The plasma membrane of young Chara internodal cells revealed by rapid freezing

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Abstract. Young elongating internodal cells of Chara globularis var. capillacea (Thuill.) Zanev. were rapidly frozen and freeze-fractured in order to observed transient events occurring within the plasma membrane. Several structures have been observed. Relatively small depressions, varying in depth, are prolific and scattered at random over the plasma membrane. Charasomes and clusters of particle rosettes are common. Arrays of intramembrane particle lines are a characteristic feature of the internodal cell plasma membrane. The charasomes and the arrays of particle lines occupy a considerable proportion of the plasma membrane. In these young cells, substantial movement must take place across this membrane and its basic structure must fluctuate accordingly. The innumerable small depressions may represent pinocytotic and secretory processes. The array of intramembrane particle lines may represent stages in fusion between the membranes of vesicles within the cytoplasm and the plasma membrane. The technique of ultra-rapid freezing allows these events and their intermediate stages to be visualised; some features of the membrane may only be seen by this method.

Key words: Chara – Charasome – Intramembrane particle (lines, rosettes) – Membrane fusion.

Introduction

Rapid freezing methods have had considerable success in visualising transient events within the plasma membrane, for example, a sequence of events during the process of membrane fusion (Chandler 1979) can be observed. The individual stages are captured by the rapid immobilisation of both membrane components and the aqueous phase. Other benefits from this particular fixation method are that it enables chemical fixation and cryoprotection to be by-passed.

The young internodal cells of the freshwater alga, Chara, elongate rapidly. In such cells much activity, involving the growth of the cell and subsequent exchanges between cell and environment, must occur across the plasma membrane. Rapid freezing provides an ideal fixation for the study of these exchanges.

Material and methods

Chara globularis var. capillacea (Thuill.) Zanev. was obtained from the Botanic Garden, Oxford. This species is corticate. The external walls of the cortical cells have an irregular and

![Diagram of Chara internode](image)

Fig. 1. Diagrammatic representation, not to scale, of part of a Chara internode in longitudinal section. The material making initial contact with the copper block would be at the top of the diagram. During fracture, the knife describes a low arc through the material to ensure the fracture line passes through the zone of best fixation. Neither the cells, nor the knife arc, are drawn to scale, however the fracture lines in the present study encompass the regions shown in the diagram. 

- cw, cell wall; 
- cc, cortical cell; 
- pm, plasma membrane; 
- ic, internodal cell; 
- t, tonoplast; 
- chl, stationary chloroplasts; 
- v, vacuole; 
- e, encrustation
Results

Several structures within the plasma membrane of the internodal cell are immediately obvious (Fig. 2). A length of thin-sectioned plasma membrane which can be correlated with this view, is seen in Fig. 3.

Small depressions approx. 0.1 μm in diameter are prolific on the plasma membranes of both cortical and internodal cells (Figs. 2, 4, 8). In appearance they vary from shallow indentations containing a few intramembrane particles to cavellae whose depth has prevented the complete penetration of the platinum shadowing. Some depressions are defined by a distinct ring (Fig. 2).

Highly reticulate, colander-like, structures, which are consistent with the sectioned images of charasomes (Crawley 1965; Barton 1965), ranging from 0.3–2.0 μm across are present on the internodal cell plasma membrane (Fig. 2) and on the plasma membrane of cortical cells (Figs. 4, 5, 6). These structures are extremely common, not appearing in any regular pattern, but occurring over the surface of the cytoplasm sometimes singly, sometimes in groups. Figs. 4 and 5 show membrane fractures at various levels within these structures, and cross-fractures through the cytoplasm in their vicinity. The conventional appearance of a charasome in thin section can be seen in Fig. 7.

A second large group of distinctive structures found on the internodal cell plasma membrane, consists of arrays of particle lines (Fig. 2). These arrays range from 0.25–2.3 μm in length. Some arrays are relatively flat, others raised onto definite mounds (Figs. 8, 10). Lines of particles which totally enclose small areas of plasma membrane had few or no single particles within their boundary (Fig. 8, arrowed). In some of the mounds the plasma membrane appears to have split open, revealing a homogeneous interior (Figs. 8, 11). Fig. 12 shows a diagram of selected lined particle arrays from two endodermal cell plasma membranes, the extra-neous particles omitted to emphasise the variety in shape and size of the arrays. Particle counts in the undisturbed plasma membrane are highly variable (Table 1, column 1). Particle counts within the lined areas, and including those particles constituting the lines, are consistently higher but equally variable. Several vesicles commonly seen in thin sections (Figs. 3, 13) lie within the size range of the lined particle arrays. In shape, these vesicles vary from small, almost spherical sacs containing...