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Exploring emergent properties in cellular homeostasis using OnGuard to model K^+ and other ion transport in guard cells[☆,☆☆]

Michael R. Blatt[a,*], Yizhou Wang[a], Nathalie Leonhardt[b], Adrian Hills[a]

[a] *Laboratory of Plant Physiology and Biophysics, University of Glasgow, Bower Building, Glasgow G12 8QQ, UK*
[b] *Laboratoire de Biologie du Développement des Plantes, UMR 7265, CNRS/CEA/Aix-Marseille Université, Saint-Paul-lez-Durance, France*

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ABSTRACT

It is widely recognized that the nature and characteristics of transport across eukaryotic membranes are so complex as to defy intuitive understanding. In these circumstances, quantitative mathematical modeling is an essential tool, both to integrate detailed knowledge of individual transporters and to extract the properties emergent from their interactions. As the first, fully integrated and quantitative modeling environment for the study of ion transport dynamics in a plant cell, OnGuard offers a unique tool for exploring homeostatic properties emerging from the interactions of ion transport, both at the plasma membrane and tonoplast in the guard cell. OnGuard has already yielded detail sufficient to guide phenotypic and mutational studies, and it represents a key step toward 'reverse engineering' of stomatal guard cell physiology, based on rational design and testing in simulation, to improve water use efficiency and carbon assimilation. Its construction from the HoTSig libraries enables translation of the software to other cell types, including growing root hairs and pollen. The problems inherent to transport are nonetheless challenging, and are compounded for those unfamiliar with conceptual 'mindset' of the modeler. Here we set out guidelines for the use of OnGuard and outline a standardized approach that will enable users to advance quickly to its application both in the classroom and laboratory. We also highlight the uncanny and emergent property of OnGuard models to reproduce the 'communication' evident between the plasma membrane and tonoplast of the guard cell.

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Introduction

A major barrier to understanding cellular physiology arises from the complexity of interactions between the transport, metabolic and buffering activities of the different cellular compartments and, at any one membrane, between the assembly of individual transporters that contribute to charge and solute movements across that membrane. Often, the most fundamental question of homeostasis can prove difficult to answer, namely which characteristics arise from these interactions, intrinsic to the assemblies, and which require independent regulatory inputs that engage transcriptional, translational or post-translational modifications. Addressing these and other questions demands a full and quantitative accounting for each contributing transporter, metabolic and buffering reaction, and generally is possible only through integrative modeling to explore the dynamics of these processes within a single ensemble that represents the cell.

In principle, constructing cellular models is not difficult. For many eukaryotic cells, there now exists a substantial body of data that encompasses most, if not all, of the essential parameter sets required for the purpose. However, integrating this information within a systematic mathematical representation can be daunting. Although the essential physicochemical relationships are simple, quantitative functions easily incorporated in a description of the cell, the recursive nature of transport across a common membrane generally defies analytical solution. For each transport process, there exists a unique set of kinetic and regulatory descriptors. However, for a majority of transporters, the process of transport itself acts on one or more of these descriptors. For example, consider the outward-rectifying K^+ channel of the guard cell. Gating of these channels is sensitive to membrane voltage as well as to K^+ concentration (Blatt, 1988, 2000b; Blatt and Gradmann, 1997; see also Benito et al., 2014; Demidchik, 2014; Véry et al., 2014). Depolarizing the membrane promotes the current, but the current carried by the channels draws K^+ out of the cytosol. As a consequence, when

Abbreviations: AHA1, plasma membrane proton pump *Arabidopsis* H^+-ATPase 1; ALMT, aluminum-sensitive malate transporter (gene family); CLC, chloride channel (gene family); [Ca^{2+}]i, cytosolic-free calcium concentration; H^+-ATPase, plasma membrane H^+ pump, ATP-dependent; H^+-PPase, vacuolar H^+ pyrophosphatase pump; H^+-VATPase, vacuolar H^+ pump, ATP-dependent; Mal, malate anion; ost2, open stomata 2 protein, identical with AHA1; R-type anion channel, rapid-gating plasma membrane anion channel; ROS, reactive oxygen species; SLAC1, slow anion channel 1 protein (localizes to the plasma membrane).

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* Corresponding author. Tel.: +44 141 330 4771.
E-mail address: Michael.Blatt@glasgow.ac.uk (M.R. Blatt).

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otherwise unchecked, the current drives the membrane voltage negative, countering its depolarization and thereby suppressing channel activity. The case of the K^+ channel is not unique. Every transport process that carries charge across a membrane will affect – and will be affected by – the voltage across that membrane, if only as a consequence of mass action and the movement of the charged ions it carries. In effect, voltage is both substrate and product of charge transport, and is shared between all of the transporters at the membrane. The problem is compounded further because, for charge-carrying transporters operating across a common membrane, these voltage dependencies are frequently non-linear in their characteristics (Blatt and Slayman, 1987; Blatt et al., 1987; Sanders, 1990; Weiss, 1996; Blatt, 2004a). Thus, the challenge becomes one of integrating each and every one of the predominant transporters in a manner that accommodates these recursive and kinetically distinct properties, and of doing so within a system that is sufficiently flexible to allow parameter modifications and substitutions for the equations representing each process between (and even during) modeling sessions.

There are many instances in which modeling has been applied to cellular homeostasis in order to explore potential functions. In plants, for example, this approach has been used effectively to test the feasibility for K^+ transport to serve as an 'energy reserve' for phloem loading of sucrose (Gajdanowicz et al., 2011). However, there are very few instances in which these methods have been applied with sufficient mathematical rigor to yield predictions of unexpected behaviors that have subsequently proven experimentally. Of the latter, dynamic models of mammalian epithelia (Lew et al., 1979) correctly predicted a transient cell shrinkage and protracted fall in tissue short-circuit current following inhibition of the Na^+/K^+-ATPase by ouabain. At the time, these experimental findings appeared to contradict the general validity of Ussing's now widely accepted, two-barrier description of mammalian epithelia (Ussing, 1982). The outputs derived from the modeling were counterintuitive, but offered substantive predictions that were confirmed experimentally (MacKnight et al., 1975a,b). More recently, similar models of the erythrocyte demonstrated unexpected connections between hemoglobin metabolism, transport and osmotic balance, both during malarial infection and in sickle-cell anemia, each of which was subsequently verified experimentally (Lew et al., 1991, 2003; Lew and Bookchin, 2005; Mauritz et al., 2009).

Most modeling efforts have been implemented on a case-by-case basis, without a standardized format, and frequently without incorporating the essential transport circuits needed for overall balance of charge, solute and water fluxes (Gradmann et al., 1993; Grabe and Oster, 2001; Shabala et al., 2006; Cui et al., 2009). Utilities such as the Virtual Cell (Loew and Schaff, 2001), E-Cell (Tomita et al., 1999), Cellerator (Shapiro et al., 2003), as well as the Berkeley Madonna Environment (BME) and similar commercial software (Abu-Taieh and El Shiekh, 2007), provide for modeling intra- and intercellular events that encompass reaction-diffusion processes in arbitrary geometries. These utilities offer standardized platforms for modeling, but many are designed for 'flow-through' (serial) networks, ill-suited to the recursive nature of membrane transport, and few have the scope to define underlying behaviors, for example as dictated by specific transport equations. Significantly, none of these utilities are flexible in their connections to physiological outputs, most important for plant cells including those of solute content, cell volume, turgor, and non-linear or anisotropic cell expansion. Some of these difficulties are illustrated by the work of Grabe and Oster (2001), who utilized BME software to explore the question of vesicular acidification during endocytosis. Their study was able to recapitulate vesicular pH driven by the V-type H^+-ATPase, but its prediction of a requirement for a voltage-gated Cl^- channel and cation pre-loading was misdirected. Indeed, more recent work has indicated that the Cl^- flux essential for vesicular acidification is mediated by CLC-type H^+–Cl^- antiporters (Smith and Lippiat, 2010; Novarino et al., 2010; Weinert et al., 2010).

These limitations are addressed in the Homeostasis, Transport and Signaling (HoTSig) libraries, an approach developed in this laboratory (Hills et al., 2012). HoTSig incorporates an open structure of expandable libraries for transporter kinetics, chemical buffering, macromolecular binding and metabolic reactions, as well as for macroscopic coupling equations such as those relating solute content, cell volume and turgor, all accessible to input and modification by the user. This open structure makes HoTSig adaptable to wide variety of single-cell systems with the potential for its expansion to multicellular situations and problems that must be addressed across scales from the cellular to whole-tissue and organ structures. The first implementation of the HoTSig libraries, in the OnGuard software (Hills et al., 2012; Chen et al., 2012), focused on guard cell mechanics and their control of stomatal aperture. OnGuard (available at www.prsg.org.uk) includes a graphical user interface for real-time monitoring of the individual transport and homeostatic processes under simulation. It incorporates a set of empirically defined equations to relate the output of solute content to cell volume, turgor and stomatal aperture. Finally, it includes a Reference State Wizard as an aid to defining a starting point for experimental simulations with sensible outputs for all known variables. This initial implementation demonstrated that an OnGuard model of the Vicia guard cell recapitulates all of the known characteristics of guard cell transport, solute content and stomatal aperture in the face of well-defined experimental manipulations; it yielded a number of unexpected and emergent outputs, among these a clear demonstration of homeostatic 'communication' between the plasma membrane and tonoplast independent of an overlay of control via signal transduction networks; and it demonstrated counterintuitive changes in cytosolic-free calcium concentration ($[Ca^{2+}]_i$) and pH over the diurnal cycle, all of which find direct support in independent experimental data (MacRobbie, 1991, 1995a, 2000, 2006; Thiel et al., 1992; Blatt and Armstrong, 1993; Willmer and Fricker, 1996; Frohnmeyer et al., 1998; Dodd et al., 2007).

OnGuard models have the power to predict physiology. This capacity is amply demonstrated by the recent study of Wang et al. (2012), who addressed paradoxical observations associated with the *Arabidopsis slac1* mutation. The *slac1* mutant lacks the plasma membrane channel responsible for Cl^- loss during stomatal closure (Vahisalu et al., 2008; Negi et al., 2008), but its absence profoundly affects both inward- and outward-rectifying K^+ channel activities and slows stomatal opening. Analysis of the *Arabidopsis* guard cell using OnGuard predicted the effect to arise from anion accumulation in the mutant, which affects the H^+ and Ca^{2+} loads on the cytosol, in turn elevating cytosolic pH and $[Ca^{2+}]_i$, both factors that regulate the K^+ channels (Wang et al., 2012). These predictions were confirmed experimentally, with the study demonstrating that experimentally 'clamping' cytosolic pH and $[Ca^{2+}]_i$ was sufficient to recover both the K^+ currents and stomatal opening kinetics. Thus, modeling with the OnGuard software uncovered an entirely unexpected homeostatic network connecting two unrelated ion channels in the guard cell. This study represents a crucial step toward using OnGuard-style modeling to guide the 'flip side' problem of reverse-engineering stomatal function; it is a very short step from OnGuard to future implementations that will enable rapid, in silico design as a guide to altering guard cell physiology, for example in improving water use efficiency during photosynthesis (Lawson et al., 2011, 2012; Blatt et al., 2013). Right now, OnGuard can be used to address a wide range of questions in guard cell biology, and further implementations of the HoTSig libraries promise to be equally powerful in enabling research scientists and students to explore similar problems in other plant cells. Here we summarize the elements of an OnGuard model as a guide to users. We provide a didactic review of the *slac1* modeling exercise, and finally, we use

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