3 Non-Invasive Microelectrode Ion Flux Measurements In Plant Stress Physiology

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3.1 Introduction: membranes and plant stress responses

Plant membranes underlie many essential cell biological processes including nutrient acquisition and compartmentation, pH and ionic homeostasis, turgor generation, metabolite distribution and waste excretion, energy transduction and signaling. According to Ward (2001), 43% of over 25,000 protein sequences in the *Arabidopsis* genome have at least one transmembrane spanning (TMS) domain, with 18% proteins having ≥ 2 TMS domains and thus associated with cellular membranes. Recent progress in electrophysiology and molecular genetics has revealed the crucial role of plasma membrane transporters in perception and signaling in response to virtually every known environmental factor (Zimmermann et al. 1999). Changes in plasma membrane potential or modulation of ion flux are amongst the earliest cellular events in response to light, temperature, osmotic stress, salinity, hormonal stimuli, elicitors and mechanical stimulation in many organisms (Blumwald et al. 1998; Sanders et al. 1999; Zimmermann et al. 1999; Spalding 2000; Knight and Knight 2001). For many, if not all the stresses mentioned above, the receptors involved were suggested to be located at one of the cellular membranes.

In addition to hosting various receptors mediating plant–environment interactions, membrane transporters always act as the ultimate effectors, enabling plant adaptive responses. In the case of salt tolerance, this may be by excluding toxic Na^+ from the cytosol via either the SOS1 plasma membrane Na^+/H^+ antiporter (Zhu 2003) or by compartmentalizing it into the vacuole by the NHX tonoplast Na^+/H^+ antiporter (Apse et al. 1999). In the case of Al^{3+} toxicity, the adaptive response includes activation of anion channels responsible for malate efflux and changes in the rhizosphere pH (Ryan et al. 2001). Osmotic adjustment includes rapid increase in the uptake of inorganic ions (Shabala and Lew 2002), while plant adaptive responses to low temperature include dramatic changes in membrane fluidity (Murata and Los 1997). Such a central role of plant membranes and membrane transport processes in plant adaptive responses to environmental conditions makes them important targets for genetic manipulations aimed to improve tolerance to a particular

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stress. To enable this, causal links between membrane-transport processes and other metabolic or physiological processes in the cell need to be understood.

Gaining such an understanding is not an easy task. It is complicated not only by the large number of transporters involved (for cations, 46 unique families are known, containing approximately 880 members in *Arabidopsis*; Maser et al. 2001), but also by the myriad of interactions and cross-talk between various transporters and signaling components. Over the last 2 decades, various state-of-the-art molecular and biophysical techniques (such as patch-clamp or fluorescence imaging) have been used to reveal some of these interactions. These techniques have been the subject of many comprehensive reviews and thus are mentioned only briefly here. However, at the same time, the inevitable consequence of such “in-depth” approaches was a decrease in the physiological reality of the transporters’ environment (Tester 1997). There are many reports (some of which are discussed in section 4) showing that activity of a particular transporter differs dramatically when expressed in a heterologous system compared with in planta conditions. This makes it very difficult (and often even impossible) to transfer the results obtained by these advanced techniques to *real* plants in their natural habitats. The more advanced our study, the bigger is the gap between physiologists/molecular biologists and the agronomists interested in plant behavior in the field.

Since the mid-1990s our laboratory has pioneered application of non-invasive ion flux measuring (the MIFE) technique in plant stress physiology. As shown in the following sections, this technique provides a unique possibility to link genetic/genomic data to cellular physiological behavior. Some of its key features (e.g. non-invasiveness, high spatial and temporal resolution) allow us to establish and quantify causal links between membrane-transport processes and other metabolic or physiological processes in the cell in almost natural conditions. In this context, the MIFE technique may be considered as a “bridging element” between molecular biologists and whole plant physiologists or agronomists.

The aim of this review is to show that in situ measurements of net ion fluxes from plant cells and tissues using the MIFE technique can provide insights into the functional genomics of plants and will significantly increase our understanding of the function of specific genes mediating plant adaptive responses to the environment.

3.2 Basic techniques for studying membrane transport in plants

3.2.1 Comparative analysis of basic techniques

A large number of techniques are available to study ionic relations and transport of nutrients and ions across cell membranes. They range from whole-plant methods (depletion experiments, radioactive tracers) to those applicable