A NEW PATTERN OF PLANT CELL ELONGATION: BIPOLAR BAND GROWTH

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INTRODUCTION

Growth in cylindrical plant cells may occur either at the tip of apical cells or along the side walls of apical and subapical cells. In cells elongating by tip growth, extension and primary wall synthesis are localized at the apical tip of the cell (1). Cells such as fungal hyphae (2, 3), pollen tubes (4), and root hairs (5, 6) elongate in this way In the case of cells whose side walls grow, cellular elongation (7, 8) and wall synthesis (9) occur throughout the cells' length

The pattern of cell elongation is correlated with the ultrastructure of the cell wall. For example, in the walls of tip-growing cells, the polysaccharide microfibrils (often cellulosic in nature) are randomly arranged and are not rearranged during growth (11). On the other hand, in cells whose side walls elongate, microfibrils in the walls are arrayed in a nonrandom fashion. Here, microfibrils are laid down next to the cytoplasm in a predominantly transverse orientation and become reoriented longitudinally during growth; this pattern of growth has been called multinet growth (11). Because microfibrillar arrangement is correlated with growth pattern, wall ultrastructure has been used to predict the growth patterns of cells whose growth pattern is difficult to measure directly (10, 11).

Although patterns of cell elongation have been studied in fungi, green algae, and higher plants, no one has yet determined the way in which red algal cells grow. Some reasons why these organisms may have been overlooked in such studies include their small cell size, relatively slow growth rates, and the difficulty of growing them under laboratory conditions While cell elongation patterns have not been directly investigated

in red algae, wall ultrastructure has received some attention In all cases studied, the microfibrils in red algal walls have been found to be randomly arranged (12, 13, 14). Such observations have led Roelofsen to suggest that red algal cells elongate by apical tip growth (11). We shall show that intercalary shoot cells of the red alga, *Griffithsia pacifica*, elongate neither by apical tip growth nor by multinet growth but by a previously undescribed type of elongation, bipolar band growth

MATERIALS AND METHODS

In these experiments we have used an unialgal isolate of the giant-celled, marine alga, *Griffithsia pacifica* Kylin (Rhodophyta, Ceramiales), which we obtained from the University of Washington, Seattle, marine algal culture collection. Culture techniques and conditions have been previously described (15). Fig. 1 shows a plant regenerated from a single shoot cell This plant has two distinct cell systems: an upright, highly pigmented shoot portion, and a prostrate, adhesive rhizoidal system. We have studied elongation in both shoot cells and rhizoidal cells.

To determine where along a cell elongation takes place, we applied markers (charcoal grains) to the surfaces of cells and photographically followed their displacement with time. Markers should move apart wherever extension occurs. For these experiments we used plants which had grown for 3–5 days from single shoot cells. To hold plants flat for photographing when shoot cell elongation was studied, plants were clamped to a microscope slide by glass rods which were cemented to one end of the slide; the slide was then immersed in a small Petri dish of enriched seawater (PES [16]). When rhizoidal cell elongation was studied, this apparatus was unnecessary as rhizoids adhered to the Petri dish bottom and thus held themselves flat. Plants were grown at 20°C on a 16 hr light: 8 hr dark photoregime at 3300 lux from "cool white" fluorescent lamps.

To study the fine structure of shoot cell walls, we excised shoot cells and removed their cytoplasmic contents by stroking the cells with a platinum wire loop. Cells were cut open to reveal the inner surface of the cell wall, and platinum-carbon replicas were made of this surface. After replicas were cleaned by floating on 70% sulfuric acid and on Chlorox, they were examined in a Zeiss EM-9 electron microscope.

Birefringence measurements were made with a Zeiss polarization microscope.

RESULTS AND DISCUSSION

Shoot and rhizoidal cells of *Griffithsia* have different elongation characteristics. In a rhizoid, only the apical cell elongates, when a cell becomes subapical, it ceases growing. In the shoot, however, elongation takes place in the upper 3-4 mm of an axis (15, 17). Individual shoot cells elongate for 6–8 days after becoming subapical; this intercalary elongation involves an 8- to 10-fold increase in cell length (15). From such observations it seems likely that these two cell types have different elongation patterns, and a priori we might predict that rhizoidal cells extend by tip growth while shoot cells elongate by multinet growth As we shall see, this prediction is only partially correct.

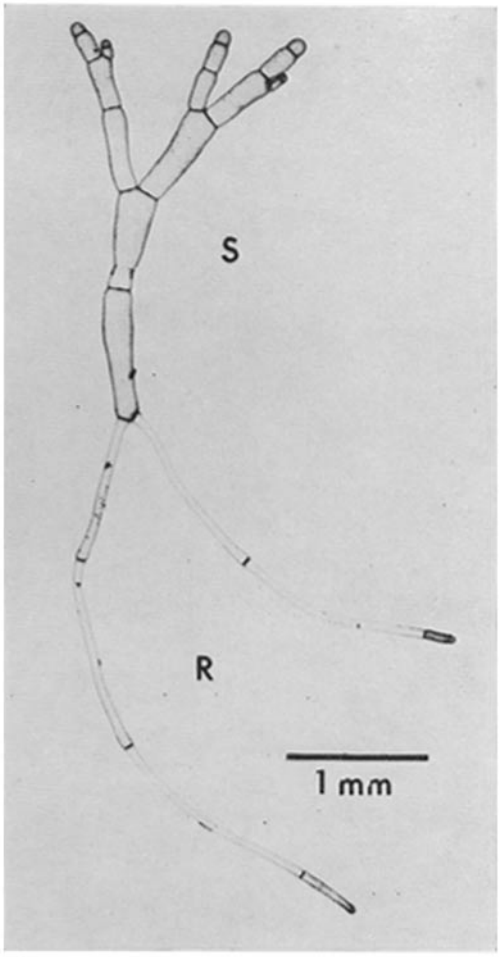

FIGURE 1 A 5 day old regenerate of *G pacifica* started from a single shoot cell. *S*, shoot portion; *R*, rhizoids. × 18.5.

Fig 2 shows the growth of a rhizoidal apical cell. Only charcoal grains at the tip of the cell are displaced; those located behind the tip do not

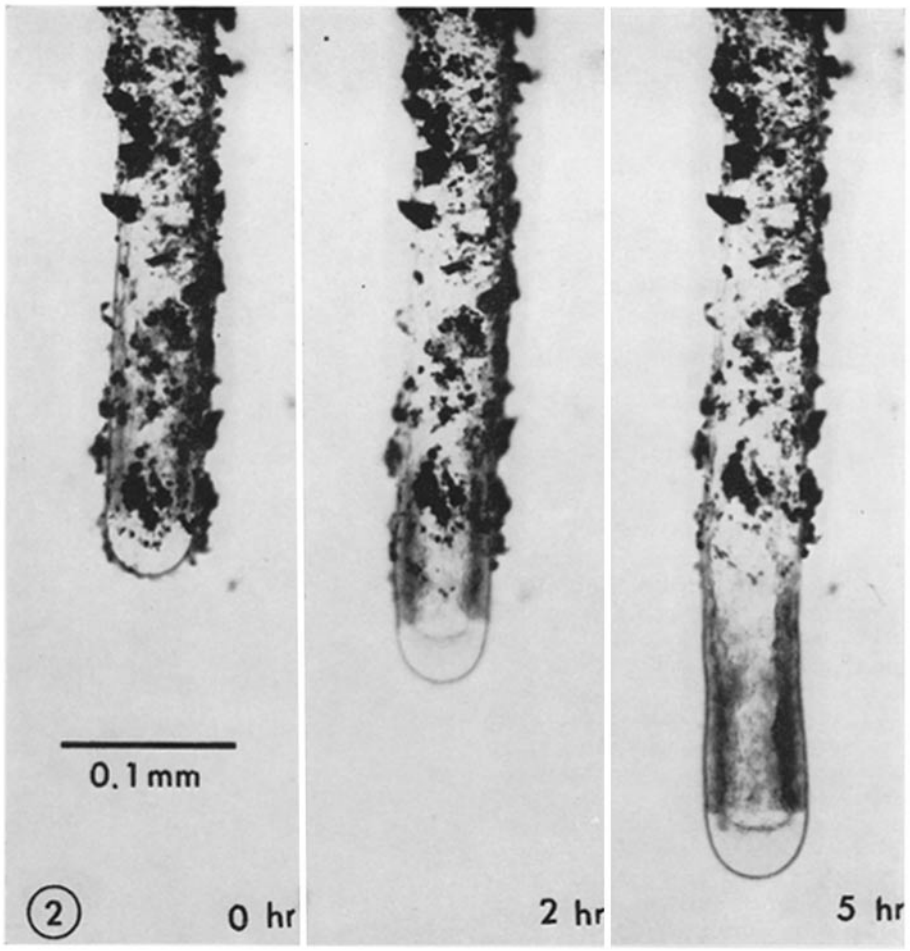

FIGURE 2 Growth of a rhizoid apical cell. Black particles are charcoal markers. × 230.

move apart. Therefore we can conclude that rhizoidal cells elongate by classical tip growth. The stratification seen in the apical cell cytoplasm (a clear zone at the very tip and basipetally a highly pigmented area containing plastids, nuclei, mitochondria, etc.) is similar to that of other tip-growing cells. In fungi this area has been shown ultrastructurally to contain vesicles thought to be involved in wall synthesis (18).

Now let us examine elongation in the cells of the shoot; here, as in the rhizoid, there are no intercalary cell divisions. In Fig. 3 we see the growth of the shoot portion of a plant over 2 days. Especially interesting is the pattern of elongation in intercalary cells Here charcoal grains have been displaced only at the tops and bottoms of cells. In the second cell, arrows mark charcoal grains originally near the upper and lower ends of the cell. The distance between the arrows remains the same even though the cell has tripled in length. Growth is confined to two bands, one at each end of the cell; the upper growing zone appears to be more active than the lower one.

To define the growing zone more precisely, we followed elongation at shorter time intervals and used higher magnification. In Fig. 4 the growth of an individual intercalary shoot cell is shown. We can see that only those charcoal grains originally near the very top of the cell have moved apart; the lower grains remain the same distance from each other. The zone on which grains move apart and therefore in which elongation occurs is just below the crosswall and is about 20 μ wide. We have confirmed this growth zone size in many other shoot cells, and it is always a 20 μ band at the top of each cell. At the base of the cell in Fig.

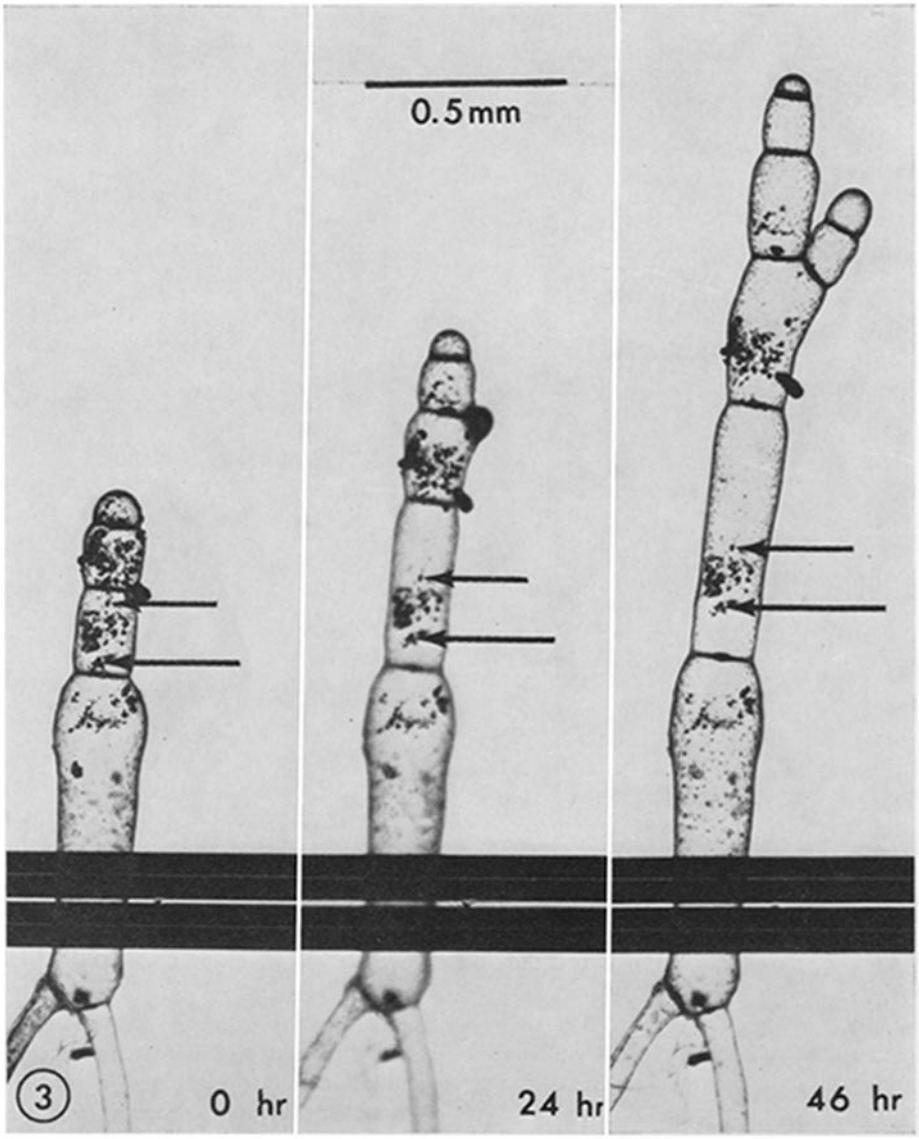

FIGURE 3 Growth of the shoot portion of a *Griffithsia* plant. Black particles are charcoal grains. Arrows indicate the positions of specific grains during the experiment. × 53.

4, we see the less active basal growth zone whose dimensions are also narrow.

We have examined the ultrastructure of the inside of the wall of such shoot cells and we find that the microfibrillar arrangement is essentially random (Fig. 5). Myers et al. (12) found that the walls of *Griffithsia flosculosa* also have random microfibrillar orientation. The shoot cell walls of *G pacifica* show a very weak negative birefringence which indicates that there is some slight orientation in the transverse direction. The birefringence appears to be uniform along the length of the wall.

In *Griffithsia*, the pattern of elongation of intercalary shoot cells is different from any other previously demonstrated in plant cells. It is unlike tip growth because growth occurs in subapical rather than in apical cells, because the side walls rather than the tip of the cell elongate, and because cells elongate at both ends It differs,

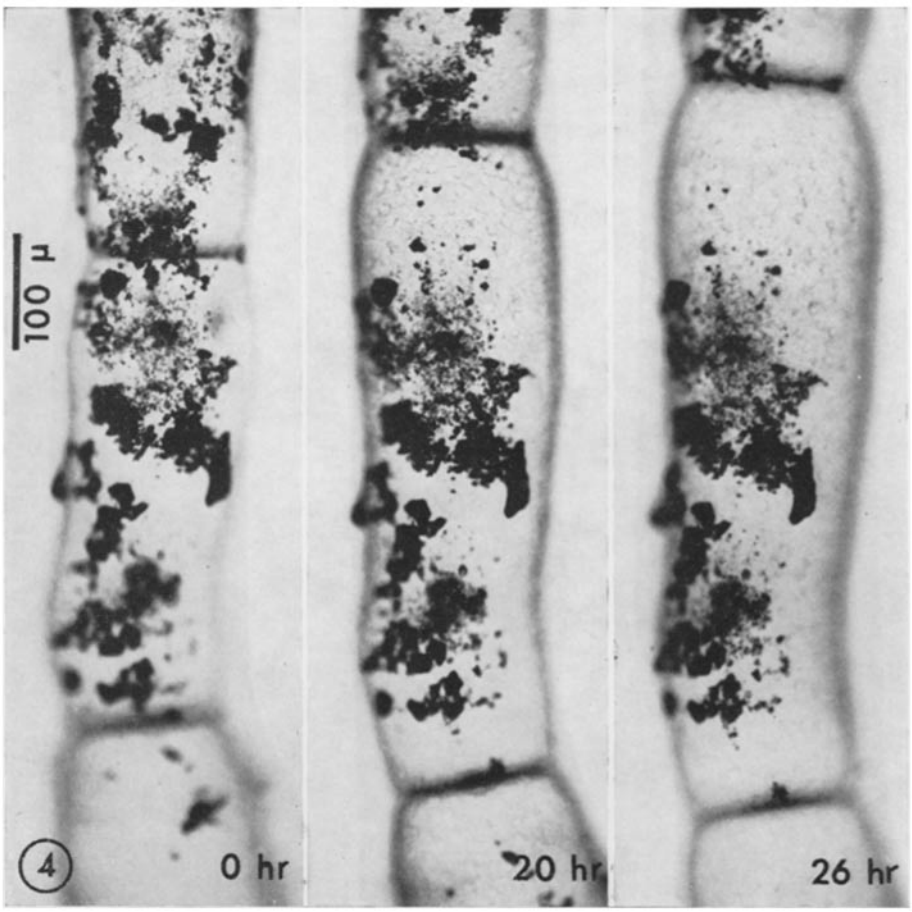

FIGURE 4 Growth of an individual shoot cell. Black particles are charcoal markers. Note the behavior of grains near the very top and bottom of the cell compared to those more centrally located. × 155.

both in the ultrastructure of the elongating wall and in the localization of extension, from elongation in all other plant cells whose side walls elongate *Griffithsia* cell walls are essentially anisotropic and their innermost microfibrils are randomly arranged, while the walls of all other elongating intercalary cells have strong transverse orientation and multinet structure (11) Extension in *Griffithsia* shoot cells is localized to specific bands rather than occurring throughout the length of the cell. Growth of a sporangium-bearing sporangiophore of the fungus *Phycomyces* does occur in a localized, subapical band, but in this case the band is much wider (*circa* 2 mm) and the growth involves a multinet-reorientation of oriented microfibrils (11)

Mühlethaler (19) and Frey-Wyssling (20) initially suggested that parenchyma cells and vascular elements elongated by "bipolar tip growth." Subsequent evidence has shown that this is certainly not the case for parenchyma (7, 9) and is probably not true for vascular elements (21). In any case, since elongation of vascular elements is intrusive, does not involve tissue elongation, and most likely involves the very tips of cells (10, 11), it is quite different from the growth of *Griffithsia* intercalary cells. On the basis of birefringence measurements, growth at both ends of subapical cells has been suggested in the green alga *Chaetomorpha linum*, but this has not been demonstrated by direct observation of elongation patterns in living cells (22).

We have shown that there are two types of cell elongation in the red alga, *G. pacifica*. Rhizoidal

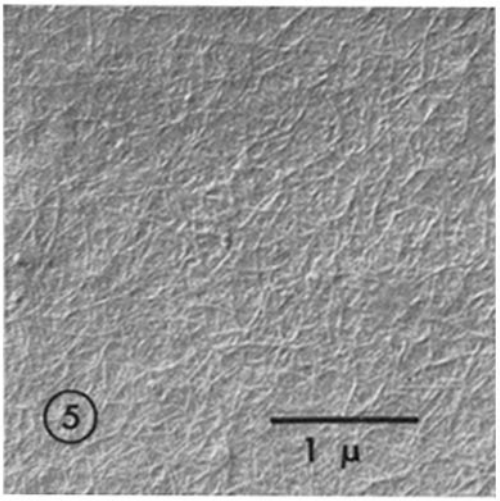

FIGURE 5 A replica of the inside (cytoplasm side) of the cell wall of a shoot cell of *G. pacifica*. Fiducial mark = 1 μ. × 19,500.

cells elongate by classical tip growth while intercalary shoot cells elongate in a pattern different from that of any other plant cell heretofore studied. This pattern is a bipolar band growth, extension being confined to two narrow bands, one at each end of a cell.

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