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Development of the intercalary meristem in *Chorda filum* (Laminariales, Phaeophyceae) and other primitive Laminariales

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SUMMARY

Development of the intercalary meristem in the terete laminarialean species *Chorda filum* (L.) Stackhouse was studied in culture using light and transmission electron microscopy as well as by tracing elongation and cell divisions in various parts of the sporophyte. Growth of *C. filum* sporophytes could be classified into three developmental stages: (i) diffuse growth; (ii) basal meristematic growth; and (iii) intercalary meristematic growth. In the diffuse growth stage, elongation and cell division frequency were almost the same in each cell. In the basal meristematic growth stage, elongation and division of cells became localized in the tissues derived from the meristematic initial cell. Cells of the basal meristematic region contained smaller chloroplasts and many small opaque vesicles. In the intercalary meristematic growth stage, there was further elongation and differentiation of cells originating from the meristematic region, and this became more active in adjacent regions below the meristem than in regions above the meristem, causing the relative position of the intercalary meristem to shift towards the tip of the sporophyte. Meristematic cells of *C. filum* contained well-developed Golgi vesicles around the nucleus (perinuclear Golgi), many secretion vesicles and many small disk-shaped chloroplasts whose thylakoids were not well developed. Sporophytes of three other terete members of Laminariales, *Chorda tomentosa* Lyngbye, *Pseudochorda nagaii* (Tokida) Kawai et Kurogi, and *Pseudochorda gracilis* Kawai et Nabata, show diffuse growth and basal meristematic growth, but no intercalary meristematic growth. This suggests that the common ancestor of the Pseudochordaceae and Chordaceae had basal meristematic growth, and intercalary meristematic growth evolved more recently in *C. filum*.

Key words: anatomy, *Chorda filum*, *Chorda tomentosa*, Chordaceae, fine structure, intercalary growth, Laminariales, meristem, Phaeophyceae, *Pseudochorda gracilis*, *Pseudochorda nagaii*.

INTRODUCTION

Sporophytes of Laminariales (so-called kelps) form the largest and the most elaborate thalli of all algae. Most of them have a characteristic intercalary meristem (growth zone) where a large proportion of cell divisions occurs. The cells originating from the meristem later differentiate into elaborate tissues. The success of Laminariales in developing these large sporophytes is considered to be due largely to this unique growth pattern, because physical damage to the thalli by wave action and drying is much less likely to cause lethal damage to the growth zone, which is usually located in the lower part of the thallus; hence, perennial growth became feasible. Such an intercalary growth pattern is rare among Phaeophyceae and its evolutionary origin is not clear. Kylin (1918) gave a detailed description of the anatomical features of the meristematic zones; however, the fine structural features and developmental processes were only poorly understood. In the present paper, we aim to describe more fully the fine structure and development of the *Chorda filum* meristem and to obtain information elucidating both the evolution of the intercalary meristem as well as the phylogeny of the Laminariales. For comparison, developmental processes in other terete Laminariales were also examined.

Within the order Laminariales, the families Phyllariaceae Tilden, Chordaceae Dumortier and Pseudochordaceae Kawai et Kurogi are considered to be primitive because of the following characteristics: presence of a stigma (eyespot) and flagellar swelling in zoospores; annual terete thallus (except Phyllariaceae); absence of mucilaginous ducts and mucilaginous tips in paraphyses; occurrence of monecious or monomorphic diecious gametophytes; and disk-shaped holdfasts (Kawai and Kurogi 1985; Henry 1987a,b; Henry and South 1987;

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Kawai and Nabata 1990). In the sporophytes of Phyllariaceae, the foliose blade and stipe are differentiated, as in more derived families (i.e. Laminariaceae, Alariaceae and Lessoniaceae), and their elongation is reported to occur mainly in the transition zone of the intercalary meristem (Norton and Burrows 1969). However, Phyllariaceae differs from other members of Laminariales in having a unique translocation system of 'solenocysts' and 'allelocysts', long, thick-walled multinucleate cells (Sauvageau 1918; Emerson *et al.* 1982; Henry and South 1987). The Phyllariaceae is therefore unlikely to be the direct ancestor of the more derived laminarialean families.

In contrast, the terete families of the Laminariales (Chordaceae and Pseudochordaceae) lack an obvious differentiation between blade and stipe. *Chorda filum*, the type species of the family Chordaceae, has an intercalary meristem that originates in the lower part of the thallus and is gradually shifted distally as the thallus develops (South and Burrows 1967). Well-developed vegetative parts of the sporophytes are composed of richly pigmented photosynthetic epidermal cells, cortical cells, and two types of inner hyphae (trumpet-shaped hyphae originating from the meristem and hyphae derived from the inner layer of the cortex). These features are similar to those in more derived families. In contrast, localized meristems have not been reported in the other terete Laminariales, *Chorda tomentosa* Lyngbye (Sundene 1963; Maier 1984), *Pseudochorda nagaii* (Tokida) Kawai et Kurogi (Tokida 1938; Kawai and Kurogi 1985) and *Pseudochorda gracilis* Kawai et Nabata (Kawai and Nabata 1990).

MATERIALS AND METHODS

Male and female clonal cultures of *C. filum* were established from zoospores released from fertile sporophytes collected at Oshoro, Hokkaido, Japan. They were inoculated into plastic petri-dishes containing *ca* 30 mL of PESI medium (Tatewaki 1966). The culture conditions used were 5°C SD (short day: 8 h light and 16 h dark), 5°C LD (long day: 16 h light and 8 h dark), 10°C SD and 10°C LD under a photon flux of 30 μmol m^{-2} s^{-1} (5°C) or 50 μmol m^{-2} s^{-1} (10°C) illuminated with cool-white fluorescent tubes. Cultures of female gametophytes or mixed male and female gametophytes were inoculated under 5°C SD and formed sexual reproductive structures. Young sporophytes derived from mixed cultures, and parthenogenetic sporophytes derived from female gametophytes were transferred to new petri dishes and later transferred to glass vessels containing 200 mL of PESI medium.

Clonal cultures of *P. nagaii* collected at Hanasaki, Hokkaido, *P. gracilis* collected at Isoya, Hokkaido, and *C. tomentosa* collected at Newfoundland, Canada were also used.

In order to characterize the functional differentiation of the meristem, we examined cell division and elongation of the cells in different parts of the young sporophytes, using a cell wall-specific fluorescent stain. For vital staining of the sporophytes, young sporophytes of various developmental stages were immersed in the staining solution (0.01% Fluorescent Brightener 28 (Sigma) in sterile-filtered seawater; Waaland 1980) for 30 min and then washed in filtered seawater. The stained sporophytes were then inoculated into culture medium without dye. After 1–2 weeks, the distance between stained walls and the number of intervening cells was measured using a Nikon EFD2 epifluorescence microscope, to assess tissue elongation and the number of cells that divided after staining (Fig. 9, top left). Preparation of sporophytes for transmission electron microscope (TEM) observations followed Kawai and Nabata (1990). All TEM observations were made using a Hitachi H-300 electron microscope.

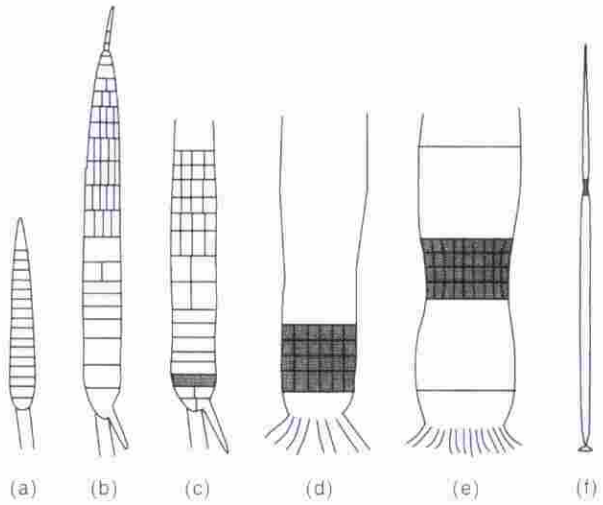


Fig. 1. Developmental stages of the *Chorda filum* sporophyte categorized in the present study. a,b. Diffuse growth stage. c,d. Basal meristematic growth stage. e,f. Intercalary meristematic growth stage.

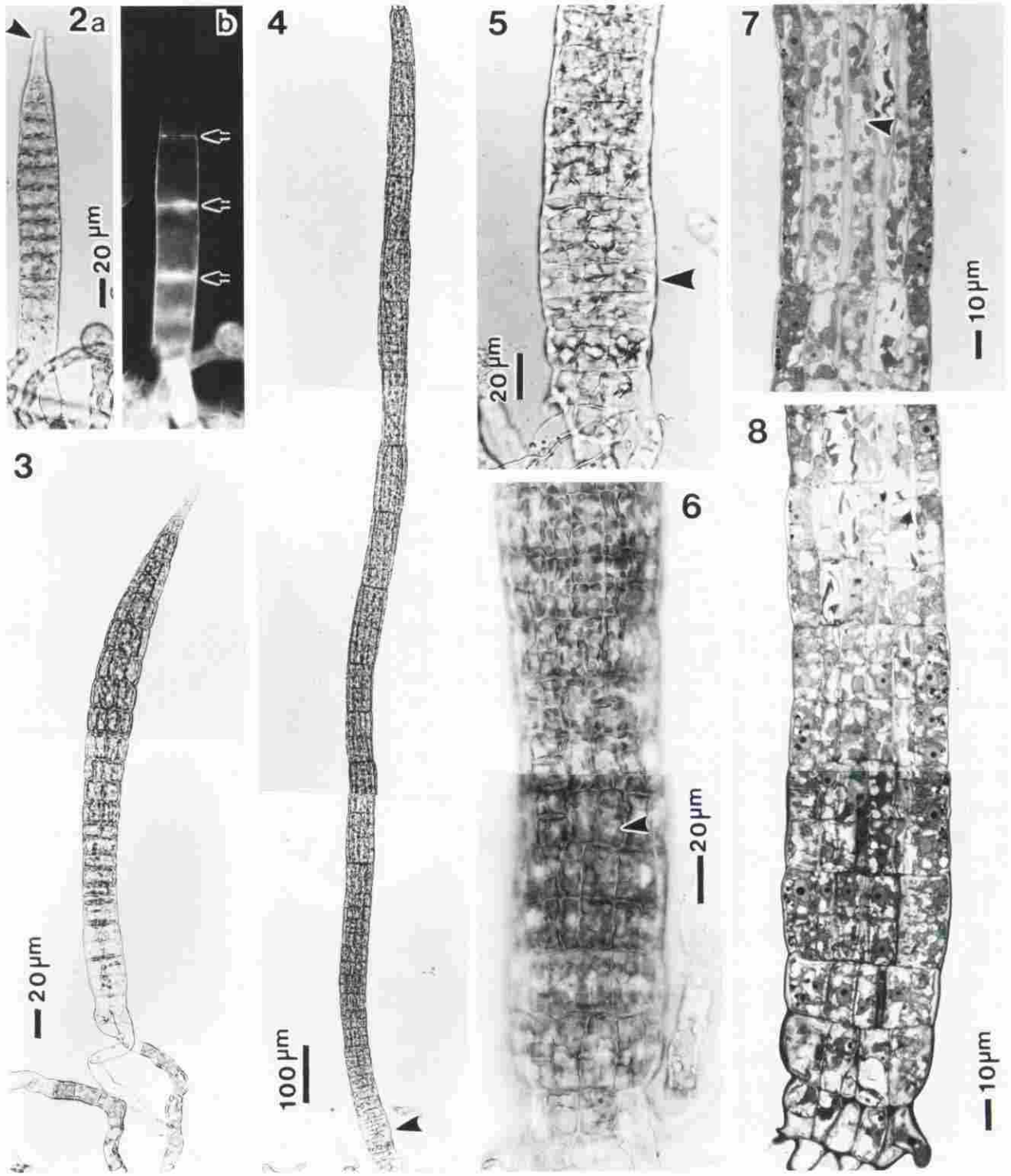
RESULTS

Chorda filum

The development of *C. filum* sporophytes can be classified into the following three stages according to the absence, formation and presence of the meristem (Fig. 1a–f).

Diffuse growth stage

Zygotes as well as unfertilized eggs developed into sporophytes attached to the mouth of oogonia (Figs 1a, 2). They developed into 12–16-celled uniseriate embryos forming cross-walls by diffuse growth. Apical hairs differentiated from the terminal cells (Figs 1b, 2, 3). At this stage, the cell length and the diameter of chloro-



Figs 2–8. Early development of the *Chorda filum* sporophyte in culture. 2. Brightfield (a) and epifluorescence micrographs (b, UV excitation) of 18-celled sporophyte, stained with fluorescent brightener when the thallus was four cells long. Stained cross walls appear as white lines (arrows) in (b) whereas walls formed after staining are not fluorescent and cannot be seen. (b) Arrowhead in (a) shows apical phaeophycean hair. 3. Young polystichous sporophyte forming rhizoids from lowermost cell. 4. Young sporophyte retaining uniseriate portion at the basal part. Arrowhead shows the area that is thought to differentiate into the meristem initial cell. 5. Basal uniseriate portion (same as Fig. 4 under higher magnification). 6. Early stage of intercalary meristem formation. Arrowhead shows meristematic initial cells. Note abundant small vesicles and smaller chloroplasts in these cells (arrowhead). 7, 8. Longitudinal section of middle (Fig. 7) and basal (Fig. 8) part of a young polystichous sporophyte. Arrowhead shows medullary initial cells.

plasts (*ca* 3 μm) were almost uniform in all parts of the thallus.

As the sporophytes became polystichous in the distal portion (Figs 3, 4), their surface cells became longer and their chloroplasts began to enlarge (*ca* 3 μm near the base and *ca* 4–4.5 μm in the upper part). The lower part of the thallus, which originated from the lowermost cell in the four-celled stage, remained uniseriate (Figs 1b, 4). In the uniseriate region, chloroplasts became a little smaller in diameter (less than 3 μm). Rhizoidal filaments were formed from the lowermost cells (Figs 1b, 3). At the end of the diffuse growth stage, segments (group of cells separated by cross-walls, which were formed prior to the formation of the longitudinal walls) became distinguishable (Fig. 3).

Basal meristematic growth stage

As the number of segments increased to 25–35 (Figs 1c, 4), abundant small vesicles became apparent in the cells of the basal uniseriate portion (Fig. 5). The cell adjacent to the cell that formed the rhizoids, which remained uniseriate the longest, finally became polystichous (Figs 11, 12) and the juvenile meristem developed from this segment (Figs 1d, 6).

The length and number of the surface cells in each segment were almost uniform before the development of the juvenile meristem (Fig. 4). However, after its development, cell divisions were localized in this region and the cells derived from it elongated mostly in the adjacent distal area (Fig. 8). Figure 9 a–c indicates the growth rate along the axis of a sporophyte in the basal growth stage, corresponding to Fig. 1d. Division of the cells was more active in the regions 0 to 7 mm from the meristem, still located very close to the base, and most active within less than 1 mm from the meristem (Fig. 9a,b). Cell divisions were more frequent in the longitudinal direction than in the transverse direction (Fig. 9b,c).

Cells of the epidermal part of the juvenile meristem divided frequently, forming shorter and broader cells than the cells in other portions (Figs 14, 15). These cells contained relatively small chloroplasts (3–4 μm in diameter) and numerous small vesicles (Fig. 13). Cells of the medullary part of the juvenile meristem divided and elongated exclusively along the longitudinal axis, resulting in the formation of bundles of densely packed simple filaments (Figs 14, 17). These cells were isodiametric, having thin cross-walls and thick longitudinal walls. Cellular connections between the filaments became rare (Fig. 14).

In the adjacent upper region of the juvenile meristem, three different tissue layers (i.e. epidermal, cortical and medullary) became distinguishable. Cells of the epidermal layer were shorter than cortical and medullary cells and contained many enlarged chloroplasts (*ca* 5.5 μm in diameter) and a few vesicles (Fig. 6). Cells of the cortical layer contained relatively large vesicles and enlarged chloroplasts, and medullary cells were more deeply stained than cortical cells (Fig. 7).

Intercalary meristematic growth stage (Fig. 1e,f)

When the epidermal part of the juvenile meristem attained 20–30 cells in length (Fig. 16), transverse cell divisions became more frequent in its upper region than in the lower, and as these dividing cells became smaller their chloroplasts enlarged. In contrast, in the lower epidermal meristematic region, longitudinal divisions became more frequent and formed narrow elongated cells (Fig. 17). In the area immediately distal to the meristem, cells of the medullary meristem elongated and differentiated into hyphae (Fig. 17). The inner part of the thallus then became hollow as a result of the frequent cell divisions in the epidermal layer and the subsequent enlargement of the cortical cells (Fig. 17).

In sporophytes in which the length below the meristem was *ca* 5 mm, elongation of cells and succeeding cell divisions became about equally or somewhat more active in the regions below the meristem (Fig. 9d–f). Cell divisions were less frequent in the central region of the meristem and most frequent in the regions 5 mm above and below the center of the meristem. Growth became less active and finally ceased in the regions more than 10 mm from the meristem. At this stage, the meristem became distinguishable, to the naked eye, as a narrower region (Figs 1e,f, 18,19). The total length of the thallus during the basal and intercalary meristematic growth stages could not be measured precisely because the distal ends of the thalli tended to become bleached and gradually lost. Epidermal, cortical and medullary layers began to differentiate in the lower region of the meristem in a similar manner as in the upper region (Fig. 20). Thereafter, the growth became much more active in the regions below the meristem (Fig. 10a–c) than in the region above it. Cell divisions were more frequent in the longitudinal direction than in the transverse direction throughout the intercalary meristematic growth stage (Figs 9e,f, 10c,d), as in the diffuse growth stage.

The TEM observations showed well-developed perinuclear Golgi (Figs 23, 24) and many secretion vesicles in epidermal meristematic cells. They also contained many small disk-shaped chloroplasts without pyrenoids, whose thylakoids were not well developed (Fig. 23). The cytoplasm of these cells stained well with toluidine blue. Extracellular mucilaginous substances were abundant around epidermal meristematic cells (Figs 21, 23). They often bore phaeophycean hairs. In cross-section, epidermal cells were only loosely attached to each other and were separated in the outer portion (Figs 21, 23), whereas they were firmly adherent in longitudinal sections (Fig. 24) and they became firmly adherent in the regions distant from meristem. Thus the terete thallus of *C. filum* has both parenchymatous and pseudoparenchymatous features in its construction.

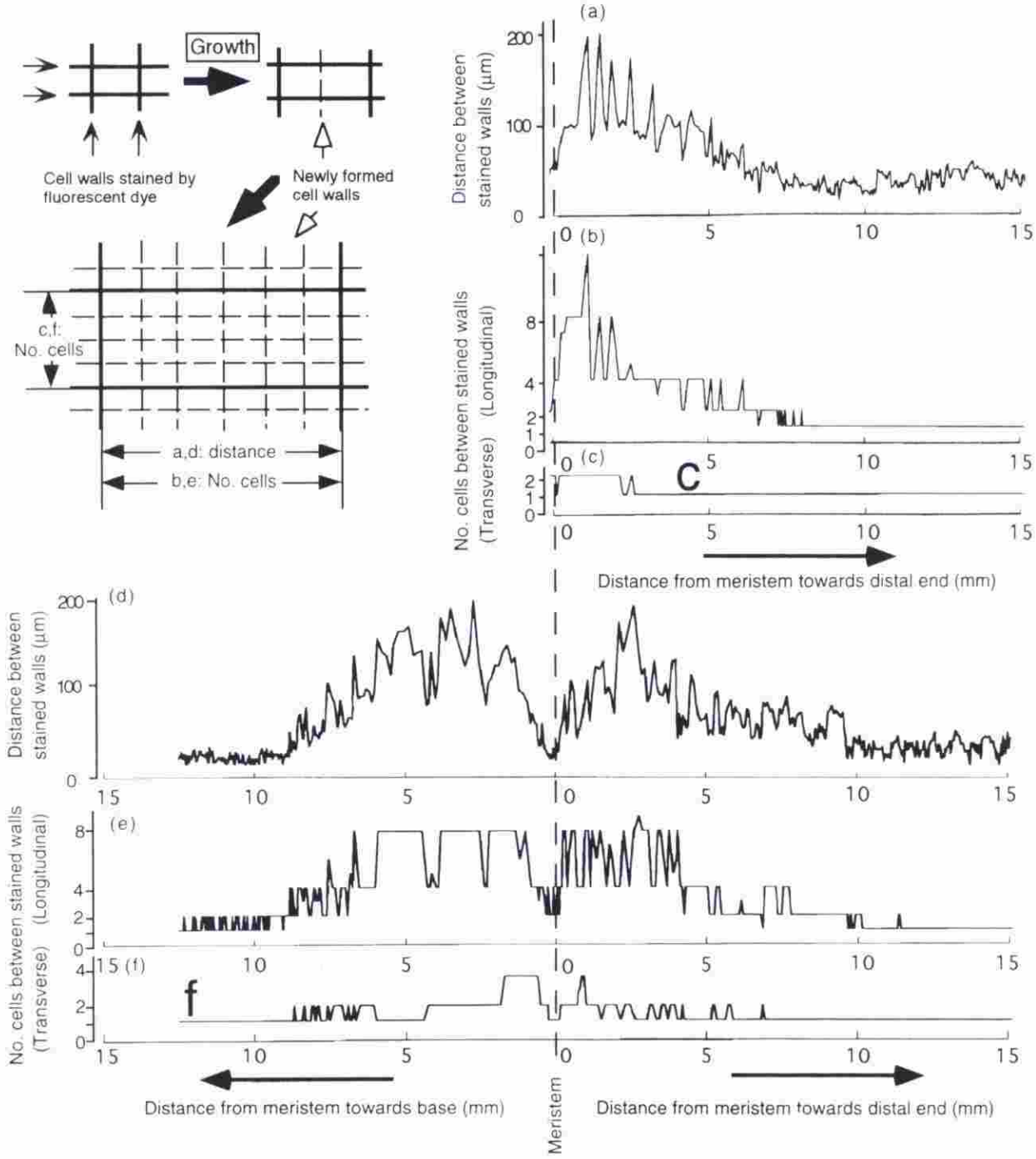


Fig. 9. *Chorda filum*. Elongation of cells and frequency of cell divisions in various parts of the thallus 1 week after fluorescent staining (a–c, basal meristematic growth stage corresponding to Fig. 1d; d–f, intercalary meristematic growth stage corresponding to Fig. 1e). Top left scheme indicates the growth of stained tissue in surface view and the parts of the tissue measured. Solid lines indicate cell walls stained with fluorescent dye, and broken lines indicate cell walls developed after staining and hence showing no fluorescence; horizontal (*x*) axes show distance of the measured area from the meristem (right towards tip and left towards base) and vertical (*y*) axes show distance between stained cells (indicating elongation, a and d), number of cells (indicating cell division frequency) in longitudinal (b and e) and transverse (c and f) directions. The vertical broken line through figures (a–f) indicates the location of the center of the meristem. Measurements were done on sporophytes whose length below the meristem was *ca* 100 μm (a–c), and 5 mm (d–f).

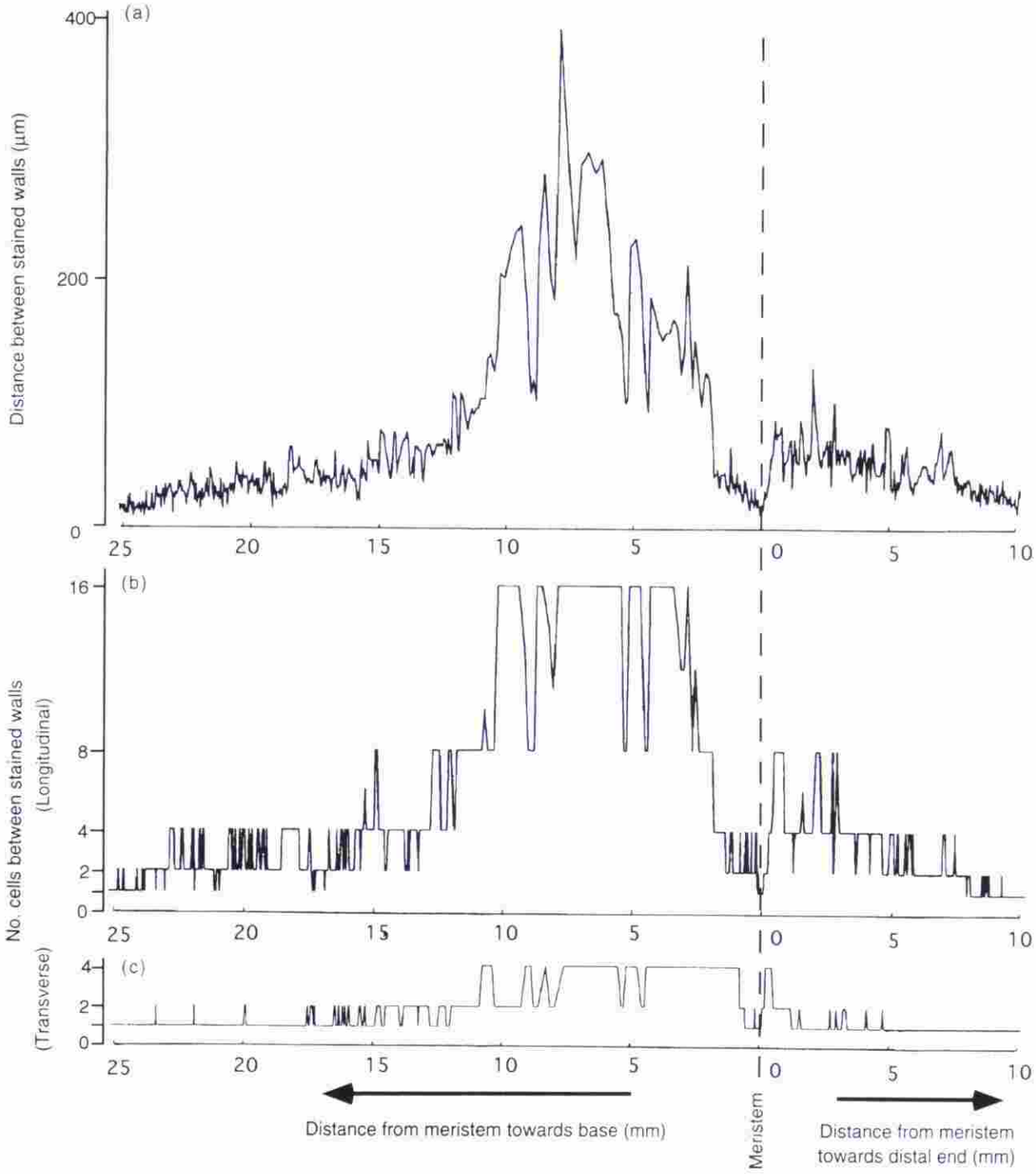
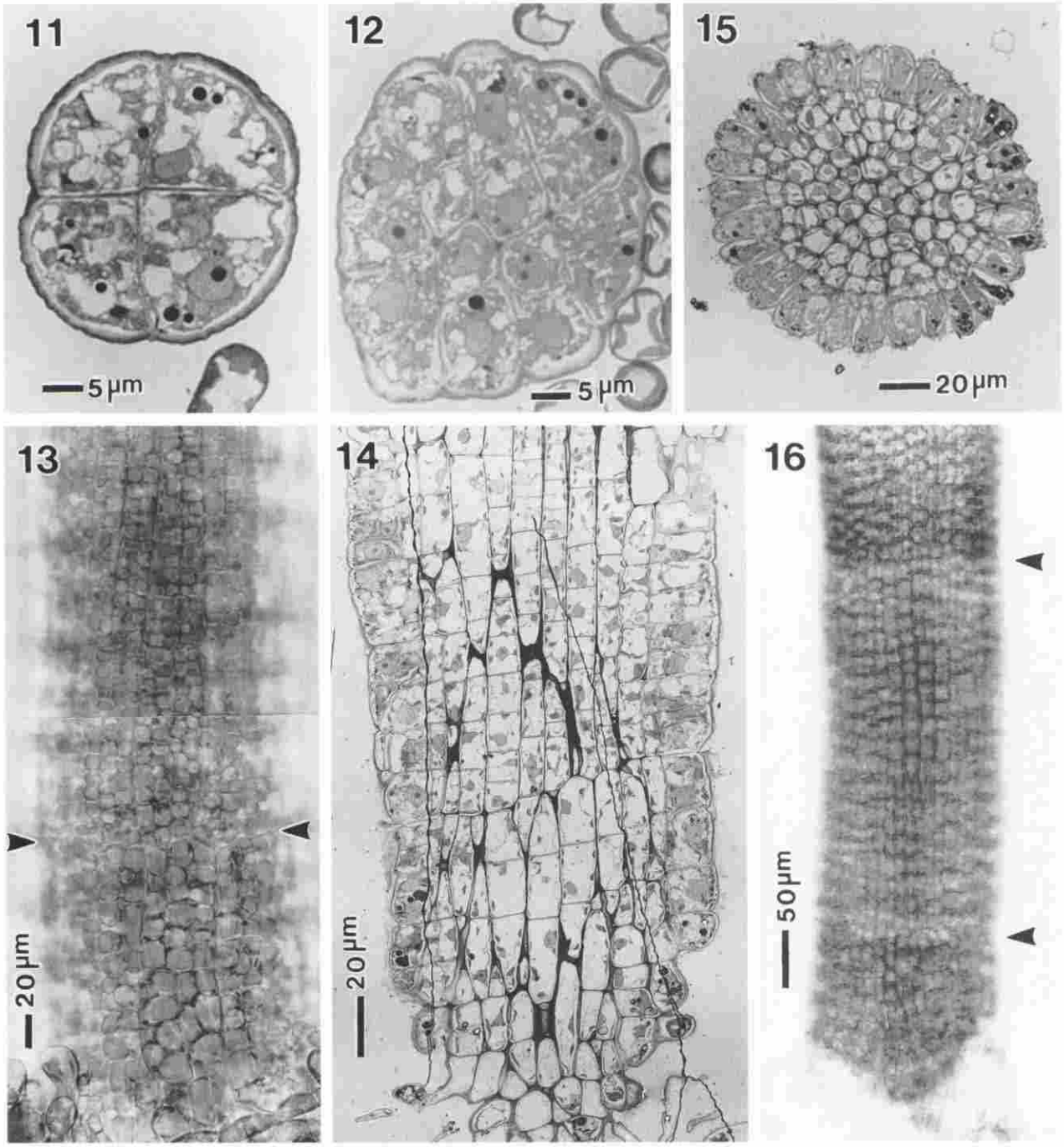


Fig. 10. *Chorda filum*. Elongation of cells and frequency of cell divisions in various parts of the thallus 1 week after fluorescent staining in the late intercalary meristematic growth stage corresponding to Fig. 1f. For detailed explanation see legend for Fig. 9. Measurements were done on a sporophyte whose length below meristem was 90 mm when stained.

Compared with the cells of the epidermal meristem, cells of the medullary meristem contained fewer chloroplasts and perinuclear Golgi bodies (Fig. 22). They were rich in cytoplasm and stained well with toluidine blue. When the thallus portion below the meristem exceeded 10 mm, cell division and elongation in the surface layer became more prominent in the region below the meristem than above. In consequence, the relative location of the meristem gradually moved upwards.

Figure 25 shows a schematic presentation of the development of the meristem in *C. filum* in longitudinal and cross-sections. In longitudinal sections, as the development of meristem proceeds, the epidermal and medullary parts of the meristem differentiate dramati-

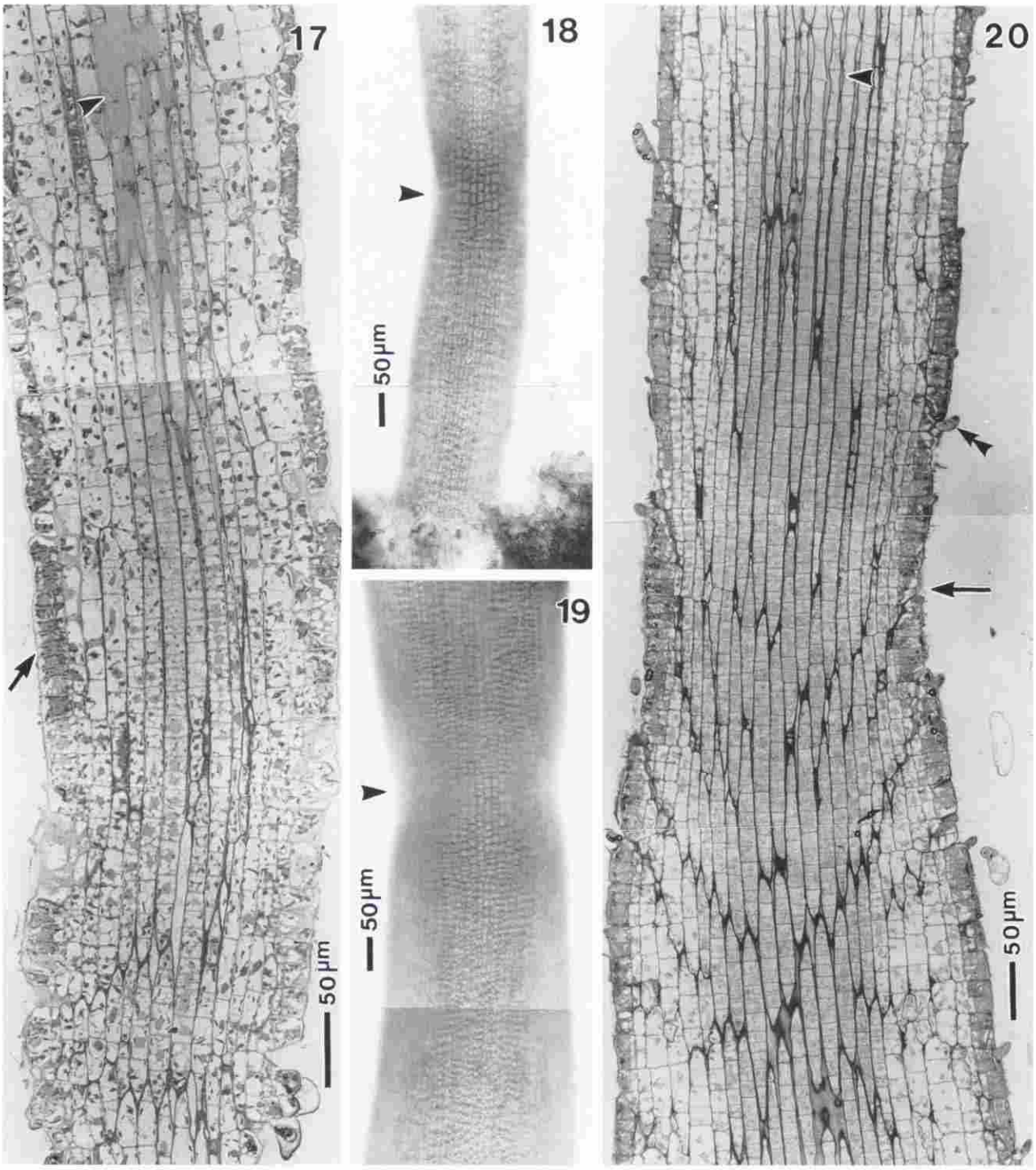


Figs 11–16. Early stages of meristem formation in *Chorda filum*. 11, 12. Cross-sections of meristematic initial cells, indicating the longitudinal wall formation. 13. Surface view of basal part of young parenchymatous sporophyte (initial epidermal meristem is 8–9 cells long). Arrowheads show lower boundary of meristem. Cells above it contain many vesicles. 14. Longitudinal section of basal part of young parenchymatous sporophyte (initial epidermal cell is 12 cells long). 15. Cross-section of juvenile meristem with longer epidermal cells. 16. Surface view of basal portion (initial epidermal meristem is 20 cells long). Arrowheads show lower and upper boundary of the epidermal meristem.

cally. The epidermal cells are formed from the short and wide epidermal meristematic cells and trumpet-shaped hyphae are formed from the medullary meristematic cells. In cross-sections, unequal divisions are seen after the 16-celled stage and then the previously mentioned differentiation in the meristem becomes apparent.

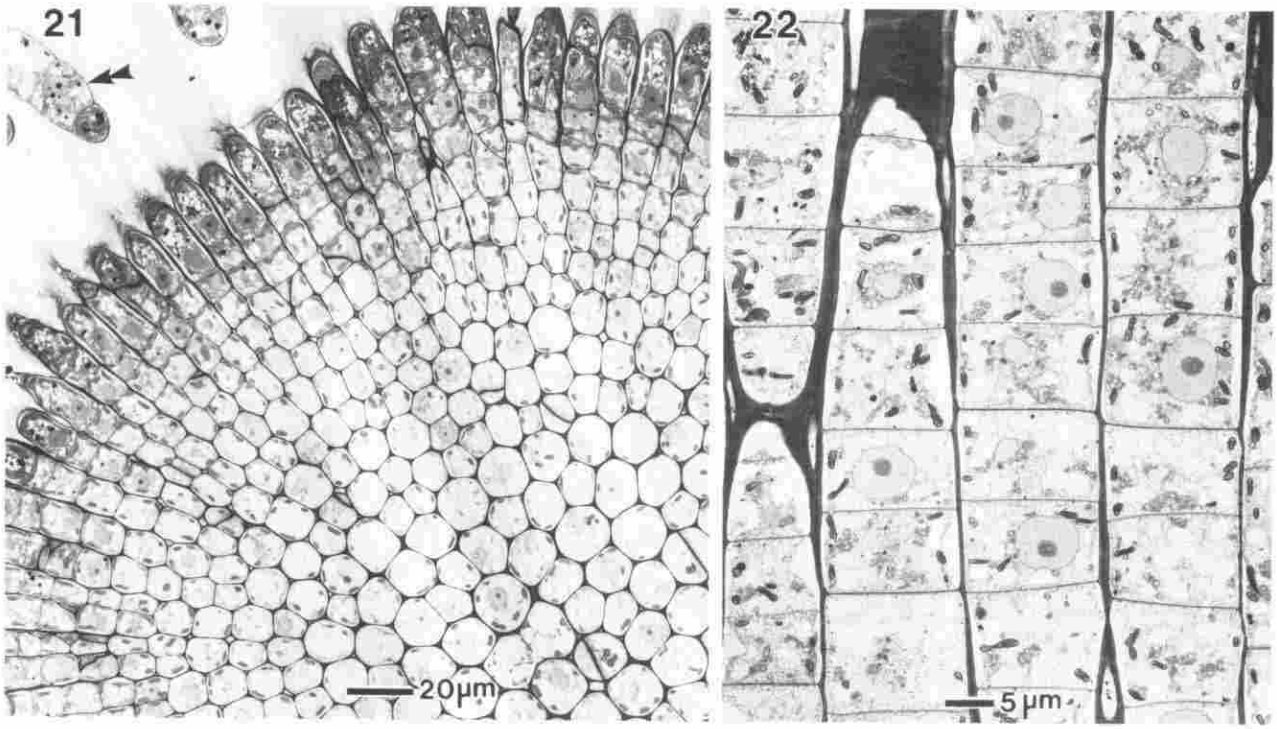
Chorda tomentosa

The early development of the *C. tomentosa* sporophyte was generally similar to that of *C. filum*. Upper and middle regions of the sporophyte became polystichous when the thallus reached a length of 16 cells. In the

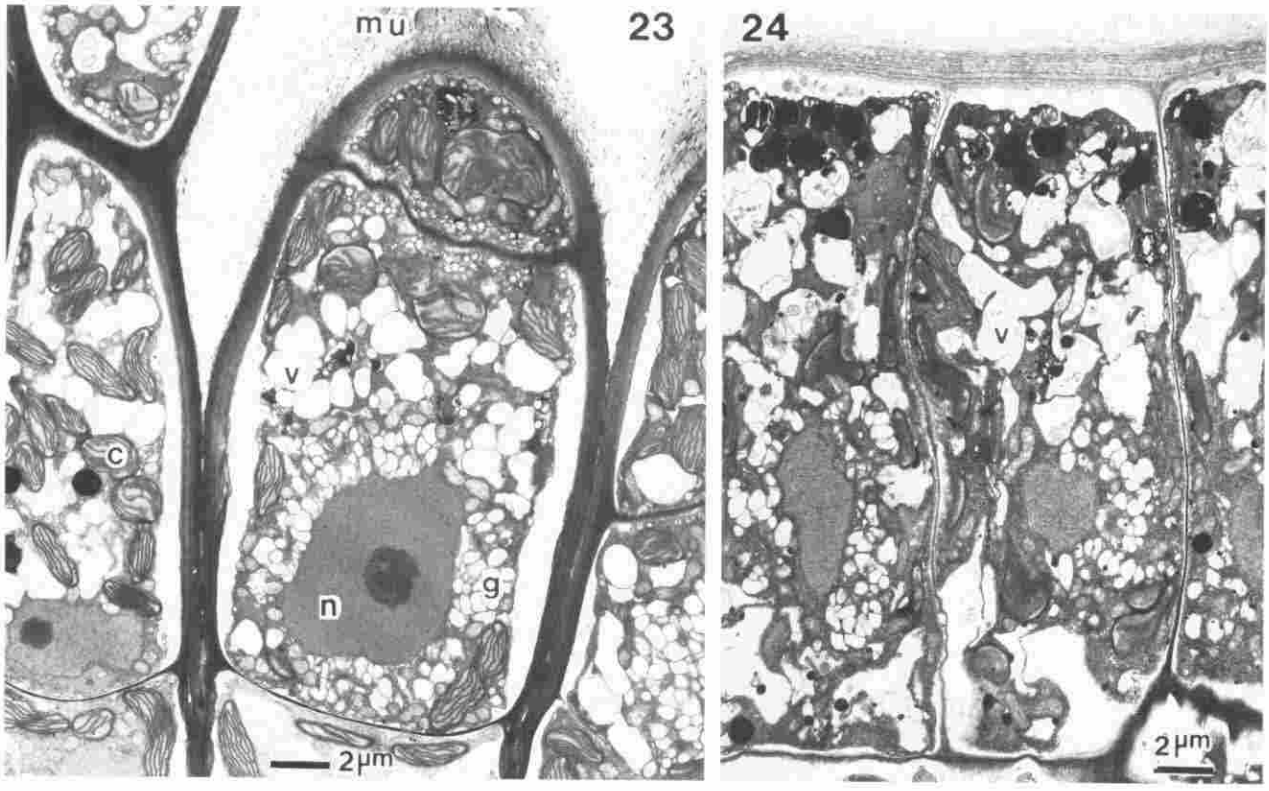


Figs 17–20. Development of intercalary meristem in *Chorda filum*. 17. Longitudinal section of the basal part of a young parenchymatous thallus differentiating the intercalary meristem. Arrow shows meristem region. Arrowhead shows hollow medullary layer. 18, 19. Surface view of a young sporophyte showing the young intercalary meristem (arrowheads). 20. Longitudinal section of a young swollen meristem (arrow). Arrowhead shows initial cells of trumpet-shaped hyphae. Doubled arrowhead shows initial cells of phaeophycean hairs.

polystichous region, chloroplasts enlarged (*ca* 5 µm in diameter), and there were no changes in the lower uniseriate region. Then, cell division and elongation became more active in the upper part of the uniseriate region, where the chloroplasts became smaller (*ca* 3 µm in diameter), reflecting the frequent divisions. When the thallus comprised 20–30 segments, the lower part of the thallus became polystichous. As shown in Fig. 26, sporophytes of *C. tomentosa* showed considerably higher cell division activity in regions within 3 mm of the base in a thallus about 20 mm in length. However, later growth activity became more or less uni-



Figs 21–22. Well-developed meristem of *Chorda filum*. 21. Cross-section of well-developed meristem, having phaeophycean hairs (double arrowhead) at the top of radially arranged dome-shaped epidermal meristematic cells. 22. Longitudinal TEM micrograph of young medullary meristem composed of bundles of simple filaments with thick longitudinal walls.



Figs 23–24. TEM micrographs of epidermal meristematic cells of *Chorda filum*. 23. Cross-section of epidermal meristematic cells. c, chloroplast; g, Golgi vesicles; mu, mucilaginous substances; n, nucleus; v, secretion vesicles. 24. Longitudinal section of surface layer of an epidermal meristematic cell.

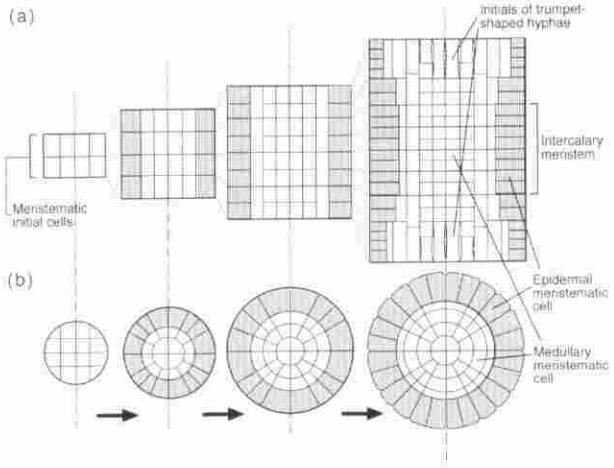


Fig. 25. A schematic representation of development of the intercalary meristem in *Chorda filum*, in (a) longitudinal and (b) cross sections. The left corresponds to diffuse growth, the middle to basal meristematic, and the right to intercalary meristematic growth stages.

form throughout the thallus and no intercalary meristem was formed.

Pseudochorda nagaii and *Pseudochorda gracilis*

Developmental processes of sporophytes in the two species of *Pseudochorda, P. nagaii* and *P. gracilis*, were very similar, although abundant hair formation was seen only in *P. gracilis*. Sporophytes became polystichous when the thalli reached a length of 8 cells; however, the lower region of the thallus remained uniseriate until the thalli exceeded several mm in length. Chloroplasts enlarged (to *ca* 4 μm in diameter) in polystichous regions, but they became smaller (less than 3 mm) in lower uniseriate regions. The cells in uniseriate regions contained many small vesicles, as was also observed in *C. filum* meristematic initial cells. As the lower regions of the thalli became polystichous, elongation and division of cells became more active in lower regions than in upper regions.

In *P. nagaii*, cell division was a little more frequent in the central part when the thallus had 20–30 transverse walls (data not shown), but later it became more frequent near the base (Fig. 27). No intercalary meristem was formed. Similarly, in a sporophyte of *P. gracilis*, whose total length was 42 mm, elongation and cell division were a little more active in regions within 3 mm of the base (Fig. 28); however, no intercalary meristem was formed later.

DISCUSSION

The early development of the *C. filum* sporophyte has previously been studied by several authors (Reinke 1892; Kylin 1918; Kanda 1938; South and Burrows 1967). South and Burrows (1967) first noted that a

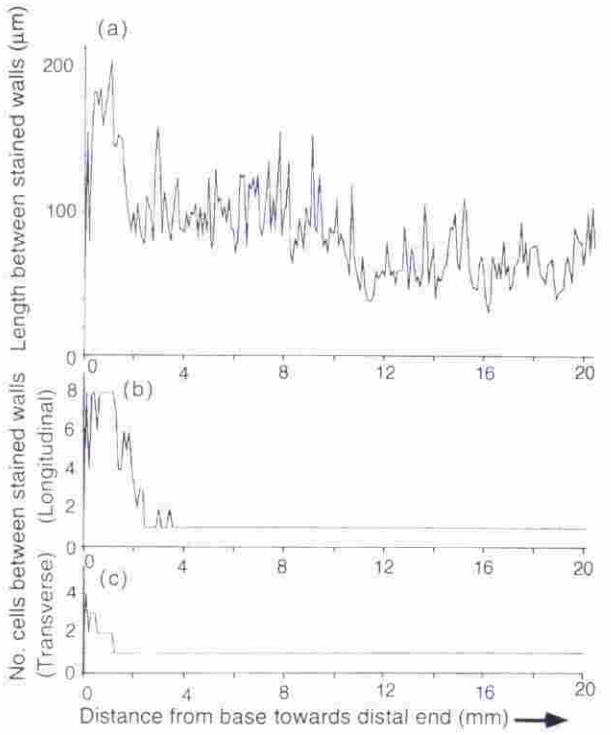


Fig. 26. *Chorda tomentosa*. Elongation of cells and frequency of cell divisions in various parts of the thallus 2 weeks after fluorescent staining. Measurements were taken from a sporophyte whose height was 1.5 mm when stained. For detailed explanations of figures see legend for Fig. 9.

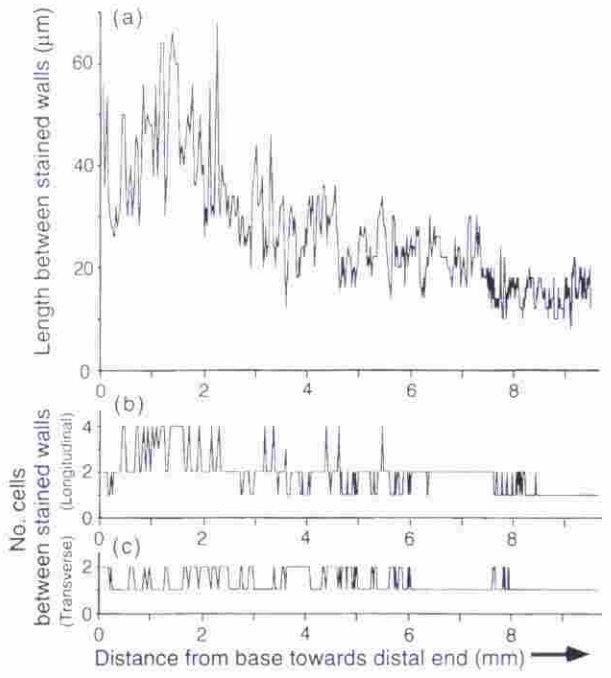


Fig. 27. *Pseudochorda nagaii*. Elongation of cells and frequency of cell divisions in various parts of the thallus 1 week after fluorescent staining. Measurements were taken from a sporophyte whose height was 25 mm when stained. For detailed explanations for figures see legend for Fig. 9.

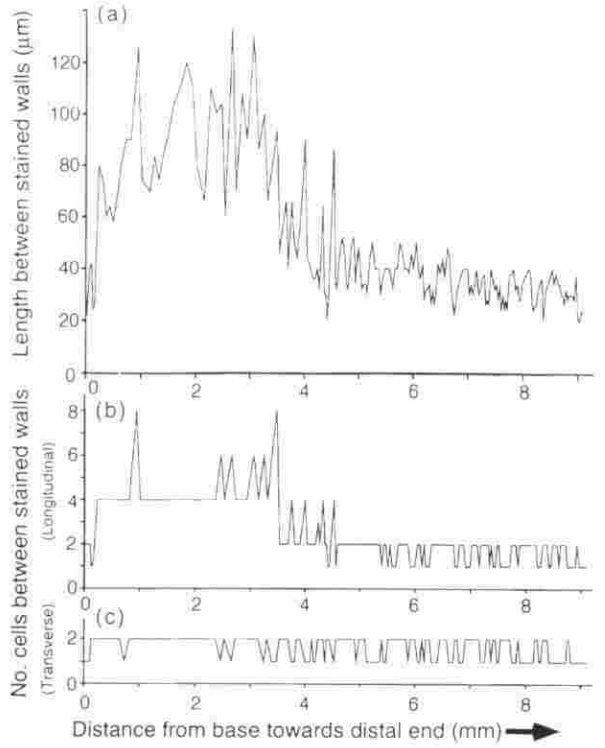


Fig 28. *Pseudochorda gracilis.* Elongation of cells and frequency of cell divisions in various parts of the thallus 9 days after fluorescent staining. Measurements were taken from a sporophyte whose height was 42 mm when stained. For detailed explanations for figures see legend for Fig. 9.

meristem arises at the base of the young sporophyte, and that the thallus becomes thicker and longer by active growth in the sub-meristematic region. Based on this finding they called the sporophyte of *C. filum*, before formation of a visible meristem, the 'primary sporophyte' (corresponding to the diffuse growth and basal meristematic growth stages in the present paper), and that having a meristem the 'secondary sporophyte' (intercalary meristematic growth stage in this paper). Our results in principle agreed with their observations, although South and Burrows (1967) did not distinguish basal and intercalary meristematic growth stages. We consider it important to distinguish these stages, because the basal meristematic growth mode can be recognized in various other taxa, within and outside the Laminariales, and possibly reflects their common phylogenetic origin.

Early developmental processes of the sporophyte in terete members of the Laminariales were, in principle, common to the four known species (*C. filum, C. tomentosa, P. nagaii* and *P. gracilis*). Localization of growth in the basal part of the sporophyte was not known before in *C. tomentosa* and *Pseudochorda* spp. However, further development of these regions into meristem having greater meristematic activity did not occur in these taxa.

In all four species, the zygotes (and parthenogenetic sporophytes) elongated and divided, forming transverse walls by diffuse growth, and then the sporophyte became polystichous, beginning from the upper or middle portions. The lower part of the thallus remained uniseriate for a considerable period, but finally became polystichous, and the basal meristem was formed from those tissues (basal meristematic growth). Sizes of chloroplasts were similar in all parts of the thallus in the diffuse growth stage, but became smaller in the basal meristem. In *C. filum*, following the development of basal meristem, active elongation of cells occurred at both ends of the meristem and later shifted to the adjacent regions below the meristem. At this stage, epidermal as well as medullary cells of the meristem showed characteristic cytological features of meristematic cells, resembling apical meristematic cells reported in some other brown algal orders (see following). Therefore, we suppose that the common ancestors of Pseudochordaceae and Chordaceae had both diffuse and basal meristematic growth stages, and that intercalary meristematic growth was newly evolved in *C. filum*.

The occurrence of both parenchymatous and pseudoparenchymatous construction in the development of the sporophyte, as seen in *C. filum* in the present study, is also seen in *P. nagaii* (Kawai and Kurogi 1985). In cultures of *P. nagaii*, spirally arranged parenchymatous tissue became firmly adherent and formed a thicker terete, hollow thallus. Such adherent construction was not seen in *P. gracilis* and the species stayed solid and smaller in diameter. In contrast, in more derived, foliose members of Laminariales the sporophytes are formed exclusively by parenchymatous development, imparting greater physical strength to these thalli. This would have enabled them to evolve even larger, more elaborate sporophytes.

In *Saccorhiza polyschides* (Lighth.) Batters, in the family Phyllariaceae, another primitive group of Laminariales, the upper two cells of four-celled sporophytes soon become polystichous and later develop into the uniseriate terminal portion of the blade, while the lower two cells remain uniseriate for a time, before the second cell from the base finally develops into the stipe and intercalary meristem (Sauvageau 1918; Norton and Burrows 1969). The developmental processes of *Saccorhiza* are similar to *C. filum* in that the lower regions of thallus remain uniseriate for a relatively long period even after the development of a polystichous region in the upper part of the thallus, and the meristem originates from the lower uniseriate region.

In more derived families of Laminariales (i.e. Laminariaceae, Alariaceae and Lessoniaceae), a structure similar to the initial meristem of *C. filum* is described, as in the young sporophytes of *Laminaria digitata* (L.) Lamouroux (Killian 1911; Fritsch 1945). However, the period during which the lower region of young thalli re-

mains uniseriate is considerably shorter compared with the Pseudochordaceae, Chordaceae and Phyllariaceae, and hence the region where the meristem differentiates cannot be demonstrated clearly (Kanda 1938; Fritsch 1945). Meristematic cells with the cytological features common to those of *C. filum* and other apical meristematic cells in other orders are not seen in more derived families.

Of the sporophytes of *S. polyschides* collected in the field, 76.5% of total elongation of the thallus takes place in the region 25 mm above the transition zone (Norton and Burrows 1969). In the region more than 75 mm from the transition zone, elongation no longer takes place. In more derived families, elongation of the sporophyte is not as localized as in *C. filum,* but maximum elongation occurs only in the upper region adjacent to the transition zone (*Laminaria hyperborea,* Kain 1976; *Macrocystis* and *Nereocystis,* Kain 1987). Therefore, we consider the elongation patterns of the blade after differentiation of the intercalary meristem to be common to all laminarialean families that have an intercalary meristem. Furthermore, the basic developmental processes of parenchymatous tissue formation, in which epidermal cells are involved in the increase of inner cell layers and medullary cells derive from cortical cells, are also common to these groups (Sauvageau 1918; Norton and Burrows 1969; Emerson *et al.* 1982).

Some groups of brown algae outside the order Laminariales are also known to have basal or intercalary meristematic regions in elaborate macroscopic thalli. Sporophytes of some members of the Desmarestiales and Sporochnales have been shown to have a meristematic region near the base (Moe and Silva 1981; Motomura *et al.* 1985). They have a pseudoparenchymatous sporophyte construction and are regarded to be rather distant from Laminariales that have parenchymatous sporophytes according to the conventional taxonomic systems (Wynne and Loiseaux 1976) that have been commonly adopted for a long time. However, their phylogenetic relationships are probably closer, as has been discussed repeatedly in regard to various other features (Clayton 1984; Maier and Müller 1986; Clayton and Ashburner 1990; Kawai 1992; Tan and Druehl 1996). It is likely that they share with Laminariales a common ancestor that had the basal meristematic type of growth in the sporophyte.

Ascoseira mirabilis Skottsberg (Ascoseirales) also has an intercalary meristem in its large, elaborate, erect thallus (Moe and Henry 1982). It has a morphological resemblance during the early development of the thallus to members of derived families of Laminariales. However, *Ascoseira* differs from the Laminariales, Desmarestiales and Sporochnales in having drastically reduced (fucalean-type) gametophytes formed in coneptacles, isomorphic gametes and the presence of unique conduction channels; its phylogenetic affinity is still uncertain.

Kawai (1986) demonstrated the close phylogenetic affinity of *Akkesiphycus lubricus* Yamada et Tanaka to Laminariales because of similar chloroplast cytological features and sporophyte early development, and suggested that this species represented an ancestral form of the Laminariales. However, our preliminary observations on the early development of the *Akkesiphycus* sporophyte did not clearly show the occurrence of a basal or intercalary meristem (data not shown).

With regard to the cytological features of meristematic cells in Laminariales, Grevby *et al.* (1989) reported the occurrence of poorly developed chloroplasts, having a lower photosynthetic capacity than normal chloroplasts in the intercalary meristem, in *Laminaria saccharina* (L.) Lamouroux. They also reported that the sizes, chlorophyll content and number of thylakoids were higher towards the distal end. In the hapteron meristoderm cells of *Laminaria* spp., Davies *et al.* (1973) reported the occurrence of poorly developed chloroplasts, having few thylakoids and containing rich Golgi vesicles. In *C. filum,* the diminution of chloroplast size and the appearance of abundant small vesicles in the cell preceded the differentiation of meristematic initial cells. The smaller chloroplasts had fewer thylakoids than normal chloroplasts. These chloroplasts enlarged and small vesicles in the cells diminished when the elongation of thalli was stopped by cutting the central and adjacent region of meristem. These features agree with the formation processes of apical meristems reported for Sphacelariales, Dictyotales and Fucales (Bisalputra and Bisalputra 1969; Neushul and Dahl 1972; Pellegrini 1979; Katsaros *et al.* 1983; Katsaros and Galatis 1985, 1988, 1990; Clayton *et al.* 1985; Klemm and Hallam 1987). The same phenomena occurred in artificially fragmented segments lacking the prospective region of the initial meristem in *C. filum* (data not shown) and in the regeneration of the apical meristem in *Halopteris filicina* (Gratel.) Kützing (Katsaros and Galatis 1990). Enlargement of chloroplasts accompanied by development of thylakoids took place in epidermal meristematic cells when the length of thallus below the meristem exceeded 40–50 mm.

There have been detailed studies of the meristematic structures in members of Sphacelariales (Prud'homme van Reine and Star 1981; Katsaros *et al.* 1983; Katsaros and Galatis 1990), Dictyotales (Katsaros and Galatis 1985, 1988) and Fucales (Moss 1967, 1969; Clayton *et al.* 1985), all of which have apical growth (see Katsaros 1995 for review). According to these accounts, apical meristematic cells generally contain many small vesicles and immature small chloroplasts, compared to ordinary vegetative cells. There have been a few studies on the anatomy of taxa that show intercalary growth (e.g. Laminariales; Kylin 1918; Fritsch 1945); however, their fine structural features and developmental processes have been only poorly understood.

In conclusion, it is now clear that all terete members of Laminariales have a basal meristematic growth stage, which is considered to be common to other members of the order Laminariales, and possibly to Desmarestiales and Sporochnales. However, in the terete Laminariales an intercalary meristem develops only in *C. filum*. The intercalary meristem of *C. filum* is unlikely to be a transitional stage from a basal meristem (seen in other primitive families) to the intercalary meristem of the more derived families in the Laminariales, because the meristematic cell of *C. filum*, referable to those of apical meristems in other phaeophycean orders, is unique in the order.

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