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The conceptual approach to quantitative modeling of guard cells

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Much of the 70% of global water usage associated with agriculture passes through stomatal pores of plant leaves. The guard cells, which regulate these pores, thus have a profound influence on photosynthetic carbon assimilation and water use efficiency of plants. We recently demonstrated how quantitative mathematical modeling of guard cells with the OnGuard modeling software yields detail sufficient to guide phenotypic and mutational analysis. This advance represents an all-important step toward applications in directing "reverse-engineering" of guard cell function for improved water use efficiency and carbon assimilation. OnGuard is nonetheless challenging for those unfamiliar with a modeler's way of thinking. In practice, each model construct represents a hypothesis under test, to be discarded, validated or refined by comparisons between model predictions and experimental results. The few guidelines set out here summarize the standard and logical starting points for users of the OnGuard software.

Stomatal pores in the epidermis of plant leaves are surrounded by guard cells, which regulate pore aperture to minimize water loss by transpiration, balancing this need with that for CO_2 in photosynthesis. Guard cells have a profound influence on global water and carbon cycles and are the focus of much effort to engineer improved water use efficiency in crops.[1,9] Equally, over the past two decades guard cells have become the premier cell model for studies of membrane transport and cell signal transduction, driven primarily by an explosive growth in quantitative studies of the various transport processes at the plasma membrane and tonoplast. We now know that stomatal opening and closing arise from the concerted transport, accumulation and release of osmotically-active solutes—mainly K^+ and Cl^-, the organic anion malate^{2-} (Mal) and sucrose—to drive water flux and cell turgor.[2,6,8,11] However, predicting stomatal behavior from this wealth of knowledge has proven beyond intuitive grasp. Testing whether the information gained at a molecular level explains stomatal function has necessitated a mathematical framework to integrate the transport, metabolic and buffering reactions of the guard cells and their link to stomatal dynamics.

We recently developed[3,6] mathematical models of the guard cell and stomatal dynamics, which we encoded in the OnGuard software (available at www.psrg.org.uk). The software encapsulates all of the fundamental properties of the transporters at the plasma membrane and tonoplast, the salient features of osmolite metabolism and the essential pH_i and $[Ca^{2+}]_i$ buffering characteristics that have been described in the literature. The Vicia[3] and *Arabidopsis*[14] guard cell models resolved to date with OnGuard successfully recapitulate a wide range of known stomatal behaviors. These behaviors include the well-characterized diurnal cycles of stomatal aperture and closure, the macroscopic dependencies on extracellular pH, KCl and $CaCl_2$ concentrations, as well as a wide range of microscopic phenomena such as diurnal changes in free cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$),[4] and the oscillations in membrane voltage and $[Ca^{2+}]_i$ that are thought to facilitate stomatal closure.[2,5,8,12] The models also

reproduce the coordination of plasma membrane and tonoplast transport that leads to shunting of K^+ and Cl^- from the apoplast through the cytosol to the vacuole during stomatal opening and the reverse of this process during closing.[7,15]

The predictive power of the OnGuard approach to quantitative modeling is amply demonstrated by its success in addressing the paradoxical observation that the *Arabidopsis slac1* mutation, which eliminates the plasma membrane channel responsible for Cl^- loss during stomatal closure,[10,13] nonetheless profoundly suppresses K^+ channel activities and slows stomatal opening. We initiated studies of the *slac1* mutant soon after publicly launching the OnGuard software at the International Plant Membrane Biology Workshop in Adelaide (September 2010). The solution proved to be that anion accumulation in the mutant affects the H^+ and Ca^{2+} loads on the cytosol, elevating cytosolic pH and $[Ca^{2+}]_i$, which in turn regulate the K^+ channels.[14] These findings uncover an entirely unexpected homeostatic network that connects two otherwise unrelated transport functions in the guard cell. They also represent an all-important step toward the application of OnGuard modeling in guiding the "flip side" task of reverse-engineering stomatal function for improved water use efficiency and carbon assimilation in the plant.

How did the OnGuard model arrive at these predictions? Indeed, how can quantitative modeling with OnGuard be used generally to explore questions of physiological relevance? Normally, formulating dynamic models of this kind begins with the definition of an initial or reference condition, a single state or set of states that represent the physiological norm, from which simulations are then begun. Resolving such a reference point—what we refer to as the Reference State or Reference Cycle[3,6]—is a laborious process that demands repeated adjustment and testing of the parameter set of a model, followed by systematic comparisons of the model outputs with known experimental data. We established a diurnal Reference Cycle for guard cells both of Vicia[3] and of *Arabidopsis*,[14] and both of these resulting models are available for download with the OnGuard software. So the user can start with these pre-packaged models and circumvent the considerable task of setting up and validating this reference point. Of course, these prepared models come with the standard proviso of a working system: while both models offer good approximations to experimental data, they do so within the bounds of the conditions and data used for validation (see Hills et al.[6] and Chen et al.[3]). It is likely that further refinements will be needed in the future as new experimental data become available that can extend these validating conditions, and we welcome users to communicate with us for this purpose.

In practice, then, it remains only to introduce one or more perturbations that represent new physiological, pathological or experimental conditions to be explored. Thereafter the OnGuard user follows the response of all system variables as they evolve over time. Simple perturbations, including the one we used to simulate the *slac1* mutant,[14] are straightforward to implement: they require the user to run the pre-packaged model, generating output equivalent to the wild-type situation; then the user has only to introduce the perturbation (for *slac1*, this amounted to resetting the effective channel population size to zero to simulate the loss of this transporter) and to run the model with this perturbation until it re-establishes stability. The final task is one of querying the simulation outputs to compare effects before and after the perturbation and to derive predictions that are experimentally testable. In the models, just as in vivo, changes in each of the model variables —including the various solute concentrations, membrane voltages, cytosolic-free $[Ca^{2+}]$ and pH, but also the rates of ion and solute flux through each of the transporters—arise through interactions between the transporters, metabolism and associated buffering characteristics. So, these variables are commonly the most helpful to identifying the emergent behaviors of the system as a whole and interpreting their origins.

A greater challenge arises when the user wishes explore reverse-engineering questions; that is, to identify and manipulate the mechanisms giving rise to a set of behaviors. For example, we might ask, "Which mechanisms are essential for solute loss during stomatal closure?" as a preface to the reverse-engineering question, "Which mechanisms need to be manipulated to accelerate stomatal closure?" The logical approach in either case is straightforward in concept, but in practice is often much more laborious. It requires a systematic testing of the model through successive cycles of perturbations, the outputs of each cycle of testing followed by comparison of the simulated outputs with experimental data. In practice, the approach is the same as was used initially to establish the Vicia and *Arabidopsis* Reference Cycles. Further validation may then include querying the simulated outputs for associated behaviors that have yet to be explored in vivo. Such additional behaviors constitute predictions, each one in effect representing a hypothesis bound with the mechanism under test, to be discarded, validated or refined by comparison with new experimental data.

The utility of any homeostatic model lies in its ability to recapitulate physiological behavior and, most important, to make experimentally testable predictions. To make the process of simulation and generating predictions as simple and intuitive as possible, the OnGuard software gives the user access to parameters "on the fly" during modeling sessions and the facility to restructure models in order to introduce (and remove) elements, including the various transporters. In effect, these structural elements serve as phenomenological "black boxes" to be opened, or reduced, whenever the internal workings become a desirable or necessary part of a modeling project. This flexibility means that OnGuard can readily accommodate the characteristics of guard cells of most species. Indeed, there appears sufficient quantitative similarity between the guard cells of many species that adapting the Vicia and *Arabidopsis* models is likely to require little more than an accounting for differences in cell geometry and the macroscopic relationships between cell surface area, volume and turgor pressure. In addition, we anticipate that the HoTSig library, on which the OnGuard software is built, will find applications in exploring other cell systems for which there is sufficient detail of transport. The modular construction of the HoTSig library[6] means

that phenomenological descriptors linking solute content, volume and turgor for any plant cell type can be "bolted" onto the library in order to generate a software package, for example for modeling cell expansion and tip growth processes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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