Structural and Functional Aspects of Stomata

I. Developmental Studies in Polypodium vulgare

R.A. Stevens and E.S. Martin
School of Environmental Sciences, Plymouth Polytechnic, Drake Circus, Plymouth PL4 8AA, U.K.

Abstract. Differential cell wall thickening in developing guard cells of Polypodium vulgare L. has been studied with particular reference to guard cell protoplast deformation and the eventual formation of the stomatal pore. Concomitant studies on the development of guard cell chloroplasts and their starch inclusions during ontogeny of the stomatal complex have provided data which have been incorporated into a model to account for the formation of the pore. Guard cell starch inclusions reach a maximum density per unit volume at the same time as the guard cell walls achieve maximum differential thickening. These events coincide with the development of the pore. It is suggested that, whilst pore formation is initiated enzymatically, the mechanical forces required to bring about the separation of the two guard cells are of an osmotic nature derived from starch hydrolysis. The development of the mesophyll in relation to the epidermis is examined in respect of the formation of substomatal chambers.

Key words: Cell walls — Ontogeny, stomatal — Polypodium — Starch — Stomata.

Introduction

The development of stomatal complexes has been extensively examined in relation to their ontogenetic derivation and final morphological form (reviewed by Fryns-Claessens and Van Cotthem, 1973), but there is little information available concerning the structure of the guard cells during their development from protodermal origins. Stomatal ontogeny in Polypodium vulgare was described by Reuter (1942). This investigation was carried out since only two comprehensive accounts exist on the structure of immature guard cells (Kaufman et al., 1970; Palevitz and Hepler, 1976), and neither of these are on fern species.

Terminologies

The terminologies employed in respect of the ontogeny of the stomatal complex and its eventual morphological form are those used by Stevens and Martin (1978).

The term 'stomatal complex' refers to the complete stomatal apparatus and includes the guard cell pair, the subsidiary cells, and the neighbouring cells. The term 'Stroma' is used in its restricted sense and is applied only to the pore which is formed between a pair of guard cells. The guard cell pair plus its associated subsidiary cells (if present) is commonly subtended by an extended air space in the mesophyll which is identified as the substomatal chamber.

Methods and Materials

The investigation was carried out on young circinately-folded pinnae obtained from P. vulgare plants which had originally been collected from a variety of sites in the Plym Forest, Devon. Whole pinna segments were used in the electron microscope studies, whilst detached epidermal strips were used for the light microscope studies.

Electron Microscopy

Pinna segments were vacuum-infiltrated with 1.5% glutaraldehyde in 25 mmol l⁻¹ cacodylate buffer (pH 7.0), followed by 2% osmic acid in cacodylate buffer for 2.5 h, dehydrated in acetone, and embedded in Spurr Resin (Spurr, 1969). Silver and gold sections were obtained with an LKB Ultratome III and a Porter Blum MT2-B Ultramicrotome, mounted on coated grids and examined with a Phillips EM 300. No post-staining was employed unless stated to the contrary in the figure legends.

Light Microscopy

Light microscopy was carried out on a Carl Zeiss Photomicroscope II. Pectinaceous materials were tested for by the Ruthenium Red
The stomatal meristemoid / guard-cell mother-cell is immediately identifiable from other protodermal cells by its dense cytoplasm, small size, and individual shape. However, we have not been able to identify the nature of the protodermal cells immediately adjacent to the stomatal meristemoid/guard-cell mother-cell as either prospective neighbouring or subsidiary cells and, therefore, it has been difficult to distinguish, with certainty, stomatal meristemoids from guard-cell mother-cells. Despite this, it is possible to infer the developmental stage of the meristemoid from its overall size, shape, and spatial relationships with adjacent cells.

The stomatal meristemoid is believed to arise from a vertical asymmetrical division of a protodermal cell very shortly after the latter cell has been cut off from the marginal meristem of the pinna. The inception of the stomatal meristemoid, in this species, is conjectural since we have not been able to observe its formation even after the examination of a large number of protodermal strips. Light microscopy indicates that the young stomatal meristemoid is sub-cuboid in shape and marginally smaller than its adjacent protodermal cells. The first division(s) of the stomatal meristemoid are also asymmetrical but do not occur in a strictly vertical plane so that the resultant subsidiary cell(s) obliquely subtend the meristemoid immediately following such division(s). At this stage, the stomatal meristemoid is sub-circular in paradermal section and lens-shaped in vertical section. The subsidiary cells quickly retract from their partially inferior positions and eventually only subtend the margin of the meristemoid.

After the (last) subsidiary cell has been cut off, the stomatal meristemoid becomes the guard-cell mother-cell and its next and final division is of a symmetrical type which gives rise to the guard cell pair. We consider that this stage is preceded by the guard-cell mother-cell reverting to its sub-cuboid shape (i.e., it is no longer partially subtended by a subsidiary cell), its increased size, and its tendency to tear away from the underlying mesophyll tissue (Fig. 2).

Electron Microscope Observations

The earliest developmental stages observed were as illustrated in Figure 1. The typical lenticular cross-section of the cell suggests that it is a stomatal meristemoid, rather than a guard-cell mother-cell, and, furthermore, it is believed that the osmiophilic ribbons present in the section are chromosomal material gathered at a pole during a mitotic event which would have led to the formation of a subsidiary cell.

It is noteworthy that the chloroplasts of the stomatal meristemoid contain prominent granal lamellae and starch inclusions in contrast to the adjoining protodermal cells. At this stage, there is little sign of vacuolation in the stomatal meristemoid, whilst adjacent protodermal cells are already showing signs of maturation in the form of quite extensive vacuolar systems.

As the stomatal meristemoid develops into the guard-cell mother-cell, the most obvious changes noted were the changes in cross-sectional dimensions and progressive vacuolation (Fig. 2). By this stage, the adjacent neighbouring/subsidiary cells are very highly vacuolated so that almost the entire lumen of the cell is occupied by a single large vacuole with the protoplast and its inclusions of organelles being restricted to the periphery of the cell. Figure 2 also illustrates the endocuticular covering of the inner face of the guard-cell mother-cell beginning to pull away from that which covers the underlying mesophyll tissue.

Immediately following the division of the guard-cell mother-cell, membranous vesicles are laid down in the plane of the future common anticlinal guard cell wall (Fig. 3). As the deposition of the cell wall elements proceeds about these vesicles, the latter enlarge and fuse together. Concomitantly, a wedge of cell wall material commences to be laid down at the junctions of the upper and lower extremities of the developing anticlinal wall with their respective periclinal walls (Fig. 4). At about the same time, the middle lamella of the common anticlinal wall becomes clearly differentiated.

During the maturation process, the wedges associated with the upper and lower extremities of the common anticlinal wall become massively developed (Fig. 5). The wedges can be seen to be formed by the sequential deposition of layers of cell wall material onto the inner face of the thickenings next to the guard cell protoplast. This build up of cell wall material causes a considerable deformation of the guard cell lumen which eventually leads to the nucleus becoming almost wedged between the thickenings of the anticlinal walls (Fig. 5). At the same time, the region of the outer periclinal walls and, to a lesser extent, that of the inner periclinal walls, associated with the wedge-shaped thickenings become depressed below the general levels of these cell walls (Figs. 5 and 9). Following the formation of the wedge-shaped