The Ultrastructure of Plasmodesmata in the Filamentous Green Alga, *Bulbochaete hiloensis* (Nordst.) Tiffany

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Summary. The ultrastructure of the plasmodesmata found in the green alga *Bulbochaete hiloensis* has been examined by electron microscopy of ultra-thin sections. Unlike most other plasmodesmata that have been described recently, there are no internal components such as a desmotubule or a derivative of the endoplasmic reticulum. Each plasmodesma consists of a cylindrical connection between the plasma membranes of adjacent cells. The cylinder is constricted at each end to orifices which may be less than 100 Å in diameter. Within the cylinder the cytoplasmic face of the plasma membrane is lined with material probably consisting of helically arranged particles. The lumen here is 400—450 Å in diameter.

The observations are discussed in relation to possible functions in intercellular transport.

Introduction

Recent publications concerned with the ultrastructure of plasmodesmata have laid emphasis on the existence of internal structures lying along the axis of the cylinder of plasma membrane that traverses the cell wall (O'Brien and Thimann, 1967; Robards, 1968). By contrast, Bisalputra (1966) has claimed that certain plasmodesmata contain no such axial components, although they are spacious enough to do so. The purpose of the present paper is to provide new information on some details of the ultrastructure of one example of the latter type of plasmodesma, and to discuss possible implications of the observations.

Material and Methods

Cultures of *Bulbochaete hiloensis* (Nordst.) Tiffany, strain 555/2 (Indiana No. 954) were obtained from the Culture Collection of Algae and Protozoa, Botany School, University of Cambridge, and maintained in soil/water media under fluorescent lamps. Fixation in 2½% glutaraldehyde in 0.025 M phosphate buffer, pH 7, proved to be satisfactory. After rinsing in buffer and post-fixation at 0 °C for 3 hrs in 2% osmium tetroxide, filaments were dehydrated in graded solutions of acetone or ethanol. Extreme difficulty was experienced in infiltrating the cells with embedding media. Numerous different resin mixtures were tried, in no case with complete success. The illustrations included here represent material that was embedded in a mixture composed of 35% nadic methyl anhydride, 15% dodecenyl succinic anhydride, 50% Epikote 812. Blocks were sectioned using a diamond knife and a
Cambridge Instruments Co., Huxley pattern, ultramicrotome, and the grids were treated sequentially with uranyl acetate and lead citrate (Reynolds, 1963), and examined using a GEC-AEI EM6B electron microscope fitted with thin metal objective apertures (C.W. French & Co.) and with the condenser 1 modification described by Meer (1968).

Observations

*Bulbochaete* (Fig. 1) is a branched filamentous green alga. Plasmodesmata interconnect the axial, photosynthetic cells of the filaments, and also these cells with the bulbous-based hairs which many of them produce (Figs. 2 and 3). Plasmodesmatal ultrastructure is the same in both types of situation.

The membranous component of each plasmodesma occupies a canal that pierces the cell wall normal to the plane of the wall. The canal is generally about 1200 Å in diameter, and its walls tend to be rather ragged, (Figs. 4—18) suggestive of a mode of formation that involves a digestive process. The length of the canal depends on the thickness of the wall (Figs. 4, 5), and, in *Bulbochaete*, this in turn depends on the number of times the cells have divided. As in plasmodesmata in other plants, the plasma membranes of the neighbouring cells are continuous. Within the wall canal they take the form of a cylinder with a uniform outer diameter of about 860 Å (Figs. 4—6). At either end of the cylinder the membrane is constricted to a comparatively small orifice (80—150 Å internal diameter) (Figs. 10, 11). Since the cylinder is slightly longer than the thickness of the wall, the plasma membranes lining the walls become raised in a dome at each end of each plasmodesma (Figs. 4—11). The pore leading into the lumen of the cylinder is near the apex of the dome, and here the plasma membrane is often convoluted (Fig. 6).

The cylindrical plasma membrane of the *Bulbochaete* plasmodesma probably owes its uniformity of diameter to the presence of a closely packed array of particles on the internal, or cytoplasmic, face of the membrane. The particles collectively comprise a cylinder of internal diameter 400—450 Å. Glancing sections sometimes include views of the surface of the particulate material (Fig. 7). In these views the individual particles appear to have electron-lucent centres surrounded by a denser zone, much as in the particles observed in thin sections of chloroplast thylakoids and thought to be lipoproteins (Weier and Benson, 1967). Many micrographs have been obtained in which the e is clear evidence that the particles are arranged in a helical pattern, although they are not resolved individually (Figs. 6, 9). The pitch suggests a two-start helix (Fig. 6). Favourable images show that the particles are closely appressed or attached to the inner (cytoplasmic) leaflet of the tripartite plasma membrane. Not dissimilar material is also occasionally seen in this location on the plasma membrane on and near the domes.