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Trends in
Plant Science

Review

What can mechanistic models tell us about guard cells, photosynthesis, and water use efficiency?

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Stomatal pores facilitate gaseous exchange between the inner air spaces of the leaf and the atmosphere. The pores open to enable CO_2 entry for photosynthesis and close to reduce transpirational water loss. How stomata respond to the environment has long attracted interest in modeling as a tool to understand the consequences for the plant and for the ecosystem. Models that focus on stomatal conductance for gas exchange make intuitive sense, but such models need also to connect with the mechanics of the guard cells that regulate pore aperture if we are to understand the 'decisions made' by stomata, their impacts on the plant and on the global environment.

Small pores with a global impact

Stomata are pores that occur between pairs of guard cells and are found on the leaf epidermis as well as other surfaces of terrestrial plants. Stomata circumvent the impermeable cuticle barrier of the plant surface to enable CO_2 entry to the air space within the leaf for photosynthesis. With some notable exceptions [1], stomata open and close to control the exchange of CO_2 gas in response to photosynthetic demand [2–4]. Open stomata also provide a pathway for water loss by diffusion of water vapor from the saturated environment of the inner air space of the leaf to the atmosphere. As a consequence, the guard cells must frequently balance the need for CO_2 in photosynthesis against the need to prevent drying of the leaf tissues, especially when access to water is limiting. Stomata can limit photosynthetic rates by 50% or more when the demand for CO_2 exceeds the capacity for water supply to the leaf [3,5], thus highlighting the tight interdependence between these often conflicting factors that regulate stomatal behavior.

Not surprisingly, stomata connect the global water and carbon cycles of the world and exert a major influence on both. Foliar transpiration has played a significant role in atmospheric modeling and weather prediction for more than a quarter of a century [4,6–8], and stomatal transpiration is at the center of a crisis in water availability and crop production that is expected to unfold over the next 20–30 years. Globally, fresh water usage has increased sixfold in the past 100 years, twice as fast as the human population, and is expected to double again before 2030, driven mainly by agriculture [9]. Even in the United Kingdom, not generally considered a region of arid climate, irrigation has risen tenfold in the past 30 years, and this trend is expected to continue [10].

How stomata respond to the environment has long attracted interest in modeling stomatal behaviors within mathematical frameworks in order to understand the drivers and their consequences for the plant and the global ecosystem. These model frameworks generally encapsulate all of the activities of plants and their genomes within a single parameter defining the stomatal aperture and its

Highlights

Stomata are pores in the epidermis of plant leaves that facilitate gaseous exchange of water and atmospheric CO_2, thereby connecting the water and carbon cycles of the world and exerting a major influence on both.

Ecosystem models generally deal with stomata phenomenologically with respect to water availability, atmospheric humidity, CO_2, and light but without connection to the physiology of the guard cells that regulate stomatal aperture.

Guard cell physiology emerges as an important component missing from ecosystem models that will be essential to advance an understanding of the decisions 'made by' stomata in regulating the pore aperture.

Guard cell models that capture the mechanics of guard cell transport and metabolism connect environmental inputs with stomatal behavior to accurately predict whole-plant gas exchange and photosynthetic carbon assimilation.

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conductance for **gas exchange** (see Glossary). As stomata mark the interface between the plant and atmosphere, such descriptions make intuitive sense, but they omit much of stomatal physiology. Berry *et al*. [4] noted a decade ago that 'the decisions 'made by' stomata emerge as an important and inadequately understood component of these models. At the present time we lack effective ways to link advances in the biology of stomata to this decision-making process.'

Clearly, bridging the gap in understanding between our deep knowledge of the inner workings of stomata and the macroscopic phenomenology of gas exchange, photosynthesis, and water use requires an overarching approach that crosses scales and draws explicitly on the molecular mechanics of the guard cell. The past decade has seen a number of significant advances that address this challenge. These advances place the mechanics of stomatal function squarely within the mathematical framework of plant–atmospheric interactions; they are certain to inform approaches to environmental modeling, and they hold considerable promise as a tool supporting future efforts toward bioengineering for enhanced vegetative and crop resilience. Here, we review the most outstanding of these advances, the experimental evidence that supports them, as well as the opportunities that they herald for the coming decade.

Modeling gas exchange

In general, effective models are those that yield experimentally testable predictions [11]. Models built on systems of elements commonly rely on the interactions between the elements to yield information about the behavior of the system as a whole. Information derived from these interactions often takes the form of emergent behaviors, characteristics that could not be anticipated from knowledge of the elements in isolation, and this information is then available for study through experiment. Model and experiment thus become complementary tools in research [12,13], each model representing a hypothesis to be discarded, validated, or refined by comparing model prediction with experimental outcome.

Macroscopic approaches to modeling of gas exchange generally treat stomata as discrete, phenomenological elements, each stoma serving as a pathway for transpirational water loss and CO_2 uptake. A very large body of information correlates stomatal aperture, expressed as stomatal conductance $\mathbf{g_s}$ of the whole leaf, with environmental inputs of temperature, atmospheric relative humidity (**RH**), light, hydraulic conductances of the stem and leaf, and the consequences for photosynthetic carbon assimilation [3,14–17]. With this information in hand, it has been possible to develop holistic approaches, although teleological in their formulation, that use mathematical descriptors to simulate gas exchange at the canopy to the ecosystems level.

The preeminent concept behind much of these modeling efforts stems from the seminal hypothesis of Cowan and Farquhar [18] that stomatal aperture should be regulated to maintain a constant water-use efficiency (**WUE**), at least over shorter periods of time. In other words, the 'water cost' of carbon associated with a change in aperture

$$\lambda = (\partial A/\partial g_s)/(\partial E/\partial g_s) \quad [1]$$

should remain constant. In effect, this hypothesis proposes that the benefit of increasing the rate of photosynthesis, $\boldsymbol{A}$, with a small increase in stomatal conductance, ∂g_s, should be offset by a proportional cost of increased water loss via transpiration, $\boldsymbol{E}$. To constrain this relationship, Cowan and Farquhar assumed that plants will maximize the gain in carbon subject to a limited amount of water. In other words, the WUE concept proposes that the plant will optimize λ, although there is no straightforward way of predicting the value of λ or how it might vary, for example with water availability.

Glossary

$\boldsymbol{A}$: assimilation rate of CO_2 as carbon fixed by photosynthesis.
Ion channel: membrane protein that facilitates the flux of selected ions across a membrane.
$[Ca^{2+}]_i$: cytosolic-free Ca^{2+} concentration.
Charge conservation: physical law that opposite charges must sum to zero.
Computational cycle: a single round of mathematical calculations, typically of a single increment in time for a model assembled of ordinary differential equations.
$\Delta\Psi$: membrane voltage (difference).
$\boldsymbol{E}$: transpiration rate of water as vapor lost as evaporation from the leaf through stomata.
hv: energy of light.
Gas exchange: exchange of water vapor and of CO_2 between the atmosphere and the inner air space of the leaf.
g_s: conductance of the leaf (or ensemble of stomata) to gaseous diffusion [$= E/(100 - \%RH_{out})$].
H^+-ATPase: membrane protein that transports H^+ across a membrane using the energy of ATP hydrolysis.
HCO_3^-: bicarbonate anion, in equilibrium with carbonic acid.
Membrane voltage: electrical potential difference across a membrane.
Model parameter: numerical descriptor that defines a non-variant characteristic of an element within a model.
Model variable: numerical output that is acted on by one or more model elements.
pCO_2: partial pressure of CO_2 in the atmosphere.
pC_i: partial pressure of CO_2 in the inner air space of the leaf.
π_e: epidermal osmotic potential.
π_g: guard cell osmotic potential.
ψ_L: soil water potential.
RH: relative humidity.
RWF: relative water feed subsumes water transport from the root to the leaf and, in OnGuard, is the ratio of the evaporative surface area within the leaf divided by the stomatal pore area.
WUE: water use efficiency, commonly defined as the amount (or rate) of CO_2 fixed by photosynthesis for each unit of water lost from the leaf via transpiration ($= A/E$).
Voltage clamp: electronic circuit used to measure current while controlling voltage across a membrane.

Our understanding of why stomata behave the way they do has been dominated by the WUE concept and its ability to explain much empirical data across species and environments [19–25]. The success of WUE optimization models rests on the simple elegance of the concept, its ability to supersede other restrictive assumptions, and its capacity to recapitulate empirical models (Table 1) that describe leaf and canopy g_s with photosynthesis [22,26–28]. The WUE concept has been extended to include soil moisture and its seasonal variations [19,29–31] as well as additional costs, for example of competition between plants and of xylem hydraulic failure [32].

In general, the key to each of these model variants rests in their encapsulating g_s within a number of phenomenological descriptors limited by common biophysical constraints [4,27,33]. These descriptors set the theoretical maxima for g_s, photosynthesis, and the availability of water. They also include teleological weightings for the cost penalty of water loss [29,30], the fitness–benefit relationship for carbon fixation, and allied costs such as those associated with competition for water [32]. Optimization and the complementary empirical models based around WUE excel in their accounting for g_s and the relationships between the sets of descriptors used to parameterize stomatal gas exchange, but they are disconnected from the biology of the guard cells. In short, they fail to explain 'the decisions 'made by' stomata' [4] and how they work in any mechanistic terms.

How stomata work

Changes in the size and shape of the stomatal pore between the guard cells arise from osmotically driven water fluxes that commonly accompany ion transport and the metabolism of organic solutes by the guard cells [2,3,34]. At maturity, the plasmodesmata that occur between the guard cells and their neighboring epidermal cells are sealed [35–37]. Thus, the guard cells are isolated from their neighbors, and all water and solute flux must take place across the guard cell plasma membrane. Even when removed from the leaf in epidermal peels, guard cells respond in a well-defined manner to an array of extracellular signals, notably light, CO_2, extracellular solutes, and hormones, including abscisic acid (ABA) and auxin, to regulate the stomatal aperture [2,38,39]. These observations demonstrate that the guard cells are competent to function independent of the other leaf tissues.

Guard cells coordinate transport at the plasma membrane with transport across the tonoplast through a complex network of controls to regulate the fluxes of the major osmotic solutes, principally K^+, Cl^-, and malate (Mal), as well as water, thereby driving guard cell turgor and stomatal aperture. Our deep knowledge of these processes, their kinetics and regulation, has made the guard cell the single, best-known cell model for studies of ion transport, homeostasis, and signaling [2]. A central feature of guard cell transport is its coordination both within and between membranes. This coordination is essential because the vacuole comprises some 80–90% of the volume of the guard cell; hence, the major fraction of solute and water accumulated during stomatal opening and lost during stomatal closing must cross both membranes [2,14].

Coordination between the plasma membrane and tonoplast arises principally because transport activities at both membranes share a common pool of solutes within the cytosol and, thus, transport of these solutes and of signaling molecules such as Ca^{2+} are affected by, and impact on, the activities at both membranes [40]. Coordination of ion transport within each membrane is tied to the driving force of the **membrane voltage**, which is shared between the overwhelming majority of transporters at each membrane and influences electrogenic transport directly and electroneutral transport indirectly. Entanglements at both levels of coordination are open to evolutionary pressures that determine the functional efficiency of stomata [41–43].

Table 1. Models of stomatal conductance and assimilation[a,b]

Approach model	Criteria/conditions	Spatiotemporal scales	Parameterization or optimization
WUE optimization			
Cowan–Farquhar [18]	$(\partial A/\partial g_s)/(\partial E/\partial g_s)$	canopy, diurnal	λ
Manzoni [29]	maximum $f(A, g_s)$ soil water deficit	canopy, diurnal to dry-down	$f(\lambda, \boldsymbol{\psi_L})$
Wolf [32]	maximum $f(A, \psi_L)$, soil water competition	canopy, instantaneous	$f(\psi_L)$
WUE empirical			
Ball–Berry–Leuning [26,28]	$f(A, RH)$	canopy, diurnal	f(**hv**, RH_o)
Biological proxy			
Buckley [61]	$f(\pi_g, \boldsymbol{\pi_e}, [ATP])$	canopy, diurnal	$f(A, [ATP], hv)$
Deans [62]	$f(\pi_g, [ABA])$	canopy, diurnal to dry-down	$f(A, [ABA])$
Mechanistic physiological			
Blatt–Hills–Lew [46,57,65,70,71]	$f([X], \boldsymbol{\Delta\Psi}, \Phi_X, A, \Sigma I=0, ...)$	molecule to canopy, instantaneous to diurnal	$f(I_x, [X], \Phi_m, hv, E, A, RH)$

[a]Modeling approaches to simulating stomatal conductance and its association with photosynthetic carbon assimilation. This list illustrates the breadth of modeling approaches rather than providing an exhaustive list of models. More complex relations are indicated as functions $f(n_1, n_2, ...)$ of parameter(s) $n_1, n_2, ...$ for simplicity.
[b]Abbreviations: A, assimilation rate; ABA, abscisic acid; $\Delta\Psi$, membrane voltage (difference); E, transpiration rate; g_s, stomatal conductance; hv, energy of light; I, current; I_x, current of ion X; λ, water cost of carbon gain; π_e, epidermal osmotic potential; π_g, guard cell osmotic potential; ψ_L, soil water potential; RH, relative humidity; Φ_m and Φ_X, metabolic and solute fluxes between compartments, respectively; [X], contents of solute X; WUE, water-use efficiency.

The importance of membrane voltage, especially, lies in its feedback on transport at the membrane. Voltage introduces an extraordinary degree of entanglement between the activities of every charge-translocating pump, channel, and carrier because physical laws dictate that the sum of all charge transport across the membrane must always be zero in the steady state [2,44,45]. Charge (ion) flux through any one transporter will affect, and will be affected by, every other transporter that moves a net charge across the membrane. As a consequence, the flux of any one ion, say of K^+ through the GORK K^+ channel, will be determined not only by the intrinsic gating characteristics of the channel but also by the sum of charge flux through every other transporter active at the membrane.

How many different transporters, whether ion pumps, carriers, or **ion channels**, are essential for guard cells to work? If we consider only the various flux characteristics, their biophysical and regulatory properties essential for stomatal movements, then a minimum count across the transported species comes to 11 different transporters at the plasma membrane and 12 at the tonoplast (Table 2), including the aquaporins at each membrane [2,46]. Obviously, the total number of transporter gene products that are important for osmotic solute flux is roughly two- to threefold higher, as the count in Table 2 subsumes redundancies in function, for example of the plasma membrane Ca^{2+}-ATPases ACA8 and ACA10 [47] and characteristics arising from heteromeric assemblies such as of the inward-rectifying K^+ channels KAT1, KAT2, and KC1 [48,49]. From a functional perspective, it is how these transport processes operate that defines how the guard cell works [2]. The identities of the individual gene products are of secondary relevance and take on importance only later, notably as foci for molecular engineering directed to manipulating the system as a whole.

For any meaningful assessment of stomatal function, we need also to add the contribution of metabolism to the osmotic relations of the guard cells, especially that of starch, sugars, and Mal [50–52]. Equally important to guard cell physiology are the essential buffering reactions, specifically of H^+ and Ca^{2+} that play physiological roles as transport substrates as well as signaling molecules in guard cells [53–56]. The predominant starch–sugar–Mal relations can be subsumed within a minimum of reactions [11,34,57]. Nonetheless, even with these simplifications the

Table 2. The minimal set of solute transporters mediating solute transport in the guard cell[a]

Transporter[b]	Representative gene product[c]	Transported ions[d]	Function[e]
Plasma membrane			
H^+-ATPase	AHA1, AHA2	H^+	Energization
Ca^{2+}-ATPase	ACA8, ACA10	Ca^{2+}, H^+	Ca^{2+} efflux
H^+-K^+ symport	HAK5	K^+, H^+	K^+ influx, high affinity
H^+-anion symport	NPF7	$Cl^-(NO_3^-)$, H^+	Cl^- influx, high affinity
Ca^{2+} channel (in)[c]	(Unknown)	Ca^{2+}	Ca^{2+} influx
K^+ channel (in)	KAT1, KAT2	K^+	K^+ influx
K^+ channel (out)	GORK	K^+	K^+ efflux
K^+ channel	(Unknown)	K^+	K^+ flux, charge balance
$Cl^-(NO_3^-)$ channel	SLAC1, SLAH3	$Cl^-(NO_3^-)$	Anion flux, charge balance
Cl^-(Mal) channel (out)	ALMT12	Cl^-, Mal	Anion efflux, oscillations
Aquaporin	PIP2;1		Water flux
Tonoplast			
H^+-ATPase	VHA1	H^+	Energization
H^+-PPase	PVA1	H^+	Energization
Ca^{2+}-ATPase	ACA4, ACA11	Ca^{2+}, H^+	Ca^{2+} sequestration
$Cl^-(NO_3^-)$ channel	ALMT9	$Cl^-(NO_3^-)$	Anion influx
Mal channel	ALMT6	Mal	Mal influx
H^+/Ca^{2+} antiport	CAX2, CAX3	Ca^{2+}, H^+	Ca^{2+} sequestration
H^+/Cl^- antiport	CLC1	$Cl^-(NO_3^-)$, H^+	$Cl^-(NO_3^-)$ sequestration
Ca^{2+} channel (in)[c]	(Unknown)	Ca^{2+}	Ca^{2+} release
K^+ channel (in)	TPK1	K^+	K^+ influx
K^+ channel	FV	K^+	K^+ leak
K^+/Ca^{2+} channel (out)	TPC1	K^+, Ca^{2+}	(Unknown)
Aquaporin	TIP1		Water flux

[a]Listed are those transporters essential for osmotically active solute and H^+ and Ca^{2+} transport. A complete description of each class of transporters can be found in Chen et al. [65] and Jezek and Blatt [2].
[b]Transporter groups by functional characteristics. Channel rectification and charge flux direction are indicated in parentheses.
[c]Representative arabidopsis gene products, where known.
[d]Channels identified physiologically and yet to be associated with a gene product.
[e]Ion (chemical) flux direction relative to the cytosol. Note that the functional significance of the voltage-dependent cation channel TPC1 remains an enigma [2,104,105].

complexity of a network comprising 23 transporters, a further 10 interconversion steps describing the metabolism of starch, sugar, and Mal, and the cellular buffering relations for Mal, H^+, and Ca^{2+} presents a major barrier to a quantitative understanding of guard cell physiology and of stomata.

Most important, guard cell physiology is dictated by the kinetic properties intrinsic to each of these processes, whether of ion transport or the metabolism of organic solutes. In the majority of cases, these kinetics are highly nonlinear, often with respect to multiple variables, including substrate concentrations and, for membrane transport, also voltage, as well as regulatory inputs that engage ligand and other post-translational controls. Combined with the entanglements intrinsic to ion transport noted earlier, these nonlinearities give rise to seemingly counterintuitive and emergent behaviors [2,40,58]. Thus, even without considering transcriptional and translational regulation, addressing how guard cells respond to environmental inputs and their coupling to the

macroscopic properties of the whole leaf is beyond intuitive grasp. It demands a full and quantitative accounting of the characteristics for the component transport, metabolic, and buffering reactions.

Modeling stomata

Over the past two decades, a few efforts have sought to encode aspects of guard cell biology and their responses to environmental stimuli within parameters defining the models, thereby connecting these physiological inputs to the output of g_s [59,60]. In general, such models have used simplified phenomenologies to develop biological proxies for the inputs with heuristic factors for scaling, and they have assumed uniform stomatal characteristics across the leaf surface (biological proxy models, Table 1). One prominent example, the hydromechanical model [61], proposed a hydroactive component to the stoma that is proportional to ATP concentration, assigning this component a pseudo-Michaelis–Menten relationship dependent on light and connecting it to the osmotic potential of the guard cell, $\boldsymbol{\pi_g}$. As a proxy for guard cell transport and metabolism, this hydroactive input was highly successful in connecting steady-state g_s with the light input, and it resolved a seemingly nonsensical negative gain in hydropassive feedback between the guard cells and surrounding epidermal cells.

A similar application of a pseudo-Michaelis–Menten relationship with ABA concentration was used to approximate the response of a gymnosperm to drought stress in the hydraulic–hormonal model [62]. This model yielded a range of g_s values that correlated with the ABA content of the tissue and, with a judicious choice of empirical relaxation constants, could also approximate the temporal transitions between states. Both the hydromechanical and hydraulic–hormonal models yield g_s values that are largely realistic, albeit principally as steady-state outputs derived from empirical correlations between g_s and the proxy, whether of ATP or ABA. What these models lack is any detail of the mechanics of guard cell solute transport, buffering, and metabolism and, hence, any insight into the dynamics of the guard cells that determines how they work [2].

The OnGuard platform is the only modeling effort to date that incorporates explicitly the mechanics of solute transport, buffering, and metabolism, and draws on the wealth of molecular and physiological knowledge available for guard cells (Table 2 and [2]). The OnGuard approach follows on similar strategies applied successfully to mammalian epithelia and red blood cells [63,64]. In the original formulation [57,65], models built on the OnGuard platform accurately simulated stomatal dynamics, its sensitivity to extracellular ions and light, and they uncovered an emergent network connecting the activity of the KAT1 K^+ channel with the SLAC1 anion channel [66]. OnGuard2 introduced foliar transpiration of the whole plant, connecting hydraulic delivery and transpiration with water and solute transport in the guard cells [46]. It utilized the idea of evaporative equilibration, introduced by Peak and Mott [67], applying the concept to the water potential in the guard cell wall, thereby harmonizing the OnGuard platform with models of liquid water delivery to the guard cells [61,68,69]. A subsequent extension incorporated apoplastic water and solute flux, and turgor 'exchange' with the surrounding epidermal cells, to accommodate the opposing mechanical pressure imposed by these cells on stomatal aperture as well as the intrinsic visco-elastic properties of the guard cell wall [70]. Finally, with the latest release, OnGuard3, CO_2 exchange between the atmosphere and the leaf was incorporated to address the feedback of CO_2 and mesophyll photosynthesis on stomatal aperture [71]. Adding CO_2 exchange has yielded new predictions, several since validated, including an unforeseen latency or 'carbon memory' in g_s kinetics with fluctuating light and $\boldsymbol{pCO_2}$ that depends on endomembrane Ca^{2+} stores in the guard cell [71].

Like biological proxy models, the OnGuard platform assumes a uniform, or averaged, behavior across the population of stomata of the leaf. However, unique to OnGuard, models built on this

platform capture the biophysical and kinetic features of metabolism and the transporters responsible for solute flux[†].

The central outputs of the models are osmotic solute content, guard cell turgor, volume, and stomatal aperture (Figure 1). These characteristics lead to descriptions of g_s as functions of light, pCO_2, and RH as well as water and solute availability. However, OnGuard outputs extend from carbon assimilation in the whole plant to the subcellular kinetics of solute flux and the molecular activities of guard cell transport, and all model components and their associated variables are available for analysis. The platform therefore yields a wealth of insights that connect the whole-plant characteristics with the underlying cellular, subcellular, and molecular behaviors. The user has access, for example, to the partial pressures of water vapor (w_p) and CO_2 (**pC_i**) within the leaf air space, the cellular compartmental concentrations of all of the major solutes, cytosolic-free Ca^{2+} concentration (**$[Ca^{2+}]_i$**) and pH, the activities of each of the transporters, and the fluxes through them, all connected to the incident light, atmospheric partial pressures of water vapor (w_{air}) and CO_2 (pCO_2), and the rate of mesophyll carbon assimilation.

Model outputs generated with OnGuard take the form of real-time kinetics, much as would be recorded in experiments, whether of transpiration and carbon assimilation rates, cellular $[Ca^{2+}]_i$, Mal content, or individual transport currents and their current–voltage curves recorded under **voltage clamp**. The OnGuard platform uses an iterative computational cycle with small increments in time to calculate the progress of solute and water flux in and out of the guard cell. This approach does not delimit an endpoint or the dynamic range of outputs. Instead, every **model variable** 'evolves' over time based on the physical laws of **charge conservation**, the equations and their parameters that define the transport and metabolic reactions of the model. Most important, this 'evolution' arises from interactions that depend on the shared variables of membrane voltage across each membrane and the common pools of substrates and products, just as they do *in vivo*.

The decisions 'made by' stomata

Interpreting the output of any modeling effort, regardless of scale, requires the modeler to interrogate the variables generated in simulation and their connections to the model elements. For the modeler to understand how a system responds to physiological or experimental perturbation, it is essential to determine how one model variable is connected to another. So, in general, the task of interpretation reduces to one of tracing and ranking the sequence of events through the network of model elements, connections, and the associated variables, for example following a trigger or a change in one **model parameter**.

We include here model outputs and experimental data from arabidopsis with a step-down in pCO_2 from 400 to 250 μbar and an overlapping dark transition to illustrate the 'choices' made between the closing stimulus of the dark and the opening stimulus of low CO_2 (Figure 2). The

[†] Models that simulate the characteristics of *Vicia*, tobacco, and *Arabidopsis thaliana* (arabidopsis) and of several arabidopsis mutants [46,66,71] are available with the OnGuard platform (www.psrg.org.uk; for full functionality, users must register and install a passcode). These models offer good starting points for users new to the platform. We welcome communication, especially in developing models for use with other species and to better understand the parameters most important to defining stomatal behaviors across species. Descriptions of the transporters, metabolic and buffering reactions, and their parameter sets for *Vicia*, tobacco, and arabidopsis are included with the publications noted earlier. The OnGuard platform assumes a good working knowledge of membrane transport and how to interpret current–voltage relations. Thus, the core parameter sets do not predefine some inputs but leave these for the user to explore and define. For example, OnGuard3 does not predetermine the actions of ABA or auxin, although the dominant targets of the hormones are well known [2,3,14,82,102,103]. These actions will be 'hard-wired' in a version of OnGuard3, to be launched in the near future, that subsumes much of the detail for transport within the inner workings of the platform. Its streamlined user interface will make the OnGuard platform widely accessible to users beyond the sphere of membrane transport physiology.

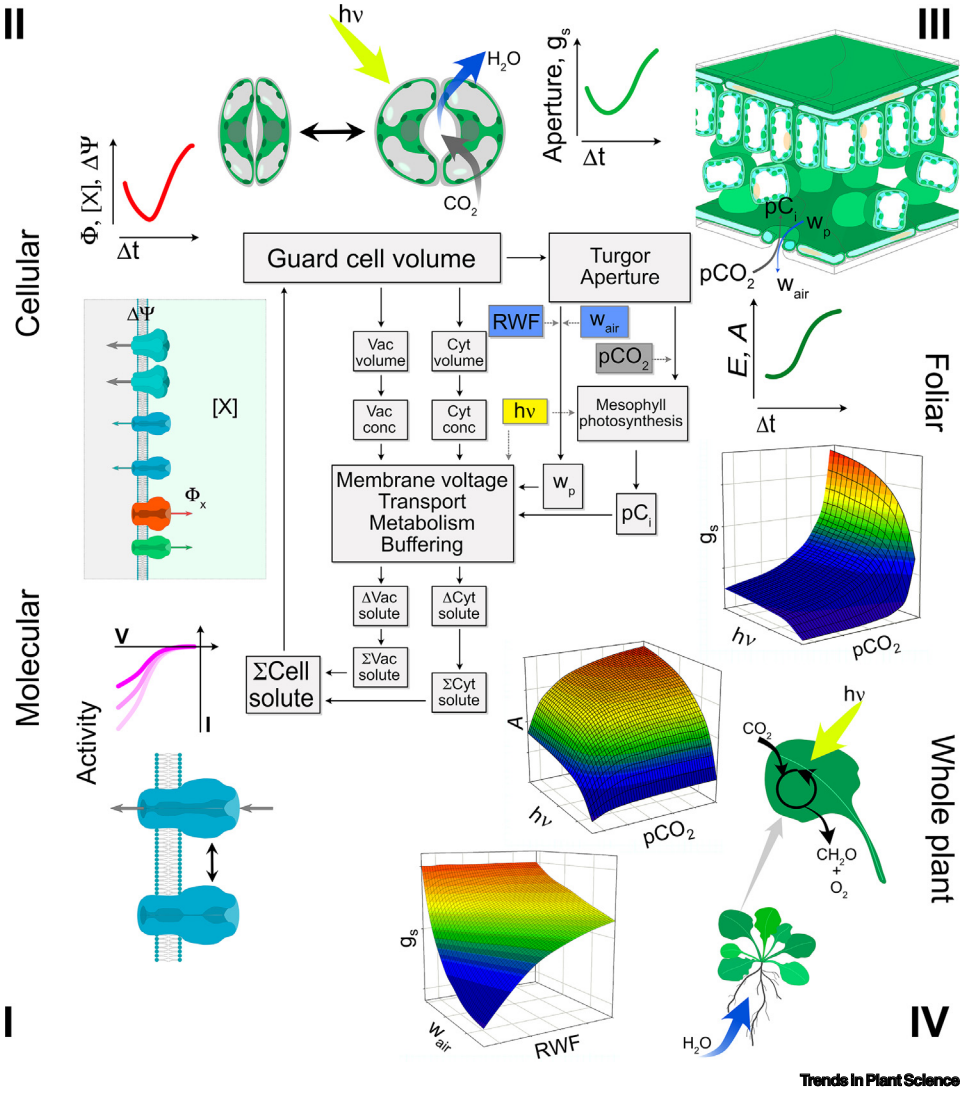

Figure 1. OnGuard3 connects the molecular events of solute flux in the guard cell to gas exchange and photosynthesis in the whole plant. **Computational cycle** (center) of the OnGuard platform surrounded by the range of model outputs available to the user. The computational cycle operates in the clockwise direction over small time increments (Δt) as indicated by the black arrows. Environmental inputs of relative water feed (**RWF**) representing water flux to the leaf, the atmospheric partial pressures of water vapor (w_{air}) and CO_2 (pCO_2) and light (**hν**) are indicated by the colored boxes and gray arrows. Model outputs evolve with time and divide between the molecular, cellular, foliar, and whole plant. Molecular and cellular outputs, quadrants I and II, comprise the activities of transport and metabolic reactions, here illustrated with current–voltage curves, the solute contents of the cytosol and vacuole ([X]), the metabolic and solute fluxes between compartments (Φ_X), and the voltages ($\Delta\Psi$) across the plasma membrane and tonoplast. These outputs determine the immediate derived variables of guard cell osmotic potential, volume and turgor, and the stomatal aperture. Foliar outputs and the steady-state surface plots of quadrants III and IV derive from the cellular outputs, the environmental inputs of RWF, light, w_{air}, and pCO_2, and the intermediates of the partial pressures of leaf water vapor (w_p) and CO_2 (pC_i) behind the stomatal pore. Water-use efficiency (WUE) optimization and empirical models address quadrant IV (Foliar and Whole Plant) characteristics. Biological proxy models extend these outputs to quadrant III to include the osmotic potential of the guard cell and stomatal aperture, albeit without mechanistic connection to the Molecular and Cellular processes. OnGuard3 makes mechanistic connections across all four quadrants and provides the user with access to all outputs. Surface plots shown in quadrant IV are modified from Wang *et al*. and Jezek *et al*. [46,71]. Abbreviations: *A*, assimilation rate; conc, concentration; Cyt, cytosol; *E*, transpiration rate; g_s, stomatal conductance; I, current; V, voltage; Vac, vacuole.

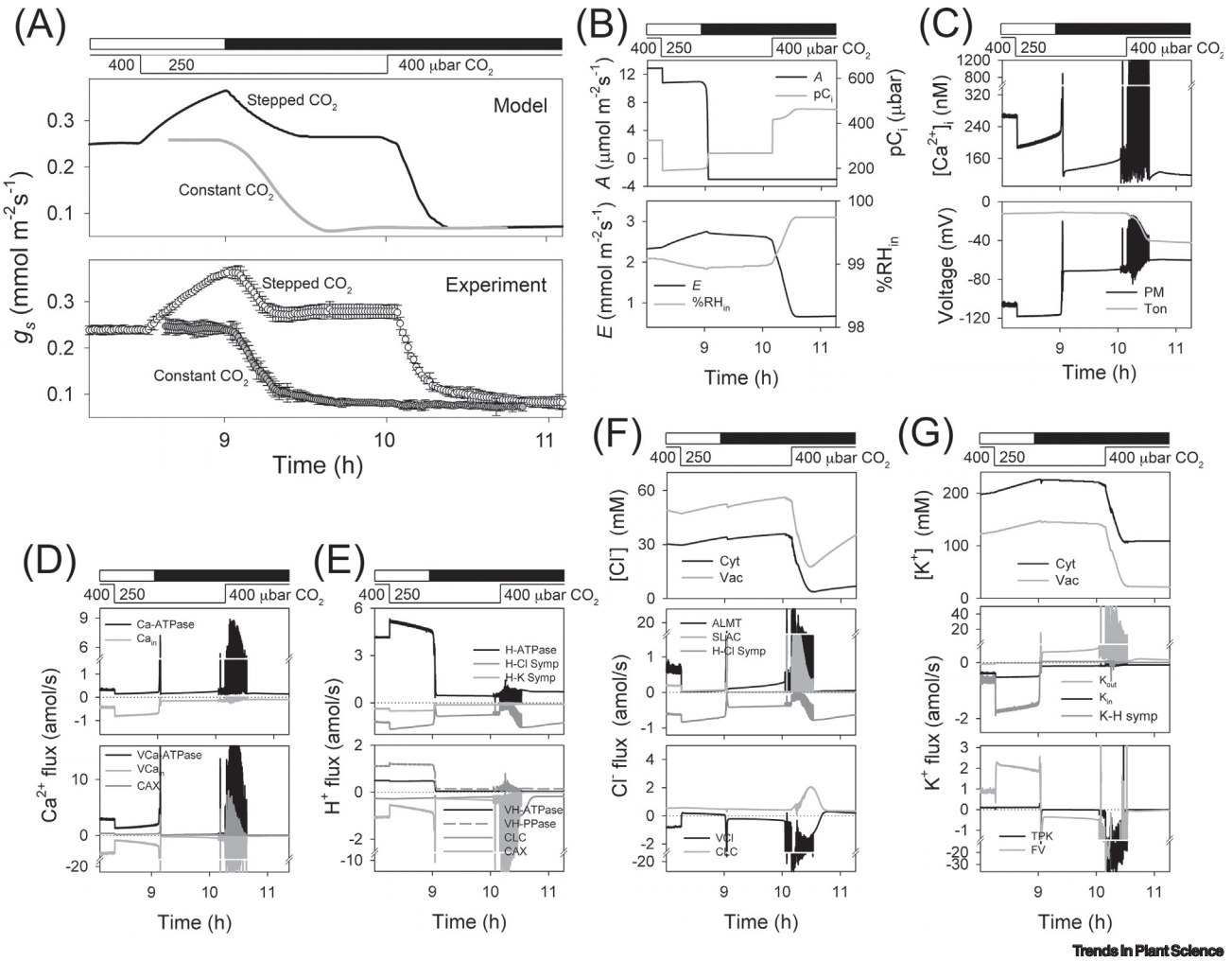

Figure 2. Reducing atmospheric CO_2 'locks' stomata open on transition to the dark, both in simulation and experiment. OnGuard3 offers a mechanistic explanation for the apparent 'choice' of stomata to remain open in the dark. With stomata open during the daytime to give a substantial stomatal conductance, g_s, simulation and experiment engage a step from 400 to 250 μbar CO_2 followed 45 min later by transition to the dark. Atmospheric CO_2 is returned to 400 μbar 1 h later. Model outputs and gas exchange data for g_s (A) include results for the dark transition without the CO_2 step (gray lines and filled symbols). A selection of OnGuard3 outputs for guard cells with the CO_2 step (B–G) show the decline and recovery of CO_2 (pC_i) and relative humidity ($\%RH_{in}$) within the leaf along with assimilation, *A*, transpiration, *E* (B), as well as changes in membrane voltages, cytosolic-free $[Ca^{2+}]$ ($[Ca^{2+}]_i$), and Ca^{2+} fluxes (C,D), H^+ fluxes (E), and the cellular contents and fluxes for Cl^- and K^+ (F,G). Fluxes here are of the ions, not the charge, relative to the cytosol; transporter fluxes (D–G) are shown in panels divided between plasma membrane (top) and tonoplast (bottom). Positive flux is defined as ion movement out of the cytosol, either into the apoplast or the vacuole. Oscillations on returning to 400 μbar CO_2 are a consequence of the corresponding oscillations in voltage and $[Ca^{2+}]_i$ that accelerate stomatal closing [44,65]. The simulation assigns pC_i, as a ligand, to regulate the endomembrane Ca^{2+}-ATPase (VCa-ATPase) and Ca^{2+} channel (VCa_{in}) [71], the reduction in pC_i thus promoting the former and inhibiting the latter to reduce $[Ca^{2+}]_i$ (C). The smaller reduction in VCa-ATPase flux (D) arises with the decline in Ca^{2+} substrate (C). All of the other flux changes are a direct consequence of the reduced $[Ca^{2+}]_i$ or its action in hyperpolarizing the plasma membrane (C) by promoting the H^+-ATPase (H-ATPase) (E) and suppressing the anion channels (SLAC, ALMT) (F). The net effect is to 'lock' the cytosolic and vacuolar contents of K^+ and Cl^- (F,G) until the return to 400 μbar CO_2.

simulation shown uses OnGuard3, as this is the only platform to handle, in native mechanistic terms, the full range of environmental inputs and generate the relevant outputs for direct comparison with experimental data. Indeed, the experimental behavior of g_s evident in these circumstances simply cannot be accommodated by WUE optimization models, nor by WUE empirical models [18,26] without the addition of an arbitrary offset [28].

The simulation with OnGuard3 shows that g_s does not decline substantially until pCO_2 returns to 400 µbar in the dark, just as in the experiment (compare also [72–74]). Interrogating the model outputs in this case shows that the decline in pCO_2 is accompanied by a hyperpolarization of plasma membrane voltage. Counterintuitively, the simulation shows a decrease in $[Ca^{2+}]_i$ and a reduced anion influx from the vacuole to the cytosol and efflux across the plasma membrane, despite the increase in the electrical driving force for Ca^{2+} entry and Cl^- efflux across the plasma membrane. In effect, then, the consequence is to 'lock' the osmotic solute content of the guard cell, preventing its efflux and stomatal closure until pCO_2 returns to the ambient level in the dark.

How are the connections made between atmospheric pCO_2 and guard cell transport? And how do these connections feedback to g_s in the whole leaf? Within OnGuard3 it has proven sufficient for CO_2 (or the bicarbonate anion **HCO_3^-** and H_2CO_3 with which CO_2 equilibrates in solution) to regulate Ca^{2+} flux through the endomembrane ACA Ca^{2+}-ATPase and Ca^{2+} channels [71]. This prediction accords with much evidence that associates an increase in $[Ca^{2+}]_i$ with elevated pCO_2 [75,76] and more generally with alterations in transport leading to stomatal closure [2]. The same prediction underpins the emergence of a 'carbon memory', subsequently validated in experiments, that affects stomatal speed and responsiveness to fluctuations in light and pC_i [71].

In the simulated output, the step-down in pCO_2 (Figure 2) reduces pC_i within the leaf, which equilibrates with soluble CO_2 and, hence, with H_2CO_3 and HCO_3^- in the guard cell. OnGuard3 predicts a substantial decline in Ca^{2+} release via the endomembrane Ca^{2+} channels (VCa_{in}) that outweighs the small reduction in transport via the Ca^{2+}-ATPase, thus promoting Ca^{2+} sequestration and reducing $[Ca^{2+}]_i$. In turn, the decline in $[Ca^{2+}]_i$ acts on all fluxes mediated by $[Ca^{2+}]_i$-sensitive transporters (some 70% of the transport activities at the two membranes [2,77]), notably enhancing the plasma membrane **H^+-ATPase** and K^+ uptake and eliminating the activities of the SLAC and ALMT anion channels facilitating Cl^- and Mal loss from the guard cell. Even on transfer to the dark, OnGuard3 predicts that reducing pCO_2 suppresses endomembrane Ca^{2+} release and, hence, prevents guard cell transport from entering the oscillatory cycles of $[Ca^{2+}]_i$ elevation, membrane depolarization, and K^+ and anion efflux that rapidly close the stoma [2,44]. Other changes in endomembrane solute flux follow a similar pattern that favors solute retention in the vacuole rather than its release. The consequence is that reducing pCO_2 maintains stomatal opening and a high g_s, even in the absence of the photosynthetic sink, until pCO_2 returns to 400 µbar and the partial pressure of CO_2 within the leaf air space rises once again.

Significantly, a number of the predictions set out in Figure 2 are amenable to experimental testing. The experimental data, and the simulation that explains these observations, indicate that the pC_i signal and its connection to guard cell Ca^{2+} transport are central to defining the overall response of the guard cells associated with the photosynthetic activity of the mesophyll. Furthermore, they show that the connection to endomembrane Ca^{2+} transport is sufficient to predict and explain stomatal physiology without adding parameters, for example to define secondary sensitivities of the H^+-ATPase [78] or of the K^+ channels to blue light [79], although adding these sensitivities may further enhance the overall response. Most important, these connections highlight the feedback between the macroscopic processes of gas exchange and the microscopic regulatory network that operates in the guard cells.

It is worth noting that both the modeling and mutant analysis [71] prove wrong the common misconception that the SLAC1 anion channel and its purported binding with HCO_3^- comprise a 'master switch' that alone is sufficient to close stomata [76,80–82]. Not least, the elevated HCO_3^- concentrations of 10–15 mM that have been reported as necessary to enhance SLAC1

activity on heterologous expression [76,80] are equivalent to 20000 µbar CO_2 or more. These concentrations bear no relation to the physiological range of pC_i, typically between 50 and 500 µbar CO_2, that regulates stomatal conductance with photosynthesis [3,26,74,83–85]. Furthermore, there is a wealth of evidence to show that stomatal movements require the highly coordinated actions of multiple transporters at both the plasma membrane and tonoplast [2]. Certainly, SLAC1 plays a part in solute efflux during stomatal closure, but its regulation alone is not sufficient to drive closure, and the evidence to date for its direct regulation by HCO_3^- is not convincing.

What, then, are 'decisions 'made by' the stomata'? And how are they made? Simulation and supporting experiments [44,46,57,65,66,71,77], including those of Figure 2, offer a truly mechanistic and overarching answer to these questions: stomatal behavior arises from the molecular characteristics of guard cell transport, buffering and metabolism, the variables that they share, and how these processes integrate those of foliar transpiration, CO_2 exchange, and photosynthesis. These mechanisms engender interactions that are deeply encoded within the network of connections determining guard cell physiology. They give rise to a wealth of emergent behaviors that we perceive as the 'decisions made' by stomata. More useful, then, is for us to recognize that the connections determining stomatal movements simply reflect a spectrum of behaviors that emerge from the ionic fluxes that play across the guard cell membranes, their entanglement and balance with the metabolic activities of the guard cells. The challenge for research is to explore the breadth of this entanglement in order to understand its consequences.

Concluding remarks and outlook

Until now, stomatal research and efforts to model stomata and their behavior have advanced along two radically different pathways that divide across scales. On one side, the physiology of the guard cell is exceptionally well described and has been modeled successfully with quantitative kinetic detail at the cellular level. These models show that stomatal behaviors can be encapsulated within a well-defined mechanistic framework, at least over the timescales pertinent to most physiological responses, but the models have been disconnected from photosynthetic carbon assimilation and related environmental inputs. On the other side, foliar gas exchange has been defined phenomenologically with respect to water availability, atmospheric humidity, CO_2, light, and photosynthesis, including teleological weightings for ancillary costs but without connection to the physiology of the guard cell.

Bridging the gap between these two scales offers a new and unprecedented set of tools with which to explore the mechanics of stomatal gas exchange within an overarching framework that operates seamlessly from the molecule to the whole plant. These same tools are sure to present many other opportunities, for example in facilitating 'reverse engineering' of stomatal traits, assimilation, and WUE, and in designing strategies based on modeling *in silico* to develop 'templates' in trials for crop enhancement. With advances toward modularizing many biochemical and physiological functions in synthetic biology [86–88], such opportunities are certain to become a central focus of much research in the near future.

Equally, mechanistic approaches such as the OnGuard platform should help to refocus large-scale modeling efforts that are relevant to understanding plant interactions with the environment. Berry *et al.* [4] noted that the conclusions drawn from global-scale modeling projects 'depend on getting our plant physiology right'. Much in these global projects rests on naive assumptions about stomata [89] and has led to some of the more controversial extrapolations in the literature [90]. Simply put, until now the absence of a detailed mechanistic platform for stomata has prevented efforts to introduce the most important developments from stomatal research in forward-looking studies of the carbon and water cycles of the planet [4,41].

Outstanding questions

To what extent are stochastic or 'patchy' behaviors among stomata important in defining the overall gas exchange dynamics of leaves? How should these behaviors be encoded within mechanistic models such as OnGuard3?

What are the predominant factors that influence the range of stomatal behaviors evident between species, and how might these be encoded in mechanistic models to accommodate gas exchange in mixed canopies?

What elements of mechanistic model platforms such as OnGuard3 are essential to yield testable predictions on canopy and ecosystem scales?

How can we introduce temporal characteristics for transcription and translation into stomatal modeling?

What are the critical transport and metabolic characteristics needed to construct mechanistic models relevant to grass stomata?

How might a mechanistic platform, such as OnGuard3, encompass the characteristics of stomata in C4 plants and the 'inverted' stomatal dynamics observed in CAM plants?

A key to the success of the OnGuard platform lies in its unique ability to model and predict temporal kinetics across a wide range of variable outputs. We view this advance as crucial to efforts going forward in any mechanistic approach to modeling foliar gas exchange. It allows the user to predict transient behaviors and to analyze their origins as they arise through the evolving interactions of elements within each model assembly. Rather than focusing on the steady-state conductance of stomata, the OnGuard platform describes stomatal physiology and its regulation, second by second, as the plant trades soil water for CO_2. As a result, the information that it yields is immediately available for comparison with measurements performed in the field.

This temporal dimension, equally, should make it possible to 'dovetail' mechanistic models, like those generated with OnGuard3, together with other dynamic platforms such as metabolic flux models [91–93] in order to explore how the mesophyll cells within the leaf integrate and respond to changes over the same time scales. Dovetailing of this kind should inform on mechanisms for redirecting assimilates among metabolic pools and their distribution within the plant. These are new directions for research that will help us to gain much greater insight, for example, into stress partitioning and yield [94–96].

Even with the mechanistic descriptions encompassed by OnGuard3, we need more extensive models of stomata with greater phylogenetic depth. There is a wealth of knowledge for guard cells, across a number of species and described in many thousands of research papers over the past half century, that has yet to be parameterized. Incorporating this knowledge within a mechanistic mathematical framework will help refine efforts in modeling and guide our understanding of stomata in the real world. There remains much still to do, too, to define the inputs of light and hormones [78,97], soil water potential, and the hydraulic controls imposed, for example, by aquaporins in the root [98,99] as well as developmental and circadian controls such as those of transcription and translation in CAM stomata [100,101], just to name a few of the challenges for the future (see also Outstanding questions).

Acknowledgments

Development of the OnGuard platform was supported with grants BB/L001276/1, BB/L019025/1, BB/M001601/1, and BB/N01832X/1 from the UK Biotechnology and Biological Sciences Research Council. We thank the many colleagues who have contributed to the OnGuard project and to formal and informal discussions over the past decade, especially Zhong-Hua Chen (Western Sydney), Yizhou Wang (Zhejiang), Tracy Lawson (Essex), Howard Griffiths (Cambridge), Anna Amtmann (Glasgow), Simon Rogers (Glasgow), Maria Papanatsiou (Glasgow), Cornelia Eisenach (Zurich), Enrico Martinoia (Zurich), Silvere Vialet-Chabrand (Essex), Diana Santelia (Zurich), Florent Pantin (Montpellier), Keith Mott (Salt Lake City), Graham Farquhar and Ross Deans (Canberra), and Tom Buckley (Davis).

Declaration of interests

No interests are declared.

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