5 Use of Non-Invasive Ion-Selective Microelectrode Techniques for the Study of Plant Development

JOSEPH G. KUNKEL,[1] SOFIA CORDEIRO,[2] YU (JEFF) XU,[1] ALAN M. SHIPLEY,[3] JOSÉ A. FEIJÓ[2]

5.1 Ion dynamics in plant development

Ion fluxes across membranes are known to have important biological roles. They exert their effect by two means: generating electrical fields and changing the local ion concentrations, thus affecting physiological processes that are dependent upon them.

Electrical fields exert force on charged particles, from molecules to organelles, and this has been proposed to lead to the movement of membrane proteins or cytoplasmic vesicles by field orientation, electrophoresis or electro-osmosis. On the other hand, cells react to the ions that carry these currents, many of which act via signal transduction pathways. If these ions have a catalytic or regulatory function, the biochemical consequences of any change in their concentration can be enough to trigger a response (Harold and Caldwell 1990; Feijó et al. 2004). Furthermore, accumulating evidence has shown that polarity, morphogenesis and many developmental steps in plant cells are defined by an intricate network of processes that often include ion distribution and concentration as major correlates. These phenomena are available for experimental manipulation and measurement during which one can seek evidence for causal relationships.

5.2 Molecular basis of ion fluxes in plants

Ionic equilibrium in plant cells is achieved, on the one hand, by the maintenance of an electrochemical gradient by proton ATPases on the plasma membrane (P-ATPases) and, on the other hand, by the flux of other ions, namely potassium, calcium and chloride through ion channels and transporters.

Turgor and volume regulation, two crucial parameters in plant physiology and development, are good examples of processes that are strictly dependent

[1] Department of Biology, University of Massachusetts Amherst, MA 01003-5810, USA

[2] Centro de Biologia do Desenvolvimento, Instituto Gulbenkian de Ciência, PT-2780-156 Oeiras, Portugal (e-mail: jfeijo@fc.ul.pt)

[3] Applicable Electronics Inc., Forestdale, MA 02644, USA

Plant Electrophysiology – Theory & Methods (ed. by Volkov)

upon ion regulation. Intracellular pressure in plant cells is achieved and maintained by the intracellular accumulation of ions and solutes, and their partition and sequestration in different osmotic biochemical forms in the vacuole (e.g. formation of salts or polymers). The turgor pressure thus created is required for cell expansion, elongation, gas exchange, transport of ions and solutes, etc.

Pivotal in the regulation is also the activity of the vacuolar proton ATPases (V-ATPases), much different from their plasma membrane counterparts both in structure and mechanism. Vacuolar pyrophosphatases (PPases) are a third active transport party in the system. The coordinated action of these three pump systems maintains the cytosol at a fairly constant and neutral pH, while keeping both vacuole and external apoplast acidic.

These pumps are the active source of energy for a number of channels and transporters (e.g. Sanders and Bethke 2000; Taiz and Zeiger 2002; Blatt 2004), many now well characterized as expressed in a number of tissues, evidenced by transcriptomics (e.g. Pina et al. 2005).

The particular ionic environment created by these conditions is cause and consequence of the ion fluxes across the cell's membranes, requiring tight regulatory mechanisms that keep calcium concentration low, potassium high and a pH neutral, among other homeostatic regulations (reviewed by Feijó et al. 1995; Holdaway-Clarke and Hepler 2003).

The study of plant ion dynamics is therefore of the utmost importance, and several laboratories have taken advantage of non-invasive microelectrode techniques, in particular scanning ion-selective probes, to approach it.

5.3 Scanning probe: technical advantages and disadvantages

In the past, we have reviewed the application of both voltage sensitive and ion-selective probes for the scanning of membrane domains underlying the development of plants, with special emphasis on a very specialized cell, the pollen tube (Shipley and Feijó 1999; Fig. 7C, D). To date, the pollen tube is probably the best-studied system in terms of ion fluxes, and the matter has been reviewed both for its occurrence and biological meaning (Feijó et al. 2001, 2004; Holdaway-Clarke and Hepler 2003). The reason for this specific cluster of applications lies in the absence of real alternatives to an extracellular scanning probe for use with plant cells. The analysis of ion fluxes in living cells has been accomplished through the use of invasive techniques such as impalement and patch clamp. These approaches usually allow one independent sample location per cell. Further sampling proceeds at the risk that prior sampling had an effect on the cell. Although results from these techniques have been remarkably important for the characterization of ion channel properties and activity in plant development, they are compromised by serious disadvantages. Since access to the plasma membrane is necessary for the