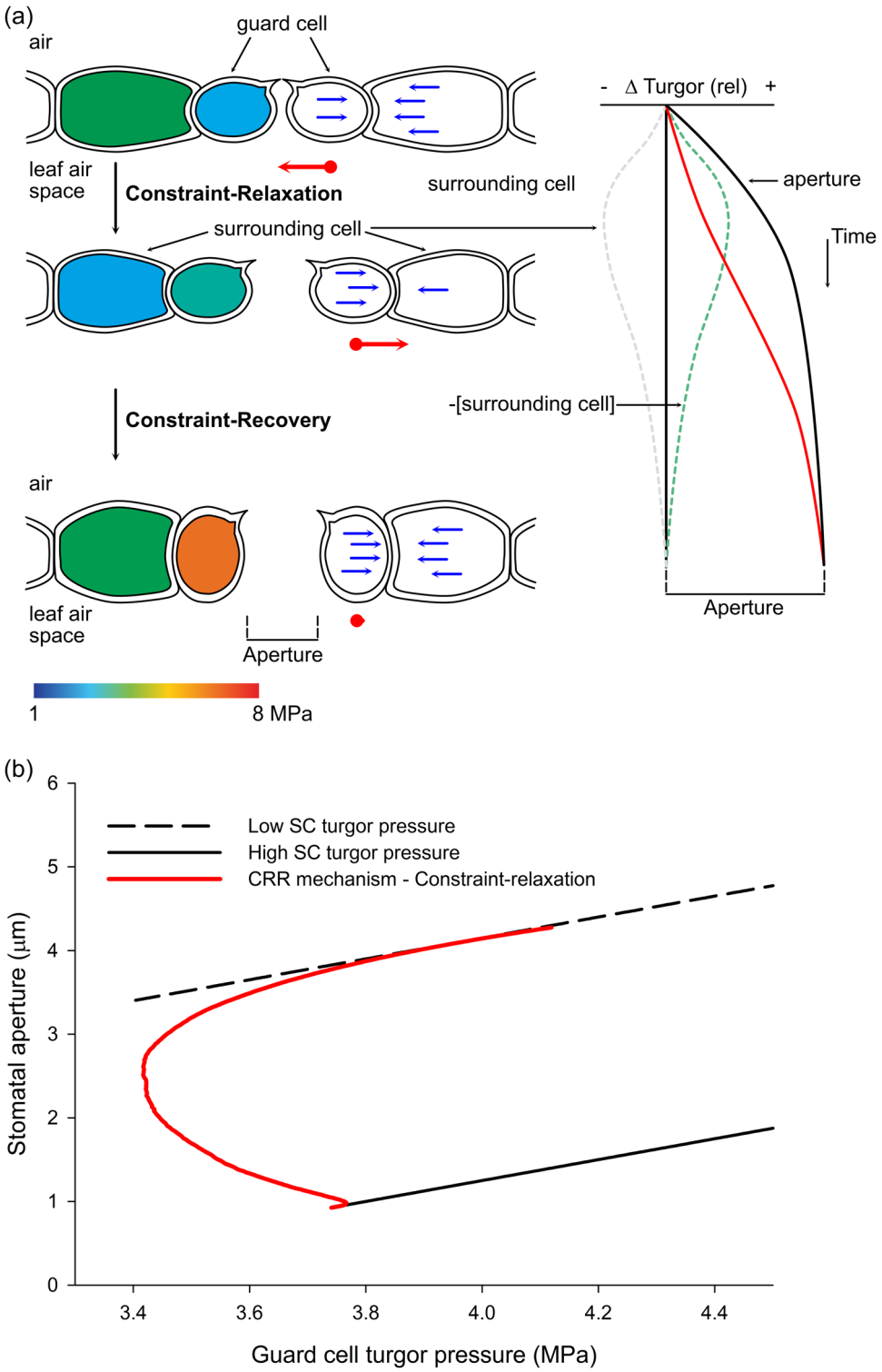

FIGURE 3 Constraint-relaxation-recovery (CRR) mechanism of stomatal movement. (a) Schematic transverse sections through the leaf epidermis (left) present guard cells and surrounding cells colour-coded to indicate the osmotic pressure. The changes in osmotic pressures and stomatal aperture are presented in temporal order from fully closed (top) to fully open (bottom). Scale 1–8 MPa. The blue vectors represent relatively the forces exerted by guard cells and surrounding cells on one another. Red vectors are the sums of blue vectors representing the direction and the net force directing the displacement of the guard cell. The right panel plots the change of stomatal aperture (black line) and the relative turgor (ΔTurgor) of the guard cell (red line) and surrounding cell (grey dashed line). The inverse of the change in relative turgor of surrounding cells (-[surrounding cell]—green dashed line) is added to yield the aperture kinetics. Adapted from Jezek et al. (2019). (b) The relationship between guard cell turgor pressure and stomatal aperture generated by the OnGuard3e platform (Nguyen, Silva-Alvim, et al., 2023) when the turgor of the SC was kept constant at low (0.68 MPa—black dashed line) or high value (3 MPa—black line). Enabling the CRR mechanism (red line) give rise to a temporal and curvilinear relationship as the turgor of the SC changes from high to low values and then recovers. For the CRR model, the maximum SC turgor pressure was set at 3 MPa, and the graph represents only the constraint-relaxation step when the SCs and GCs reciprocally change their turgor pressures. [Color figure can be viewed at wileyonlinelibrary.com]

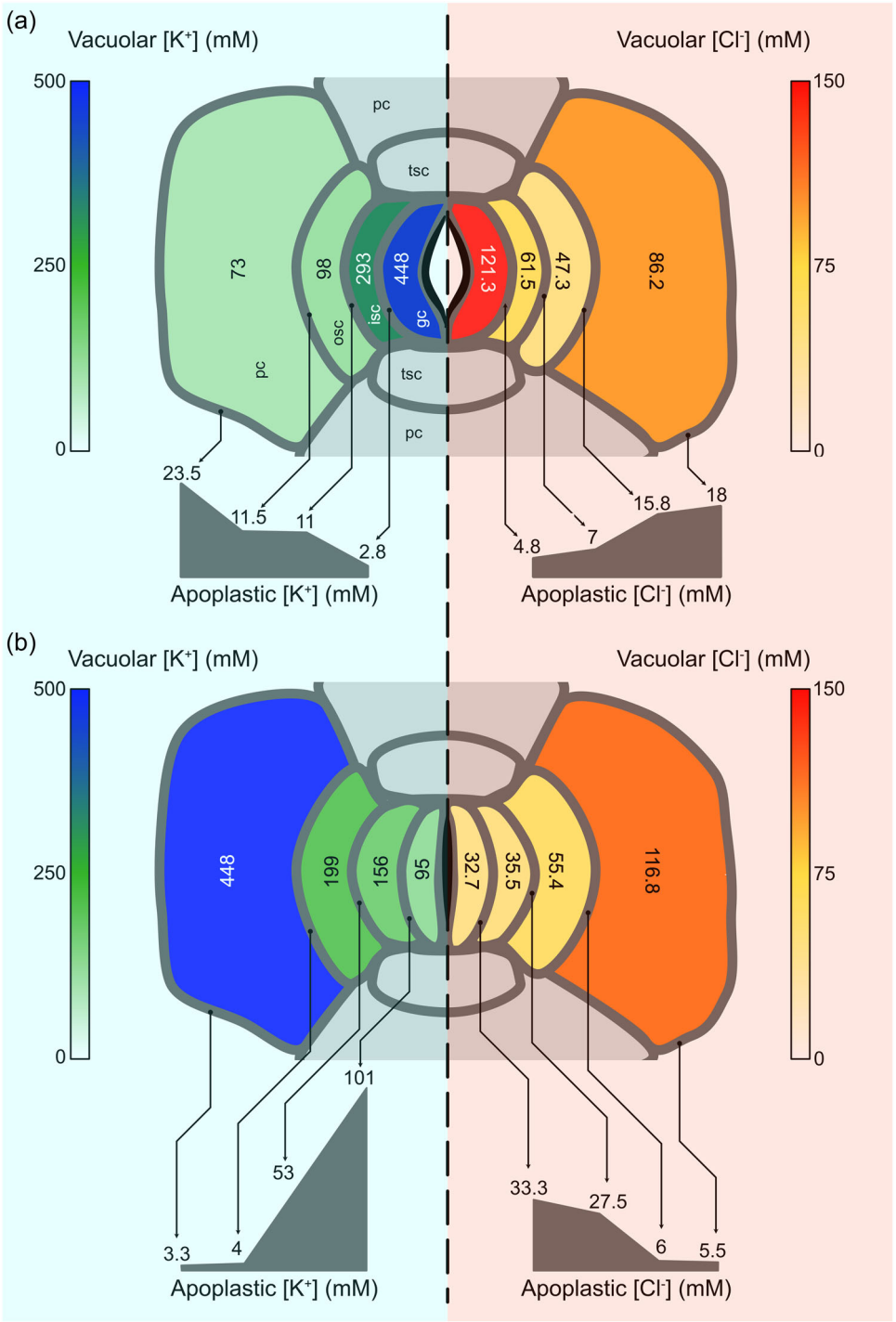

FIGURE 4 K^+ and Cl^- shuttling between surrounding and guard cells in the *Commelina communis* stomatal complex. Schematic of K^+ (left-hand side) and Cl^- (right-hand side) contents in the vacuole and apoplast of cells in the *C. communis* stomatal complex during stomatal opening (a) and stomatal closure (b). Graphics adapted from the data of Penny and Bowling (1974), Penny et al. (1976) and Bowling (1987). Each stomatal complex is formed by a pair or kidney-shaped guard cells (gc) surrounded by a set of six surrounding cells including two inner lateral surrounding cells (isc), two outer lateral surrounding cells (osc) and two terminal surrounding cells (tsc). The whole stomatal complex is surrounded by four to five epidermal pavement cells (pc) of much larger size. The ion contents of the guard cells, lateral surrounding cells and lateral pavement cells are colour-coded to indicate the vacuolar $[K^+]$ ($[K^+]_{vac}$) and $[Cl^-]$ ($[Cl^-]_{vac}$). Scale 0–500 mM for $[K^+]_{vac}$ and 0–150 mM for $[Cl^-]_{vac}$. Black lines link the apoplast of each cell to its respective K^+ or Cl^- concentrations (below). [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 List of ion channels and transporters found in surrounding cells.

Transporter	Gene products	Locus	Species	Transported substrates	Function	Verification method	Pavement—guard cells	Refs
K^+ channel	AKT2	AT4G22200	Arabidopsis	K^+	K^+ influx	GUS	Yes—Yes	Lacombe et al. (2000)
	AtKC1	AT4G32650	Arabidopsis	K^+	Silence subunit	GUS	Yes—Yes	Nieves-Cordones et al. (2022); Pilot et al. (2003)
	ZMK1	Zm00001eb359320	Maize	K^+	K^+ influx	RT-PCR	Unknown—No	Büchsenschütz et al. (2005)
	ZORK	Zm00001eb280210	Maize	K^+	K^+ efflux	RT-PCR	Unknown—Yes	Büchsenschütz et al. (2005)
	OsK5.2	Os06g0250600	Rice	K^+	K^+ efflux	GUS	No—Yes	Nguyen et al. (2017)
	OsK2.1/ OsKAT2	Os01g0210700	Rice	K^+	K^+ influx	GUS	No—Yes	Hwang et al. (2013)
	OsK2.3/ OsKAT3	Os02g0245800	Rice	K^+	K^+ influx	GUS	Yes—Yes	Hwang et al. (2013)
K^+ transporter	KUP9	AT4G19960	Arabidopsis	K^+	K^+ influx, high affinity	GUS	Yes—Likely	Yamanashi et al. (2022)
	OsHAK12	Os08g0206400	Rice	Na^+	Na^+ influx	GUS	Yes—Yes	Zhang et al. (2021)
H^+-ATPase	AHA2	AT4G30190	Arabidopsis	H^+	Energization	GUS	Yes—Yes	Fuglsang et al. (2007); Ueno et al. (2005)
Anion channel	SLAH3	AT5G24030	Arabidopsis	Cl^- (NO_3^-)	Anion efflux	GUS	Yes—Yes	Negi et al. (2008)
ABC transporters	ABCB14	AT1G28010	Arabidopsis	Ma^{2-}	Malate influx	GUS	Yes—Yes	Lee et al. (2008)
Ca^{2+}-ATPase	ACA8	AT5G57110	Arabidopsis	Ca^{2+}	Ca^{2+} efflux	GUS	Yes—Yes	George et al. (2008); Schiøtt et al. (2004)
	ACA10	AT4G29900	Arabidopsis	Ca^{2+}	Ca^{2+} efflux	GUS	Yes—Yes	Brown et al. (2012); Schiøtt et al. (2004)
	ACA13	AT3G22910	Arabidopsis	Ca^{2+}	Ca^{2+} efflux	GUS	Yes—Yes	Iwano et al. (2014); Yu et al. (2018)
Cyclic nucleotide-gated channel	CNGC6	AT2G23980	Arabidopsis	Ca^{2+}	Ca^{2+} influx	GUS	Yes—Likely	Brost et al. (2019); Gao et al. (2012)
	CNGC14	AT2G24610	Arabidopsis	Ca^{2+}	Ca^{2+} influx	GUS	Yes—Likely	Brost et al. (2019); Gao et al. (2012)

Abbreviation: RT-PCR, reverse transcription polymerase chain reaction.

transporter, CST1, appears to be the first glucose transporter specific for subsidiary cells (Wang, Yan, et al., 2019). Mutation of this gene in maize reduced stomatal opening, suggesting the importance of glucose transport in the surrounding cells for stomatal function. However, the qualitative differences in transporter gene expression between guard cells and their surrounding cells identified to date do not offer many clues to a reciprocity in ion shuttling.

It is likely that differences in how ion transport is regulated between the two cell types contributes to ion shuttling. Indeed, the effects of ABA and cytosolic Ca^{2+} ($[Ca^{2+}]_i$) on anion transport in the surrounding cells of maize have been reported to complement what that of guard cells (Mumm et al., 2011). Both ABA and elevated $[Ca^{2+}]_i$, which are known to activate anion channels in guard cells (Brandt et al., 2012; Chen et al., 2010; Demir et al., 2013; Garcia-Mata et al., 2003; Geiger et al., 2011; Grabov et al., 1997; Maierhofer et al., 2014; Scherzer et al., 2012; Thiel et al., 1992), reduced the anion conductance in subsidiary cell protoplasts (Mumm et al., 2011). How these changes arise remains unknown, however. SLAC1 and SLAH3 are activated in various cell types by Ca^{2+}-dependent kinases, including several CPKs and CIPK23 (Brandt et al., 2012; Demir et al., 2013; Dubiella et al., 2013; Geiger et al., 2010, 2011; Gutermuth et al., 2013; Maierhofer et al., 2014; Scherzer et al., 2012; Sun et al., 2021). By contrast, only two phototropin-related kinases, CBC1 and CBC2, are known to reduce the activity of SLAC-like anion currents in Arabidopsis (Hiyama et al., 2017), and these are not Ca^{2+}-dependent. So, it is likely that other Ca^{2+}-dependent inhibitors have yet to be identified.

Mumm et al. also observed an inverse behaviour in the response of the membrane voltage between surrounding and guard cells, with the surrounding cells depolarising and guard cells hyperpolarising on transition to light (Mumm et al., 2011). These changes may arise from differences in H^+-ATPase regulation with corresponding consequences for the voltage-sensitive K^+ channels. Depolarisation of maize surrounding cells might be expected to facilitate K^+ release via the ZORK channel, with hyperpolarization promoting K^+ uptake in the guard cells. The changes in membrane voltage were transient, whereas prolonged voltage changes would be required for net changes in ion contents in each case. Whether the voltage changes reflect differences in H^+-ATPase activity is similarly unclear; the observations of subsidiary cell acidification in the dark and alkalinization in the light are more readily understood if the pumps were activated by the light to promote H^+ efflux from the cells, much as in guard cells.

2.3 | Perspectives

The diversity in the structures of the cells surrounding the guard cells of the stomatal complex suggests that plants have adapted the function of these cells through evolution to optimise their stomatal function. Some of the relationships to stomatal function are known (Cowan, 1972; Mott et al., 1999; Rand et al., 1982) but others have yet to be explored in any detail. It is notable that an analysis of the stomatal kinetics in 15 different plant species (McAusland et al., 2016) focused on guard cells and carbon assimilation without reference to the contributions of the surrounding cells. The seven grass species used in this study divide between two groups based on their stomatal kinetics, a slower group that included oat (*Avena sativa*) and wheat (*Triticum aestivum*) coincides with dome-shaped surrounding cells, and a faster group that consists mostly of species with triangular-shaped surrounding cells. However, this comparison is, at best, rough and ready, and other factors may also need consideration. In short, a reassessment is needed, especially in relation to stomatal kinetics (Blatt et al., 2022; Jezek et al., 2019).

Equally, attention to ion transport in surrounding cells is also needed to provide greater depth to our understanding of their characteristics and regulation in coordination with the guard cells in the control of stomatal movements. Such understanding is certain to help nuance efforts towards engineering stomatal function (Blatt & Alvim, 2022; Horaruang et al., 2022; Nguyen, Lefoulon, et al., 2023; Papanatsiou et al., 2019). The availability of new research tools, for example, optogenetics (Cosentino et al., 2015; Papanatsiou et al., 2019; Reyer et al., 2020; Zhou et al., 2021) and mechanistic modelling platforms (Blatt et al., 2014; Hills et al., 2012; Jezek et al., 2019; Nguyen, Silva-Alvim, et al., 2023), clearly will aid in exploring the properties and functions of surrounding cells. The knowledge gained from these studies is certain to facilitate our knowledge of how plants accommodate the wide range of environments in which they survive.

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DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no data sets were generated or analysed during the current study.

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