8 Electrophysiological Characterization of Plant Cation Channels

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8.1 Introduction

Cation channels are macromolecular protein pores in bio-membranes that catalyze passive cation influx and efflux (MacKinnon 2004). They do not use ATP energy to transport cations as opposed to active transporters such as pumps and carriers. Since cation channels are not limited by the rate of metabolic interactions, they saturate at much higher concentrations than active transporters and demonstrate low Q_{10} coefficients (<2.0). Cation channels consist of several transmembrane alpha helices that are also called transmembrane spans or transmembrane domains. These transmembrane domains form a pore region with a selectivity filter that selects cations over anions. Rearrangement of transmembrane domains causes pore opening (activation) or closing (deactivation). Different cation channels have different activators and inhibitors, including membrane voltage (V_m), H^+, divalent cations, G-proteins, ATP, cyclic nucleotides, hormones, ROS, amino acids, stretching and gravity. Specific chemical sites in the channel macromolecule are responsible for interactions with activating and inhibiting factors. Some cation channels have fixed anion surface charges outside and/or inside of the channel entry. These charges increase a local cation concentration and modify voltage-dependence, gating and selectivity of the channel (Green and Anderson 1991; Miedema 2002). Protons and divalent cations effectively screen surface charges and cause significant changes in the channel function. Cation channels are sensitive to a range of specific and non-specific blockers. Experiments with blockers, or so-called pharmacological analysis, are necessary for the selection between several groups of channels. For example, tetraethylammonium (TEA^+) is a specific blocker of K^+ channels that does not affect other cation channels (reviewed by Demidchik et al. 2002a). Blockers can be of "natural" origin, such as Ca^{2+}, Mg^{2+}, Zn^{2+} or H^+, or xenobiotics, for example Ba^{2+}, TEA^+, Cs^+, lanthanides, dihypropiridines, phenylalkylamines

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among others. Analysis of blockage provides important information about molecular determinants of the channel (Hille 1994).

Cation channels play multiple physiological roles in plants. They catalyze nutritional uptake of N (taken up as NH_4^+), macronutrient and micronutrient cations such as K^+, Ca^{2+}, Na^+, Fe^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+} and Mn^{2+}. Cation channels are responsible for the generation of negative resting V_m and action potentials. This is necessary for maintaining structural and functional integrity of the membrane, signaling processes and polarity. Cation channels are directly involved in osmotic balance and regulation of the turgor. This property of cation channels underlies stomata opening and closing. Calcium-permeable cation channels trigger Ca^{2+} signaling in plants that is involved in tissue and organ coordinated growth, development and stress responses. ROS, amino acids, purines, elicitors, hormones, gravity, different stresses and stretching act through activation of cationic channels. Having multiple physiological roles in plants, cation channels have been a subject of extensive study. Different physiological and molecular techniques have been employed for examination of physiology and structure of cation channels. Unfortunately, the crystal structure of plant cation channels remains unknown.

Significant progress has been achieved in our understanding of the molecular nature of cation channels in the last decade (reviewed by Davenport 2002; Demidchik et al. 2002a; White et al. 2002; Véry and Sentenac 2003). Many genes encoding plant cation channels have been identified and characterized molecularly. Analyses of knock-out plants and plants over-expressing K^+ channels showed for the first time physiological consequences of the lack or abundance of the particular channel. Nevertheless, molecular studies do not provide information regarding physiological characteristics of cation channels in intact cells. Electrophysiological techniques should be employed to establish channel properties in in vivo conditions. Here, we briefly review some of the most important electrophysiological techniques and provide examples of their use for studies of cation channels in plants.

8.2 Overview of electrophysiological techniques

The main question of the physiology of cation channels is how cations are transported through the channel and how this transport is regulated by internal and external factors. To investigate this, electrical currents or net fluxes mediated by cation movement through the channel should be measured.

There are two main types of electrophysiological recordings in plant cells: extracellular and intracellular recordings. Techniques for extracellular electrophysiological recordings include extracellular electrodes and “microelectrode ion flux estimation” (MIFE®). Intracellular recordings can be performed by impaling a cell with one or more fine-tipped electrodes (so-called “impalement techniques”), or by sticking a cell to the glass micropipette (so-called