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Distinct fluorescent pattern of KAT1::GFP in the plasma membrane of *Vicia faba* guard cells

Ulrike Homann[1], Tobias Meckel[1,2], Jennifer Hewing, Marc-Thorsten Hütt[3], Annette C. Hurst*

Institute of Botany, University of Technology Darmstadt, Schnittspahnstrasse 3-5, 64287 Darmstadt, Germany

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Abstract

The organisation of membrane proteins into certain domains of the plasma membrane (PM) has been proposed to be important for signalling in yeast and animal cells. Here we describe the formation of a very distinct pattern of the K^+ channel KAT1 fused to the green fluorescent protein (KAT1::GFP) when transiently expressed in guard cells of *Vicia faba*. Using confocal laser scanning microscopy we observed a radially striped pattern of KAT1::GFP fluorescence in the PM in about 70% of all transfected guard cells. This characteristic pattern was found to be cell type and protein specific and independent of the stomatal aperture and the cytoskeleton. Staining of the cell wall of guard cells with Calcofluor White revealed a great similarity between the arrangement of cellulose microfibrils and the KAT1::GFP pattern. Furthermore, the radial pattern of KAT1::GFP immediately disappeared when turgor pressure was strongly decreased by changing from hypotonic to hypertonic conditions. The pattern reappeared within 15 min upon reestablishment of high turgor pressure in hypotonic solution. Evaluation of the staining pattern by a mathematical algorithm further confirmed this reversible abolishment of the radial pattern during hypertonic treatment. We therefore conclude that the radial organisation of KAT1::GFP depends on the close contact between the PM and cell wall in turgid guard cells. These results offer the first indication for a role of the cell wall in the localisation of ion channels. We propose a model in which KAT1 is located in the cellulose fibrils intermediate areas of the PM and discuss the physiological role of this phenomenon.

Keywords: Potassium inward rectifier; KAT1; Cell wall; Cellulose fibres; Cytoskeleton; Latrunculin B; Propyzamide; Turgor pressure; Membrane domains

Introduction

Structural conditions which cause a heterogeneous distribution of membrane proteins are believed to be important factors for the regulation of numerous signalling and transport events, especially at the plasma membrane (PM). So far a number of different mechanisms that induce a heterogeneous distribution of

*Corresponding author. School of Biomedical Sciences, University of Queensland, St. Lucia, QLD 4072, Australia. Tel.: +61 7 3346 1224; fax: +61 7 3365 1766.
E-mail address: a.hurst@uq.edu.au (A.C. Hurst).
[1]These authors contributed equally to the work.
[2]Present address: Institute of Physics, Leiden University, Niels Bohrweg 2, 2333 CA Leiden, The Netherlands.
[3]Present address: School of Engineering and Science, Jacobs University Bremen, Campus Ring 1, D-28759 Bremen, Germany.

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membrane proteins have been described mainly in mammalian cell lines and yeast.

These mechanisms include the lateral separation of specific membrane lipid species (i.e. mainly cholesterol and glycosphingolipids) which leads to the formation of specialised microdomains, so-called lipid rafts. Certain proteins accumulate in these microdomains (e.g. glycosyl phosphatidyl inositol (GPI)-anchored proteins) while others are not affected. This accumulation can be explained by a slowdown of protein mobility by a factor of ~2 (Dietrich et al., 2002) which results in a heterogeneous distribution of proteins in the PM. Many proteins involved in signalling cascades are found in lipid rafts (Brown and Rose, 1992). This provided the first hint on the physiological significance of microdomain formation. The slowdown and accumulation of proteins in lipid rafts is believed to increase the probability of dimer, multimer and cluster formation which is important for many signalling events at the PM. Nevertheless, the exact role of lipid rafts in cellular signalling, trafficking, and structure has yet to be determined.

Lipid rafts and raft-associated proteins have also been identified in plants (Borner et al., 2005; Mongrand et al., 2004). However, like for lipid raft formations in mammalian and yeast cells, the physiological meaning of these microdomains remains to be confirmed.

A second factor that was found to limit free diffusion of membrane proteins is the cytoskeleton. It can determine the localisation of PM proteins via direct attachment to the protein or indirectly. A direct connection to the cytoskeleton is for example of particular importance for the localisation and functioning of the cellulose synthase complex in the PM of plant cells (Gardiner et al., 2003). In the case of certain mechanosensitive ion channels the attachment to the cytoskeleton is also proposed to function as a signalling component in mammalian cells (Barritt and Rychkov, 2005; Ghazi et al., 1998). Apart from the direct connection to PM proteins it is believed that the actin cytoskeleton can confine the movement of proteins with enlarged cytosolic domains by generating ''fenced'' microenvironments without the direct attachment to the diffusing components (Ritchie and Kusumi, 2004). While such a mechanism has so far not been described in plant cells, a cortical cytoskeleton—the only determinant of this effect—is also present in plant cells (Staiger and Lloyd, 1991).

The third factor that can contribute to heterogeneous distribution of PM proteins is the extracellular matrix (ECM). It is able to directly influence the distribution of proteins in the PM of eukaryotic cells (Arnold et al., 2004). For plant cell walls, which can be viewed as the plant ECM, a role in distribution of PM proteins has not yet been described.

PM ion channels which play an important role in signal transduction have been implicated to be distributed non-homogenously in the PM of plant and animal cells (Tester, 1990; Deutsch, 2002). In animal cells lipid rafts have been described as an important factor for the localisation of ion channels in certain domains in the PM (Martens et al., 2004). For plant ion channels mechanisms which determine their localisation in microdomains have not been identified.

Recently Sutter et al. (2006) demonstrated that the K^+ inward rectifier KAT1 from *Arabidopsis thaliana* is localised in clusters in the PM when transiently expressed in tobacco epidermal cells. The authors detected KAT1 protein in a 'moderately' detergent-resistant fraction, indicating its association with lipid rafts. The KAT1 cluster showed nearly no lateral mobility. Investigations of the role of SNAREs (soluble NSF [*N*-ethylmaleimide-sensitive factor] attachment protein receptors) on trafficking of KAT1 indicated that SNAREs are involved in cluster formation and mobility of KAT1 (Sutter et al., 2006). However, the mechanism which anchors KAT1 in the PM remains to be determined.

KAT1 plays an important role in guard cell functioning. We therefore analysed turgid guard cells transiently expressing KAT1 fused to green fluorescent protein (GFP). KAT1::GFP was organised in clusters in the PM similar to what we previously described for guard cell protoplasts (Hurst et al., 2004). In addition we found a radial distribution of KAT1::GFP clusters which was dependent on a close contact between the PM and the cell wall. In animal cells contacts of the PM with the ECM are mediated by substrate adhesion molecules such as fibronectin, vitronectin, collagen, and others, via the short amino acid sequence Arg-Gly-Asp (RGD) (D'Souza et al., 1991) that interacts with integrins. The integrins in turn link the ECM to the cytoskeleton (Ruoslahti, 1996). For plant cell walls plant biologists are just beginning to understand how cell wall-to-membrane interactions are established to acquire cell- and tissue-specific characters and how this affects cell function and polarity and cell-to-cell interactions. So far, in plants only few homologues of classical adhesion molecules, e.g. β-integrin or fibronectin that revealed a RGD-mediated membrane matrix adhesion have been identified (Canut et al., 1998; Faik et al., 1998; Gens et al., 1996; Pellenc et al., 2004). This points to a similar interaction between the PM and the ECM or cell wall of animal and plant cells, respectively. In addition a number of plant PM proteins have been proposed to directly bind to both the PM and extracellular carbohydrates and may thus anchor the cell to the cell wall (for review see (Kohorn, 2000)). Among these is the cellulose synthase complex (Kohorn, 2000), which also requires cortical microtubule arrays for normal localisation in the PM (Gardiner et al., 2003). This indicates that a continuous cytoplasm–cell wall scaffold is essential to control key events in plant development and growth.