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Polarity and Growth of Caulonema Tip Cells of the Moss *Funaria hygrometrica**

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Abstract. In the caulonema tip cells of *Funaria hygrometrica*, chloroplasts, mitochondria, and dictyosomes have differences in structure which are determined by cell polarity. In contrast to the slowly growing chloronema tip cells the apical cell of the caulonema contains a tip body. Colchicine stops tip growth; it causes the formation of subapical cell protrusions, redistribution of the plastids, and a loss of their polar differentiation. Cytochalasin B inhibits growth and affects the position of cell organelles. After treatment with ionophore A23187, growth is slower and shorter and wider cells are formed. D_2O causes a transient reversion of organelle distribution but premitotic nuclei are not dislocated. In some tip cells the reversion of polarity persists; they continue to grow with a new tip at their base. During centrifugation, colchicine has only a slight influence on the stability of organelle anchorage. The former polar organization of most cells is restored within a few hours after centrifugation, and the cells resume normal growth. In premitotic cells the nucleus and other organelles cannot be retransported, they often continue to grow with reversed polarity. Colchicine retards the redistribution of organelles generally and increases the number of cells that form a basal outgrowth. The interrelationship between the peripheral cytoplasm and the nucleus and the role of microtubules in maintaining and reestablishing cell polarity are discussed.

Key words: Caulonema – Cell growth (tip) – *Funaria* – Microtubules – Organelle modification – Polarity.

* Dedicated to Prof. Dr. A. Pirson on the occasion of his 70. birthday

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Abbreviations: DMSO = dimethylsulfoxide; CB = cytochalasin B

Introduction

The tip cells of the caulonema of the moss, *Funaria hygrometrica*, are a highly polar system. The chloronema is the juvenile form of the protonema of a moss, the caulonema the secondary form. The latter consists of cell filaments with brownish cells walls, oblique cross walls, few usually spindle-shaped chloroplasts, and, under the light microscope, a clearly visible nucleus (Bopp, 1961).

We studied, with both light and electron microscopy, the morphological polarity of the tip cells and their growth, under normal conditions and in protonemata treated with inhibitors and centrifugation, to determine whether it is possible to change or even to reverse the polarity or its manifestation. We especially tried to trace whether microtubules, microfilaments, and a Ca^{2+} gradient are involved in the realization and maintenance of polarity.

Material and Methods

The methods have been described in detail by Schmiedel (1978). The protonema of *Funaria hygrometrica* Sibth. was grown on cellophane films, covering agar (Difco-bacto-agar with Knop-solution) in Petri dishes.

Inhibitors were added to the solidifying agar. We used dimethylsulfoxide (DMSO) as the solvent for cytochalasin B (CB) and the ionophore A 23 187. To stabilize the protonemata during centrifugation 2% nutrient agar (38° C) was poured over them. They were then excised, transferred to a slide, wrapped with a cellophane film, and placed in a centrifuge tube filled with 3% solidifying (38° C) agar. The specimen was centrifuged usually for 20 min with 600–4,000 rev min^{-1} (60–2,700 *g*). Thereafter the protonema cells were fixed either for light or electron microscopy or cultivated under standard conditions.

For electron microscopy, the fixative (2.5% glutaraldehyde in 0.06 M PIPES buffer, pH 8.0, 20° C) was cautiously poured

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over the protonemata. After 1 h they were covered with solidifying agar (2% in 0.06 M PIPES, pH 8.0), postfixed (1% OsO_4 in 0.06 M PIPES, pH 6.8, in an ice bath, 1 h, Salema and Brandão, 1973), and embedded.

Results

Normal Structure and Growth

The tips of 14 to 17-day old protonemata have the typical features of the caulonema (Fig. 1 and 2). The cell length is 280–300 µm (200 µm or less in the chloronema), and the growth rate is about 48 µm h^{-1}. The growth zone is restricted to the very tip as shown by labeling with latex particles. The tip cells elongate from 140 µm (postmitotic) to 400 µm (premitotic) and divide every 6–7 h, but the divisions are not synchronous. During growth the distance between the tip and the nucleus is constant (120 µm). The caulonema growth rate remains constant during nuclear division. After cytokinesis the nucleus of the new tip cell soon reaches its normal position. Under unfavorable conditions, "intercalar divisions" (Knoop, 1973) occur.

The apical region in the tip cell contains a tip body which is free of plastids (Fig. 1, 2 and 3). It consists of a clear cap (about 4 µm long), primarily containing different types of Golgi vesicles, for forming a tip body and a subsequent granular zone (about 20 µm long); dictyosomes are concentrated here. The subapical region (plastid zone) is characterized by an abundance of plastids and a dense cytoplasm which extends to the nucleus. The basal part of the cell contains a voluminous vacuole and extends during cell growth (up to 280 µm). The peripheral cytoplasm includes only a few organelles. Microtubules are found in a subplasmalemmal position, as well as in the interior of the cell, where they often are associated with the nuclear envelope, mitochondria, and plastids. They are lacking in the apical vesicle zone (Fig. 4).

The chloronema tip cells grow more slowly (4–10 µm h^{-1}) and have no distinct tip body.

Cell polarity is not only expressed by the unequal distribution of cell organelles but also by their differentiation. There is a group of about 10 roundish, large (diameter about 5 µm) amylochloroplasts with many large starch grains in the tip region (Fig. 1, 2 and 3). The chloroplasts in the more basal parts of the plastid zone have less starch and more thylakoids. In the vacuole zone the chloroplasts are spindle-shaped and greener; their starch content is low (Fig. 1 and 2). The polar distribution and differentiation of plastids is also found in the second and third cell of the caulonema tip, although less distinct (Fig. 1).

The mitochondria in the apical region are short and often even globular. In the basal part of the cell they are elongated and reach a length of 10 µm.

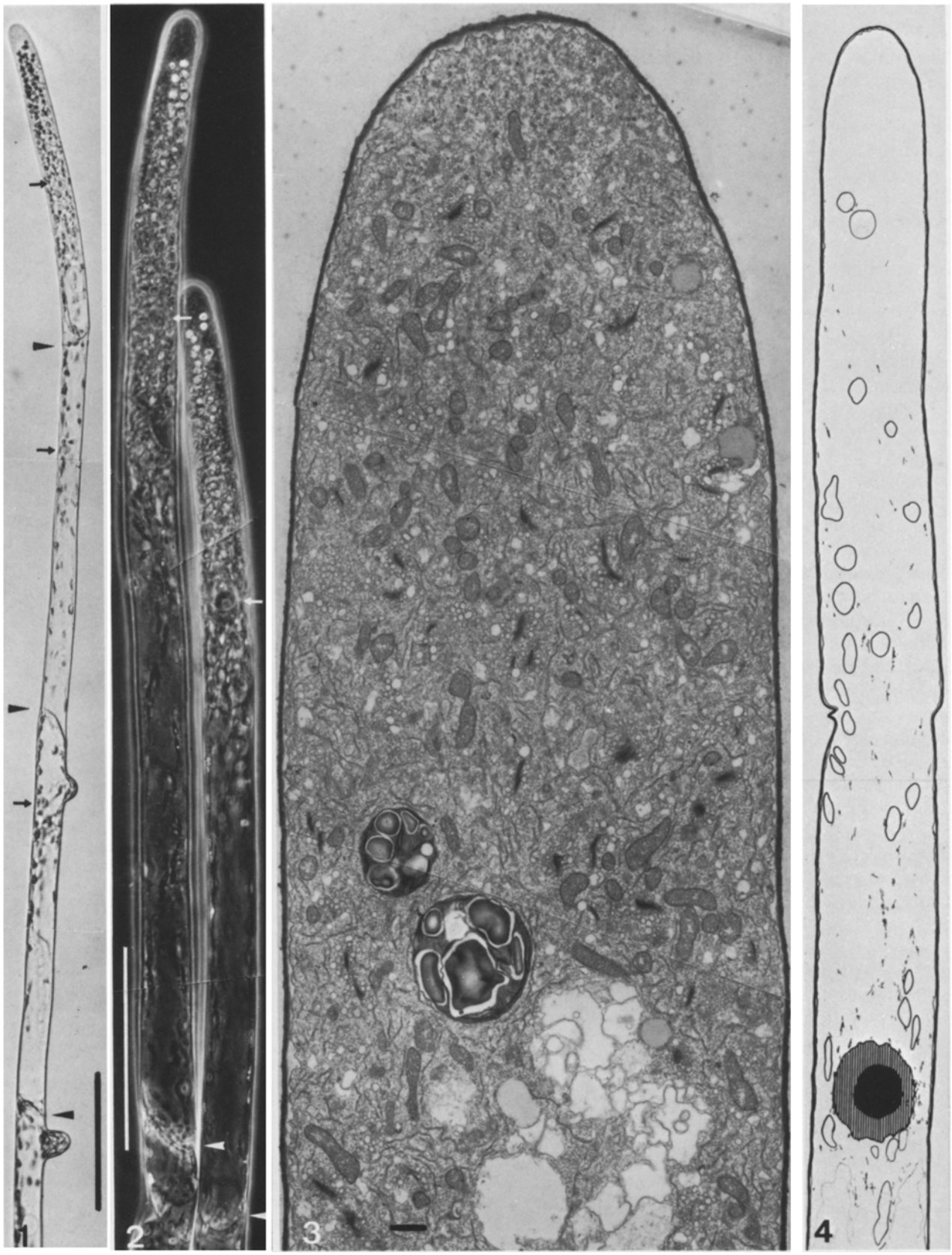
In the apical region, the dictyosomes consist of up to 9 Golgi cisternae instead of the 4–6 in the more basal parts of the cell. The vesicle production does not seem to be considerably higher in the tip region.

The Effects of Inhibitors on Structure and Growth Colchicine

We applied colchicine in concentrations of from 0.025% to 0.3%, mainly 0.1%. About 10 min after colchicine application the nucleus of the tip cell ceases movement; it often drifts backward somewhat. The cell does not continue to grow. After about 30 min nearly all microtubules disappear; only very few cortical microtubules persist somewhat longer. After 24 h on colchicine agar the first microtubules reappear. Within 45 min the tip body is reduced drastically and is often displaced laterally. Its composition of different types of vesicles, as well as the activity of the dictyosomes, do not seem to change. The cell apex begins to inflate irregularly. Plastids assemble into small groups and tend to concentrate around the nucleus. Later, the activity of the dictyosomes is reduced. After a few hours, the cells form subapical irregular protrusions, at one or more points (Fig. 5), which soon cease growing (Fig. 7). The cell also becomes vacuolated in the apex which remains free of plastids. More or less, the organization of the cytoplasm into an organelle-rich apical zone and a vacuole-rich basal one is maintained, as well as the general distribution of the plastids. The gradient in plastid modification, however, appears to be lost, although the plastids continue to retain considerable quantities of starch for a certain time.

D_2O. Depending on the concentration, D_2O interrupts the growth of *Funaria* caulonema cells for a certain time (50%: about 12 h, 80% and 96%: about 40 h, 100%: absolutely). Thereafter, growth is continued but at a reduced rate, and intercalar divisions occur. To test the effects of D_2O on the polar organization of the tip cell in short-time experiments, we used mainly 80–90% D_2O in the substrate.

Growth stops and the polar zonation breaks down soon after the transfer of the caulonema to D_2O. The tip body disappears, the dictyosomes become reduced in size, and the apical amylochloroplasts intermingle (Fig. 8 A) with the other organelles in the anterior part of the cell. The nucleus moves backward at a rate of 26 µm h^{-1} and becomes positioned near the basal cross wall (Fig. 8 A). Plastids are also dis-



Figs. 1 and 2. Tip region of a caulonema filament. Polar distribution and differentiation of organelles. Arrowheads: cross walls; arrows: nuclei. **Fig. 1:** Bright field, **Fig. 2**: Phase contrast; marker bar 100 μm

Fig. 3. Tip of an apical cell with "vesicle zone", "Golgi zone", and the distal part of the "plastid zone" (with 2 amylochloroplasts). Marker bar 1 μm

Fig. 4. Drawing of the tip region of an apical cell (the same as in Fig. 3) demonstrating the distribution of microtubules. Plastids have a closed, vacuoles a dotted outline, the nucleus is hatched

placed to the basal end of the cell and gradually lose their starch. The reversal of the polar distribution of the cell organelles is completed after about 6 h, with the exception of some very long-tip cells which are obviously in a stage just prior to cell division; they appear to become multinucleate.

In the subapical cells the polarity is likewise sometimes reversed and the nucleus moves backward, but more often the organelles become distributed irregularly throughout the cell. In these cells, too, nuclei, just before or during mitosis, are not dislocated.

The cell wall becomes thickened in the cell apex with a conspicuous irregular wall layer (Fig. 10) which includes vesicular structures and callose. The number and structure of the plasmodesmata is not changed.

The mitochondria and the plastids often seem to be affected transiently (Fig. 10). The microtubules do not seem to increase. The nucleus, present in the basal accumulation of cytoplasm, is surrounded with only a few microtubules.

In most tip cells the reversal of polar distribution of cell organelles is not stable. After about 9 h the nucleus begins to migrate to its former position, which is reached not earlier than after 12 h (Fig. 8B). The nucleus' movement is associated with bundles of microtubules which occur especially in cytoplasmic strands between the nucleus and the basal cross wall. They are particularly numerous in grooves in the basal part of the nucleus. The plastid gradient is restored but more slowly and more incompletely. The characteristic tip amylochloroplasts are not found before growth has been resumed (Fig. 10). The growing tip is usually formed slightly subapically (Fig. 9).

The reversal of polarity persists in some filaments. Some apical cells divide intercalarly in their basal region. Other apical cells develop a new growing tip from the cell base which often even grows in a backward direction, i.e., toward the center of the protonema, with a rate of about 8 μm h^{-1} (Fig. 9); later this outgrowth divides regularly. In some cases, however, the basal growth is transient and takes place only as long as the nucleus has a basal position; it is visible by a local apposition of wall material (Fig. 6). After cell division, the former tip of the apical cell also continues to grow.

Cytochalasin B. 1 μg ml^{-1} CB reduces the growth rate of caulonema tips to 10–14 μm h^{-1}. Two hours after CB application the apical zone is reduced, and the plastids come close to the cell tip. Later their starch content diminishes. The nucleus moves foreward (until 80 μm from the tip).

Ca^{2+}-Ionophore A 23 187. In ionophore concentrations of $2 \cdot 10^{-6}$ M and $2 \cdot 10^{-5}$ M the DMSO content of the substrate is 0.02 and 0.2%, respectively, and does not affect the cells. 2% DMSO, the solvent for $2 \cdot 10^{-4}$ M ionophore, inhibits growth slightly but does not modify the structure of the cell remarkably. Higher concentrations of Ca^{2+} in the substrate do not influence the effects.

Ionophore treatment reduces the growth rate. After 1 h the cell apex broadens, the "apical zone" extends down to the side walls. As a result the cell diameter is increased from about 24 μm to about 34 μm (Fig. 11). The cell length is reduced correspondingly so that the cell volume remains constant. At first, the caulonema cells retain the polar distribution of cell organelles.

In concentrations of $2 \cdot 10^{-5}$ M and $2 \cdot 10^{-4}$ M the ionophore induces the plasmalemma to become irregular, especially in the apical region. The vesicular zone is only affected after longer treatment whereupon it disappears. The cell wall is thickened irregu-

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Fig. 5. Terminal thickening of the tip after 1.45 h colchicine (0.04%). Marker bar 100 μm

Fig. 6. Formation of a local wall thickening (asterisc) after D_2O treatment (90%, 48 h) near the basal cross wall. Marker bar 10 μm

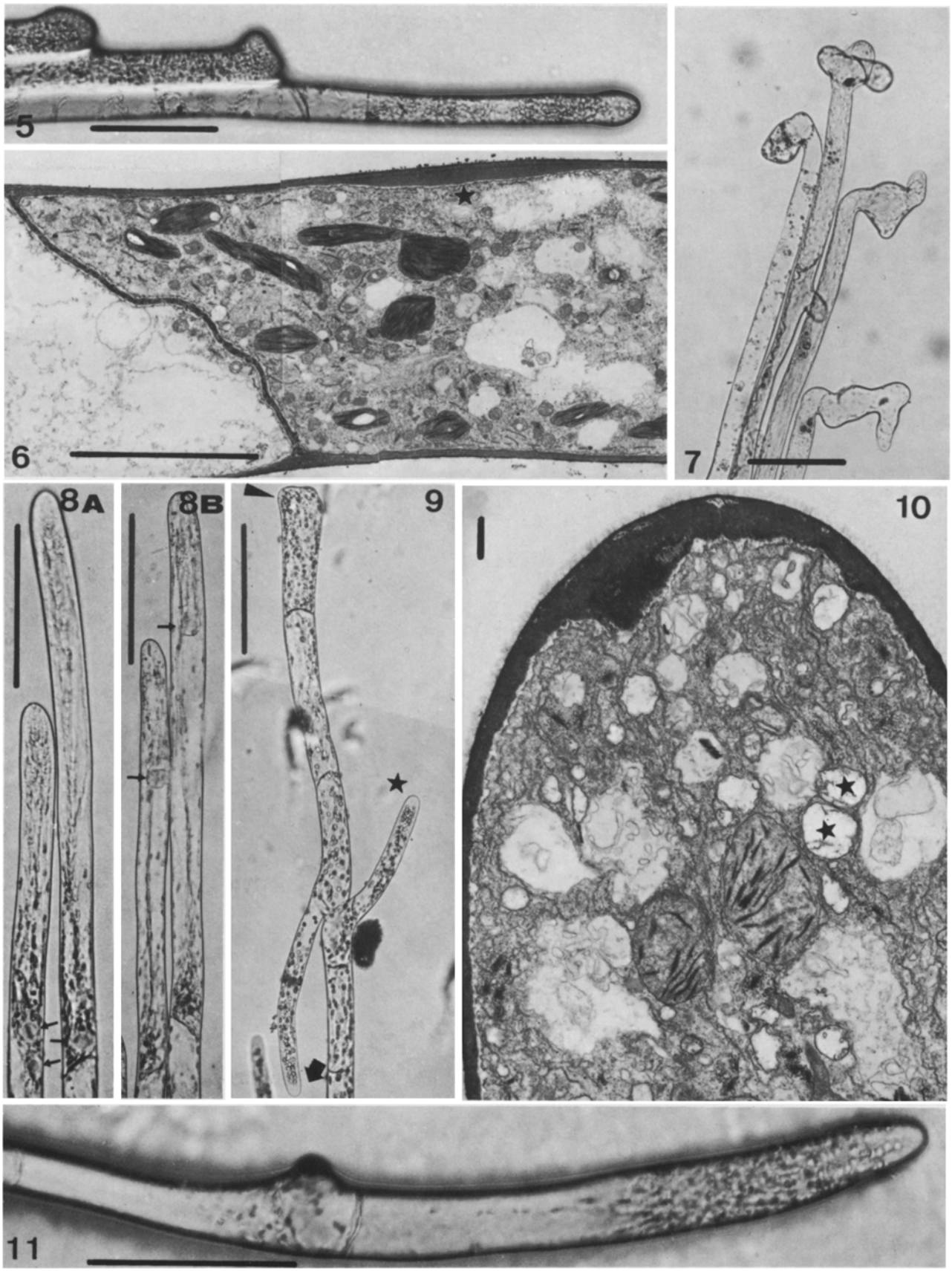
Fig. 7. Irregular malformations in the tip after 3 days colchicine (0.1%). Marker bar 100 μm

Fig. 8A. Apical cells after D_2O treatment (90%, 6 h). The nuclei (arrows) and the bulk of the cytoplasm are displaced and lie near the proximal cross wall. One cell has become binucleate. Bright field; marker bar 100 μm. **B** Apical cells after long D_2O treatment (90%, 24 h). The nuclei (arrows) have remigrated into their former position whereas the bulk of the cytoplasm lies proximally. Marker bar 100 μm

Fig. 9. Reversion of polarity after D_2O treatment (100%, 34 h, thereafter 15 h normal substrate). At the base of the former apical cell (which by then has divided intercalarly) a new tip with caulonema character is formed which grows in reversed direction (arrow). The former second cell has produced a side branch (asterisc) which is caulonematic rather than a (chloronematic) side branch. Arrowhead: subapical formation of a new growth zone at the former tip. Marker bar 100 μm

Fig. 10. Tip region after D_2O treatment (80%, 8 h). Irregular wall thickening; the plastids have lost their starch, mitochondria are swollen (asteriscs). The apical vesicle zone is lacking; no polar distribution of organelles. Marker bar 1 μm

Fig. 11. Increase of filament diameter after ionophore treatment ($2 \cdot 10^{-6}$ M, 20 h). On the left side the initial diameter is seen. Bright field; marker bar 100 μm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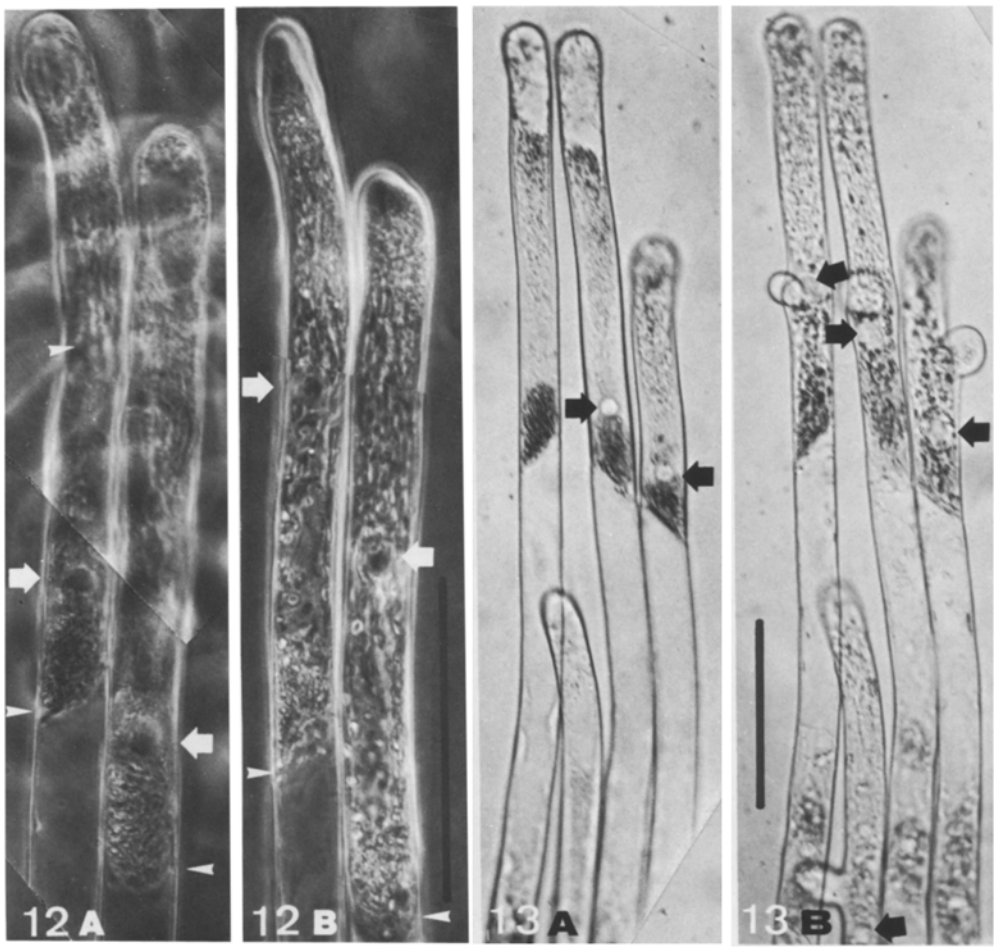

Fig. 12 A and B. Development of short apical cells after centripetal centrifugation (2,700 *g*, 20 min). Immediately after centrifugation (**A**) the bulk of cytoplasm lies in the basal part of the cell; after 80 min (**B**) the remigration of the cytoplasm has started and tip growth continues. Arrow: nucleus, arrowhead: cross wall. Phase contrast; marker bar 100 μm

Fig. 13 A and B. Inhibition, by colchicine (0.1%), of remigration after centrifugation (1,500 *g*, 15 min). **A** 30 min, **B** 9 h after centrifugation. Short outgrowths are formed near the nuclei (arrows) in the proximal part of the cells. Bright field; marker bar 100 μm

larly, especially in the tip region. The polar organization of the tip cell is affected only after 24 h. The plastids lose their starch and the gradient in polar modification disappears. Their thylakoidal system is reduced and the mitochondria appear to swell in part.

Centrifugation Experiments

After centrifugation in a basal direction at 4,000 rev min^{-1}, the heavy apical amylochloroplasts become crowded at the basal cross wall followed by the plastids possessing less starch (Fig. 12A, 13A and 14A). The nucleus is positioned above the plastid zone, while the granular cytoplasm containing mitochondria, dictyosomes, and vesicles is located above the nucleus. The apical portion of the cell is occupied by the vacuole.

The centrifugal forces needed to relocate certain organelles differ. With low forces (600 rev min^{-1}) the vesicle zone is affected and growth ceases. Speeds higher than 600 rev min^{-1} are necessary to move the amylochloroplasts, and still higher speeds are required to displace the chloroplasts. The apical plastids usually cannot pass the nuclear region and remain above the nucleus. To shift the nucleus, more than 2,000 rev min^{-1} are required.

Colchicine (0.07–0.1%) reduces the stability of organelle anchorage slightly. Without colchicine, 2,000 rev min^{-1} are necessary to displace the cytoplasm in about 50% of the cells; with colchicine, 1,000 rev min^{-1} have the same effect.

After basipetal centrifugation (4,000 rev min^{-1}, 20 min) (the reactions after acropetal centrifugation are similar in principle), a short tip cell (Fig. 12B),

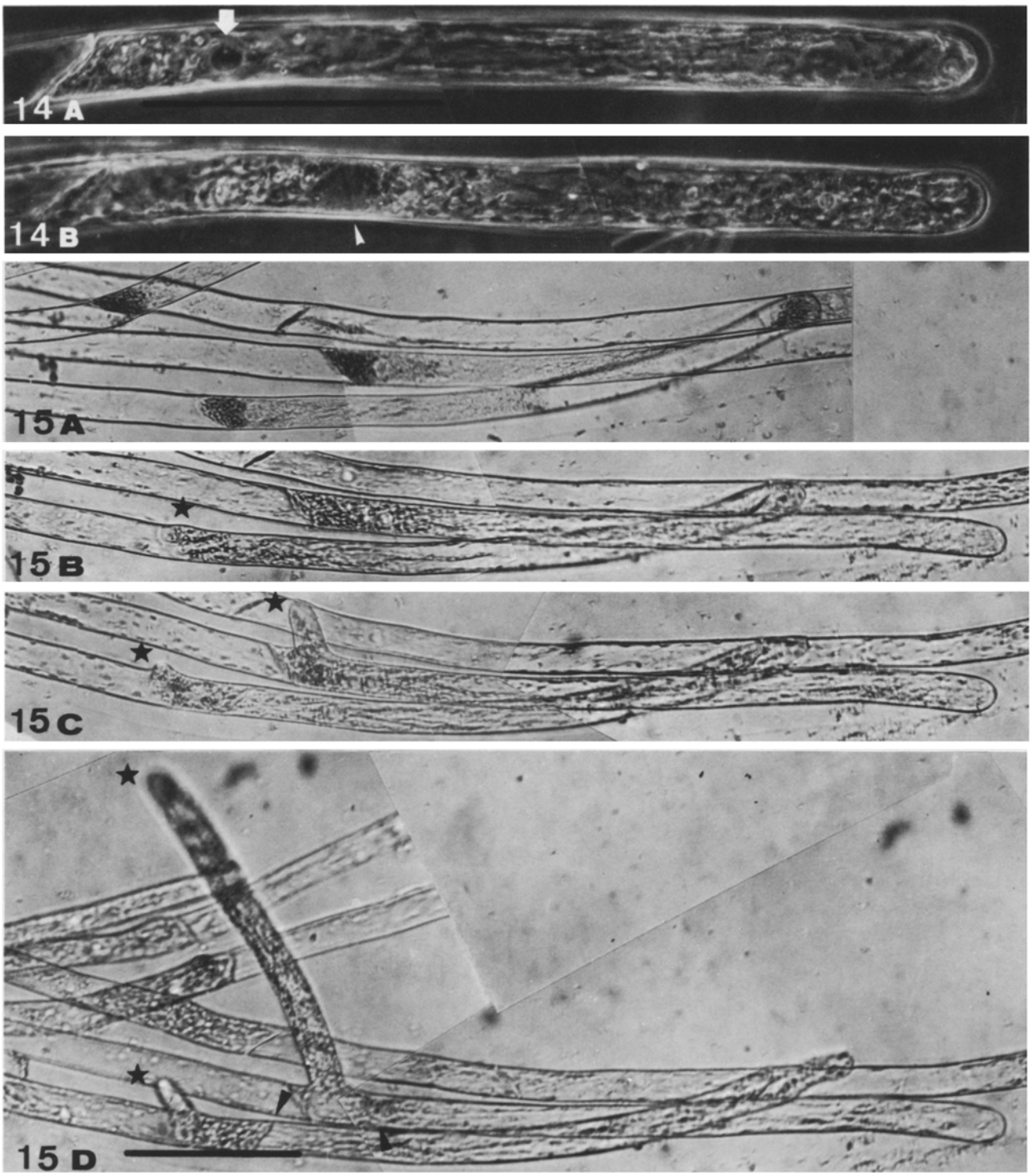

Fig. 14A and B. Development of a long apical cell after centripetal centrifugation (2,700 *g*, 20 min). **A** immediately after centrifugation; **B** 6 h after centrifugation. The remigration of the nucleus (arrow) is retarded; the cell plate (arrowhead) is formed in the proximal part of the cell. Phase contrast; marker bar 100 μm

Fig. 15A–D. Reversion of polarity after centrifugation (2,700 *g*, 20 min). In long apical cells a new tip (asteriscs), growing in reverse direction, develops in the proximal part of the cell. **A** immediately after centrifugation, **B** 4 h, **C** 8 h, **D** 13 h later. Arrowheads: apical cross walls of short cells which are cut off from the apical cells. Bright field; marker bar 100 μm

i.e., one which is in interphase, reconstitutes the apical vesicle zone within one hour. The nucleus returns to its typical position, 120 µm from the cell tip, at a migration rate of about 42 µm h^{-1}, which corresponds with the rate of movement in normally growing cells. The cell tip continues to grow before the plastids have regained their typical arrangement. Amylochloroplasts are observed after 4 h in their former shape and arrangement. The normal distribution of organelles, however, is not restored before the next cell division. This division is, in general, only slightly retarded. As a consequence of growth interruption, the newly formed subapical cell is shorter than in non-centrifuged filaments.

In long tip cells (about 400 µm) (Fig. 14 and 15) the course of events is different. Cytoplasm centrifuged to the basal cross wall remains aggregated for a longer time than in short cells and the nucleus does not remigrate. Subsequently, two different developmental ways can be followed.

In the majority of these cells, the nucleus and cell divide soon after centrifugation to form a very short (70 µm) cell at the base of the tip cell (Fig. 14). The new wall has an oblique orientation characteristic of a caulonema cross wall. This basal short cell produces a new tip which often grows with reversed polarity toward the center of the protonema. The filament has a new caulonema-like tip with a tip body (a side branch would have been chloronema-like and would lack a tip body). The apical long cell formed simultaneously often continues to grow with its original tip.

Less frequently, but also demonstrating the reversion of polarity, the apical cell produces a new tip at its base without having undergone a division (Fig. 15) and grows towards the center of the protonema. After mitosis, one of the daughter nuclei moves into the newly developed filament, whereas the other remains in the old cell.

Colchicine inhibits the remigration of cellular organelles remarkably (Fig. 13). Within 3 h after centrifugation, the agglomerated cytoplasm at the basal cross wall becomes only slightly less compact. In the next 6–10 h new tips are initiated. The formation of these tips (with a restricted growth as characteristic of colchicine-treated cells) usually takes place close to the nucleus (Fig. 13B).

Discussion

Our observations confirm the concept that cell polarity is based partly on the peripheral cytoplasm (Quatrano, 1978) and, thus, cannot be reversed by centrifugation. The plasmalemma, cortical microtubules, and perhaps also other membrane-associated elements should be taken into consideration when interpreting cellular polarity.

Electrical potential differences, intracellular gradients of ions and molecules, and diverse transport systems could function as "effectors" at the molecular and organelle level to realize and control the polar morphological cell organization (e.g., the position of the nucleus) and polar growth.

However, the effects of centrifugation and heavy water also show that this concept is too simple, at least for the *Funaria* caulonema cells. Usually, the cortical cytoplasm induces the dislocated nucleus to remigrate to its former position. In a premitotic stage, however, nuclear movement is inhibited, possibly because of changes in the nuclear envelope which may no longer allow attachment of potential transporting structures (possibly microtubules, microfilaments). In this case the other organelles likewise fail to remigrate.

Inhibition of remigration could also be based on a transient reorientation of the transporting system in the preprophase, or on a temporary disorientation of the control mechanism within the plasmalemma as a consequence of a reorientation of microtubules in preprophase. In any case, microtubules appear to be involved. The effects of colchicine and the close contact between microtubules and certain organelles (cf. Kiermayer, 1972; Heath and Heath, 1978) also indicate a role of microtubules in the reorganization of organelle distribution. It is important to note in this connection that the nucleus tends to move backward after colchicine application, whereas it migrates foreward when cell growth is inhibited by cytochalasin B. Obviously, microtubules and microfilaments (Hepler and Palewitz, 1974) function antagonistically.

If the nucleus has occupied a new position for a period of time, it is no longer efficiently controlled by the peripheral cytoplasm. Then, on the contrary, the nucleus apparently exerts control upon the peripheral cytoplasm and on other cell organelles and, depending upon its position, may *revert the polarity of the cell.* Similarly, Czaja (1930) could change the polarity in *Cladophora* but only after repeated centrifugations. The capacity of the nucleus to control local cell growth is also demonstrated by the localized development of cell protrusions after colchicine treatment. It remains unclear in which way the nucleus influences the surrounding cytoplasm. The suggestions drawn from the present results are supported by observations of side-branch formation (Schmiedel and Schnepf, 1979a, b).

An interruption of growth and a disorganization of polar cytoplasmic structure by inhibitors, i.e., their effects on manifestation of polarity, are not necessa-

rily a result of a suspended or reversed polarity but could likewise be caused by disturbances at the levels of the "effectors". In contrast to e.g. growth of pollen tubes (Franke et al., 1972), but similar to other protonemata (Stockwell and Miller, 1974), colchicine induces swelling in the cell apex. Since microtubules do not occur in the vesicle zone proper, and growth processes are disorganized rather then inhibited, it is improbable that microtubules are directly involved in polar transport of Golgi vesicles.

Internal gradients in Ca^{2+} ion distribution are also demonstrated for *Funaria* caulonema tip cells (Reiss and Herth, 1979b). The calcium ionophore A 23 187 primarily affects wall formation as it does in pollen tubes (Reiss and Herth, 1979a), but blocks polar growth and disturbs polar cell organization only after strong and long application. The role of Ca^{2+} gradients in polarity of *Funaria*, hence, is not yet clear.

It is known that D_2O influences microtubules (Hardham and Gunning, 1978) but also has many other effects (Uphaus et al., 1975). Its influence on organelle localization remains unexplained. Presumably it interferes, like colchicine, with the antagonistic vectorial systems in the cell.

The polar organization of the caulonema tip cells is directly related to the polarity of growth. The tip body, the occurrence of which was questioned by DeMaggio and Stetler (1977) in protonemata, is the expression of the higher growth rate of the caulonema tips as compared with the chloronema cells. The variations in starch content of the chloroplasts (cf. Wada and O'Brien, 1975) is connected with their position and, as shown by the effects of dislocation, does not indicate a permanent differentiation.

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