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Radial widening of the Casparian strip follows induced radial expansion of endodermal cells

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Abstract The Casparian strip, the barrier to apoplastic transport that is located at the endodermis in roots and stems, is formed by individual endodermal cells and is constructed as a highly organized mesh within the primary wall. Since little is known about the mechanism of formation of the strip, we tried to obtain morphological evidence for the existence, prior to suberization and lignification, of some regulatory system at the expected site of the strip. Endodermal cells in etiolated pea stems were induced to expand in the radial direction by piercing the stems through the cortex before formation of the strip. The radial width of the strip increased significantly with the expansion of the radial walls of these endodermal cells. The expansion of the cells occurred before the formation of the strip. However, strips that had already been formed when the stems were pierced did not increase in width despite an induced expansion of the radial walls. These observations suggest that some positional information exists in the radial wall of endodermal cells that defines the future site of formation of the strip and its width.

Keywords Casparian strip (band) · Cell expansion · Endodermis · Lignin · *Pisum* (Casparian strip) · Positional information

Introduction

The Casparian strip, which is present in the radial walls of the endodermal cells in roots of vascular plants, plays an important role in retention of solutes in the stele (Clarkson and Robards 1975; Haas and Carothers 1975; Peterson et al. 1981, 1993; Shreiber et al. 1999). The cell wall of the Casparian strip is impregnated with hydrophobic substances such as suberin (Zeier et al. 1999a, b), and the plasma membrane adheres very strongly to the cell wall at the Casparian strip (Bonnett 1968; Karahara and Shibaoka 1992). As a result, the apoplastic flow of ions is restricted at the endodermis (Nagahashi et al. 1974; Peterson et al. 1993).

The Casparian strip must be continuous to function. However, it is totally unknown how the precise deposition of the strip is regulated, what signals are responsible for this very localized deposition, and how each unit is precisely located adjacent to the next. As an initial attempt to define these mechanisms, we assumed that there might be some positional information indicating the future site of the strip. If there is such information, we anticipate that the strip would be widened after radial expansion of endodermal cells.

Plants develop a starch sheath or, in some species, an endodermis with a Casparian strip at the innermost layer of the cortex in shoots. This cell layer has been a focus of interest recently since it plays an important role in shoot gravitropism (Fukaki et al. 1998; Fujihara et al. 2000). Etiolated stems of pea, which develop an endodermis with a Casparian strip (Priestley 1926; Sack 1987; Karahara and Shibaoka 1994), are more suitable for surgical treatments than roots. In the present study, endodermal cells were induced to expand radially by injuring the nearby cortex. The effect of this wall expansion on the development of the Casparian strip or modification of a mature strip was followed.

Materials and methods

Plant materials

Seeds of Alaska pea (*Pisum sativum* L. cv. Alaska; Carolina Biological Supply Company, Burlington, N.C., USA) were soaked in distilled water overnight and sown on moist vermiculite. Seedlings were grown in darkness at 25 °C. The ages of seedlings were expressed in terms of the number of days after imbibition of seeds. Seven-day-old seedlings with a hook on the third internode were used for all experiments. Only seedlings in which the distance between the base of the internode and the bending point of the hook
ranged from 40 to 60 mm were selected for experiments. The third internode of each seedling was marked with black oil paint at 2-mm intervals from the bending point of the hook to a point 40 mm proximal. Seedlings were selected and marked under dim green light.

Treatment of stems with a needle

Seven-day-old, marked seedlings were pierced at a site 4, 10 or 40 mm below the bending point with a sewing needle 200 μm in diameter and then the needle was pulled out. Seedlings continued to grow in darkness for 1–3 days. Marked, non-pierced seedlings were used as controls.

Measurement of the width of the Caspian strip and the radial wall of endodermal cells

Segments (2 mm long) were cut from the region that had been pierced with a needle. Cross-sections, 50 μm in thickness, were prepared as described previously (Karahara and Shibaoka 1992). They were first examined under a bright-field microscope to determine the site of injury. The only specimens that were used were those in which the needle had passed through the cortex near the endodermis. Since the cell wall at the Caspian strip emits autofluorescence under ultraviolet (UV) light because it is impregnated with lignin and suberin (Brundrett et al. 1988), we observed the strip under an epifluorescence microscope (BX-60; Olympus, Tokyo, Japan) equipped with a filter assembly for excitation by UV light (U-MWU: excitation filter, BP330–385; dichroic mirror, DM-400). Only endodermal cells that had expanded outward radially were selected for measurements. Photographs were taken on T-Max 400 films (Eastman Kodak Co., Rochester, N.Y., USA) and digitized using a film scanner (Coolscan, Nikon, Tokyo). Digitized fluorescence pictures were displayed as reverse images on which the width of the strip could be measured easily and accurately. The width of the strip and the width of the radial wall were measured with computer software (NIH Image). The Mann-Whitney U-test was used to determine whether changes in width of the strip were statistically significant.

Results

In 7-day-old pea seedlings, the Caspian strip was not observed above a point 32 mm from the bending point of the hook at the start of the treatment. Cells in the process of developing the strip were arrayed in sequence above this point (Karahara and Shibaoka 1998). There was no Caspian strip at a position marked 10 mm below the bending point at the start of the experiment (not shown) but 3 days later a normal strip had developed at this marked position in untreated stems (Fig. 1c). By piercing the stem 2 mm below the bending point at the start of the experiment, cells of the central cortex, but not those of the endodermis, were induced to expand outward radially (Fig. 1b) compared with those of an untreated stem (Fig. 1a). However, when the cortex was pierced near the endodermis 10 mm below the bending point, the endodermal cells as well as the strip expanded significantly in the centrifugal direction (Fig. 1d; Table 1), compared with those in the untreated stem (Fig. 1c). This analysis was repeated 4 mm below the bending point and similar results were obtained (Table 1).

The relationship between the width of the radial wall of endodermal cells and the width of the Caspian strip was examined using Spearman’s correlation coefficient.