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Endocytosis in Guard Cells

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Abstract Stomatal movement requires large and repetitive changes to cell volume and consequently surface area. These alterations in surface area are accomplished by addition and removal of plasma membrane material. Recent studies of membrane turnover in guard cell protoplasts using electrophysiology and fluorescence imaging techniques implicate that exocytosis and endocytosis are sensitive to changes in membrane tension. This may provide a regulatory mechanism for the adaptation of surface area to osmotically driven changes in cell volume in guard cell protoplasts as well as turgid guard cells. In addition guard cells also exhibit constitutive membrane turnover. Constitutive and tension-driven membrane turnover were found to be associated with addition and removal of K^+ channels. This implies that some of the exocytosis and endocytic vesicles carry K^+ channels. Together the results demonstrate that exocytosis and endocytosis are essential for stomatal movement and thus gas exchange in plants.

1 Introduction

Guard cells mediate opening and closing of the stomatal pores which regulate gas exchange in plants. Accumulation of K^+ salts and subsequent water influx lead to swelling of guard cells and opening of the stomatal pore. The reverse process closes the pore. Thus, during stomatal movement, guard cells undergo large osmotically driven changes in cell volume and consequently surface area over a period of minutes. These large changes in surface area of up to 40% (Raschke 1979) cannot result from stretching of the existing membrane as the maximum possible stretching of membranes is limited to about 2% (Wolfe et al. 1986). In addition, the large turgor pressure of up to 5 MPa (Franks et al. 1998) prevents the guard cell plasma membrane from maintaining infoldings that could provide excess surface area. Therefore, alterations in surface area must be accomplished by addition and removal of membrane material to and from the plasma membrane, respectively. However, the mechanisms underlying these osmotically induced changes in surface area are largely unknown. Recently, the application of new cell biology techniques, namely patch-clamp capacitance measurements and microscopical imaging of membranes stained with fluorescent styryl dyes, has led to a more detailed understanding of the processes occurring during opening and closing of the stomatal pore. Results from these studies are summarised here.

2
Osmotically induced Exocytosis and Endocytosis in Guard Cell Protoplasts

2.1
Investigation of Exocytosis and Endocytosis by Patch-clamp Capacitance Measurements

Osmotically driven and pressure-driven changes in surface area of guard cell protoplasts have been investigated extensively by patch-clamp capacitance measurements. This technique allows the examination of exocytosis and endocytosis in single living protoplasts. Recordings can be performed with a resolution that is high enough to detect fusion and fission of vesicles with a diameter as low as 60 nm and a temporal resolution on the order of some 10 ms (Neher and Marty 1982; Kreft and Zorec 1997). Measurements of exocytosis and endocytosis via capacitance recordings are based on the fact that a biological membrane can be viewed as a capacitor. The capacitance of this capacitor depends on its surface area. For a number of plant protoplasts, including guard cells, such a linear relationship between membrane capacitance and surface area has been demonstrated, and a specific capacitance (capacitance per unit surface area of membrane) between 7.5 and 8.1 mF m^{-2} has been calculated (Zorec and Tester 1992; Thiel et al. 1994; Carroll et al. 1998; Homann 1998). Under the valid assumption that the specific capacitance remains constant during the time of observation, the changes in plasma membrane surface area resulting from exocytic and endocytic activity can be monitored by measuring membrane capacitance. In principle, the membrane capacitance of a cell is determined from the current measured in response to a voltage command which is applied to the cell (Gillis 1995; Homann and Tester 1998; Thiel et al. 2001). When measurements are carried out in the so-called whole-cell configuration, the cytoplasm is rapidly dialysed by the pipette solution. This allows control of cytoplasmic composition and the introduction of potential regulators of exocytosis and endocytosis.

The high temporal resolution and the potential of manipulating the cytosolic composition via the patch pipette make patch-clamp capacitance measurements a powerful tool for studying exocytosis and endocytosis. The main limitations of patch-clamp capacitance measurements are the general limitations of patch-clamp measurements: the requirement of an accessible membrane (measurements are generally carried out on protoplasts) and possible loss of endogenous substances that affect exocytosis and endocytosis during cell dialysis.