MORPHOGENESIS AND THE FINE STRUCTURE OF CULTURED CARROT CELLS

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SUMMARY

A study has been made of cultured cells from the root of Daucus carota at defined stages along
the route from quiescent cells to the globular embryos known to produce plants. The salient
stages were: (i) the quiescent cells in tissue explants exposed only to a basal medium; (ii) cells
variously induced to grow by prescribed growth-promoting supplements; (iii) clusters of cells
whether attached to a proliferating explant or free in a liquid medium; (iv) stock cultures routinely
maintained in an incipiently embryonic condition in the form of small cell clusters; and (v)
cultures in which, as pro-embryonic globules, embryogenesis has been released at will. The
fine structure of the cells along this morphogenetic route has been investigated and related to the
causal and controlled conditions by which the stages in question were achieved. A range of
distinctive cytoplasmic inclusions that occur in cells at these specified stages has been described;
these inclusions are not virus particles per se and their occurrence and form is an obvious func-
tion of the cultural conditions. The morphogenetic propensity of the cells that give rise to embryos
is associated with cytoplasm that is notably free of identified inclusions, that are otherwise
conspicuous, but this cytoplasm is rich in microtubules and has some other distinguishing
characteristics that are described.

INTRODUCTION

As the cells of the secondary phloem differentiate from the cambium, they enlarge
and reach a static, quiescent condition in the storage root, and normally they would
not grow again. When isolated aseptically in the form of small explants, however, they
may be induced to grow again and they then exhibit, as explants, various degrees of
activity in accordance with the nutrients and supplementary growth-promoting sub-
stances in the ambient medium (Steward, 1970).

Some of the changes in organelles and inclusions which accompany the recrudescence
of growth and development in the cultured explants have already been described
(Israel & Steward, 1966, 1967). These are: (1) The mitochondria become more numer-
ous and are often long and irregular, with coarse cristae, in contrast to their more
rounded form and sharply defined cristae in the resting tissue. (2) The plastids also
change their form and, in the light, develop into chloroplasts as their thylakoids arise
from a characteristic dense inclusion, the prethylakoidal body, which forms especially

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in the dark. (3) The ground cytoplasm becomes densely granular due to the numerous ribosomes, which may be free or attached to membranes or present in orderly clusters. (4) The Golgi apparatus (dictyosomes) shows visible evidence of activity as the cells pass from the quiescent to the growing state. (5) Also microtubules appear, especially at the outer cell surface, adjacent to the plasmalemma.

The proliferating explants may also release free cells and small clusters, which may then grow in the ambient liquid medium and furnish inocula for subcultures. By suitable means, these subcultures may be caused to grow in an organized, even embryonic, manner and eventually develop into plants (see Steward, Mapes & Ammirato, 1969).

The cells in liquid cultures, particularly the isolated cells, depart drastically from the quiescent condition of their progenitors in situ (Steward, 1961) and they become almost unrecognizable as the counterparts of the quiescent cells as they exist in the storage organ. In fact, there is a striking resemblance among the cells cultured from different plants as they occur free in liquid media of the same composition and under the same conditions (Steward et al. 1969, cf. Fig. 14).

Cultures generated in this way from explants of the secondary phloem of carrot root have been adapted to the study of morphogenesis (Steward et al. 1969 and references there cited). Along the route from isolated tissue explants to proembryonic globules, which may so develop that they resemble zygotic embryos, definitive events occur at which the course of growth and development is responsive to both environmental conditions, nutrients and growth stimuli in the medium. The details of the conditions which are conducive to these events cannot be dealt with fully here but the appearance of cells under the treatments that have been used will be stressed. Thus, the present fine-structural study extends the others that have been made (Israel & Steward, 1966, 1967), for it describes the range of inclusions that visibly occur in carrot cells subjected to different media and stimuli which variously induce growth and eventual somatic embryogenesis.

MATERIALS AND METHODS

Recent developments on the effects of darkness and total concentration (Ammirato & Steward, 1971) now permit cultured cells of explants from the secondary phloem parenchyma of the carrot (Daucus carota) root that are morphogenetically induced, to be reversibly arrested and later released in their development. To accomplish this the cells are first stimulated to grow by the use of various culture media supplemented by combinations of growth-regulatory substances or systems (Degani & Steward, 1969; Steward & Degani, 1966) and are subjected, sequentially, to different environments and/or stimuli (Steward, Kent & Mapes, 1967).

In the present work 2 basal culture media were routinely used. They consisted either of a medium (designated Bw) after White (1943) or one (designated B3) after Murashige & Skoog (1962). The medium Bw, in contrast to B3, has lower total and potassium concentrations and contains all its nitrogen as nitrate, in contrast to the ammonium which is present in the medium B3. The supplementary growth factors used were whole coconut milk (CM) (or water, i.e. the liquid endosperm from the central cavity of mature nuts) used at a concentration of 10%, v/v; inositol at 25 ppm; indoleacetic acid (IAA) at 0.5 ppm; naphthaleneacetic acid (NAA) at 2-5 ppm; and zeatin used at 0.5 ppm.

Ordinarily the experiments were carried out for 18 days in constant diffuse light at 22 °C and under the standard conditions used in this laboratory (Steward, 1970). For the purposes of this study, the aseptically cultured plant materials were obtained under the specified conditions listed.
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as (a) to (e) below. These conditions present cells in different states along the sequence of events which are intermediate between tissue explants and embryos and are described in Figs. 1–31.

(a) In quiescent explants exposed only to B_w, which neither promotes rapid growth nor organization (Figs. 1, 2).

(b) In tissue explants variously induced to grow in B_w supplemented by inositol (Fig. 3), by IAA + zeatin (Fig. 4), by IAA + zeatin + inositol (Fig. 5), by CM (Fig. 6), or by CM + inositol (Fig. 7).

(c) In growing cultures on B_ils (Figs. 8, 9) or in cultures of free cells on this medium + NAA (Figs. 10–12), as well as in cultured tissue explants on B_w + CM if this also contained NAA (Figs. 13, 14).

(d) In organizing cell clusters, either as nodules from cultured explants on B_ils + NAA (Figs. 15–18), or in stock cultures grown on the same medium as potentially embryonic units consisting of 30–50 small dense cells (Figs. 19–26).

(e) In proembryonic globules derived as above on B_ils + NAA but released to grow in an organized manner by the sequential removal of the NAA by transfer to B_ils alone (Figs. 27–31).

The cultured cells and tissues so obtained were fixed for later examination under the electron microscope in phosphate-buffered (pH 7.2) glutaraldehyde (2%–5% %) for 3 h. Proliferated explants were cut into suitably small segments and the faster growing cultures with smaller cells were best fixed at the lower concentrations. The glutaraldehyde-fixed specimens were washed for 1 h with the buffer and then treated for 1–2 h with buffered osmium tetroxide (1–2 %). All these operations were done at 7–8 °C. The fixed stained specimens were embedded in Epon 812 and thin sectioned with diamond knives for examination in a Philips EM 200. The micrographs reproduced in the figures are representative of the treatments and conditions described.

To assess the frequency of given inclusions in response to cultural treatments many micrographs were prepared from each of the replicated samples harvested and fixed and from which numerous preparations were made for electron microscopy. At a typical magnification of x 20,000 only about 0.01 of the larger vacuolated cells that did not divide may be seen in a single microscopic field, whereas the smallest proembryonic cells approximately filled a field. Full use was made of the very large range of material available from the 2 cultural treatments most used as controls in our experiments (about 30 experiments in which standard explants were grown on the basal medium, B_w, with and without coconut milk). For all other treatments enough replicate samples were fixed, sectioned and examined to provide many micrographs from different experiments using different carrot clones to ensure that the observations as recorded reflected the effects of the treatments.

EXPERIMENTAL RESULTS

Carrot cells possess many well recognized organelles that are so necessary for the vital activities of living cells that they appear at all levels of biological activity. Even so, certain of these, e.g. mitochondria, plastids, Golgi, etc. do show some changes in number and form in response to conditions which regulate the activity of the cells in which they occur. However, Figs. 3–26 show that some other inclusions are also greatly affected in their abundance and form by factors which induce growth and morphogenesis. Although these inclusions are not essential organelles, they become more obtrusive as attention is directed to the changes that accompany the transitions from resting cells of the storage organ to the proembryonic state. These inclusions occur under varied conditions in a range of forms and, since their nature, function and nomenclature are neither clear nor generally agreed upon the descriptive terms listed below will be used.

'Rotate inclusions' (or 'pinwheels') consist of whorled sheets borne on cylindrical laminated 'stalks' from which, in different planes of section, may be derived the several forms (Figs. 3–7) that these inclusions take. Inclusions, which possess both the
‘pinwheel-like whorled head’ attached to a laminated or ‘multifibrillar stalk’ with its base often attached to a membrane (Fig. 4) have been adequately depicted in such diagrams as those of Edwardson (1966) and Edwardson, Purcifull & Christie (1968).

‘Cratile inclusions’ appear as dense compact rectangular grids (Figs. 8–14) and have all the structural regularity of crystalline bodies. Similar structures have also been described by Israel & Ross (1967), Petzold (1967) and Villiers (1968).

‘Multifibrillar bundles’ appear as rather massive inclusions and consist of tight bundles of fibrils (Figs. 17, 18, 23–26). Comparable structures seen in developing phloem have been described by Cronshaw & Esau (1967); Parthasarathy & Mühlenhaher (1969); Wergin, Gruber & Newcomb (1970); Palevitz & Newcomb (1971); Konar, Thomas & Street (1972); and Thomas, Konar & Street (1972).

All of these bodies may be different forms of the same material but on present evidence this can be no more than a presumption based upon the repeated observations that when the one type of inclusion is abundant the others are not.

Table 1 summarizes the conditions (a–e) which produced cells in the different states noted and the fine-structural features described; it also indicates the range of materials surveyed of which the figures are held to be representative. When phloem explants were cultured for 18 days, without much growth, on Bw (condition a: Figs. 1, 2) they did not contain the inclusions here referred to as rotate, cratile or multifibrillar. When the explants were variously stimulated to grow in Bw to a limited extent by inositol and zeatin, singly or in combination, or more rapidly by CM with and without additional inositol (conditions b: Figs. 3–7), the characteristic inclusion that appeared in the cells was that which has been termed rotate. A different basal medium, BMS, allowed cells attached to proliferating explants to acquire the grid-like inclusions here termed cratile; similar inclusions were elicited in freely suspended enlarged cells on this medium plus NAA and in cells on proliferating explants on Bw + NAA + CM (conditions c: Figs. 8–14). But, in localized nodular centres produced on growing explants by BMS + NAA (conditions d1: Figs. 15–18) and in stock cultures of globular units, composed of small cells, from which organized growth could be elicited, and derived via the same conditions (category d2: Figs. 19–26), the rotate or cratile inclusions were not seen, whereas those termed multifibrillar bundles were prominent. Moreover, when the culture medium was changed sequentially (BMS + NAA → BMS) to release organized development, the proembryos which first emerged from the globular units were composed of dense cells free of the rotate, cratile or even multifibrillar inclusions, although microtubules were their conspicuous feature (category e: Figs. 27–31).

In the transition from the rather unorganized proliferative growth of attached cells on explants, with their large vacuoles, thin cytoplasm, rotate and cratile inclusions seen in Figs. 1–14, to the more embryonically induced cells of attached nodules and loose clusters, with their developing vacuoles, dense cytoplasm and multifibrillar bundles seen in Figs. 15–18, certain cytoplasmic organelles such as Golgi bodies, endoplasmic reticulum and mitochondria, as well as the microtubules, became conspicuous. Vesicles of presumed Golgi origin tended to permeate the entire cytoplasm (Fig. 16) and in Figs. 17 and 18 they are shown congregated near to microfibrillar bundles. Although Golgi bodies were still apparent as cells reached the proembryonic globular stage, the
<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Status of cells or culture when fixed</th>
<th>Pertinent figures</th>
<th>Characteristic inclusion†</th>
<th>No. of clones or expts</th>
<th>No. of micrographs used¶</th>
<th>Approximate no. of cells examined</th>
<th>Frequency of occurrence of characteristic inclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Large (100–150 μm), highly vacuolate, quiescent cells in explants which, on the basal medium (Bw), neither undergo cell division nor morphogenesis</td>
<td>1 and 2</td>
<td>None</td>
<td>31</td>
<td>&gt; 150</td>
<td>Very large</td>
<td>Special inclusions absent</td>
</tr>
<tr>
<td>b</td>
<td>Cells (30–50 μm) in cultured explants variously stimulated to grow on basal medium (Bw) by inositol (25 ppm); IAA (0.5 ppm) + zeatin (0.5 ppm); coconut milk (10%, v/v) + inositol</td>
<td>3 to 7</td>
<td>Rotate</td>
<td>34</td>
<td>&gt; 500</td>
<td>Very large</td>
<td>Very many per cell, fewer in presence of coconut milk or balanced growth factors (order of 0.5 to 1%, of field area)</td>
</tr>
<tr>
<td>c</td>
<td>Cells (15–30 μm) in cultured explants growing on basal medium (Bw) and the larger free cells on Bw + NAA Cells in cultured explants on Bw stimulated by both CM and NAA</td>
<td>8 to 12</td>
<td>Cratile</td>
<td>7</td>
<td>120</td>
<td>30000</td>
<td>No. ranging from 4 to 50 per cell; more in the free cells on Bw + NAA, order of 1%, of field area</td>
</tr>
<tr>
<td>d</td>
<td>Small (15–30 μm), dense cells in localized, nodular, growing centres attached to cultured explants or in free nodules on Bw + NAA (2 ppm)</td>
<td>13 and 14</td>
<td>Multi-fibrillar</td>
<td>4</td>
<td>130</td>
<td>4800</td>
<td>Order of 1 per cell</td>
</tr>
<tr>
<td>dii</td>
<td>Similar dense cells (10–20 μm) in small globular units grown free on Bw + NAA in the propagation of incipiently embryonic stock cultures</td>
<td>15 to 18</td>
<td>Multi-fibrillar</td>
<td>2</td>
<td>75</td>
<td>50000</td>
<td>Order of 1 per cell</td>
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<tr>
<td>e</td>
<td>Somewhat larger (20–50 μm) cells in proembryonic globules (dii) but after their release to develop in an organized way on Bw minus NAA</td>
<td>19 to 26</td>
<td>Microtubules</td>
<td>4</td>
<td>250</td>
<td>30000</td>
<td>Microtubules abundant, other special inclusions absent</td>
</tr>
</tbody>
</table>

* The experiments were carried out under the standard conditions in diffuse light at 22°C for 18 days of growth.
† This table refers only to the occurrence of the special inclusions listed.
¶ The micrographs used were representative of a large number of cells which ranged from over 100,000 in the most frequently sampled experimental conditions (a) and (b), but they were never less than 10 to 20,000 cells for the less-frequently applied treatments.
proliferation of vesicles seemed less evident (Fig. 22). Conspicuous endoplasmic reticulum in these cells was characteristic of their transformed state and this feature also occurred in the globules from which proembryos could develop. While mitochondria exhibited no special morphological features in the cells of attached nodules or loose clusters, they were seen in the cells of the globular units as drawn out (Fig. 21), circular (Fig. 22), or cup-shaped (Fig. 21) forms, depending upon the plane of sectioning. Microtubules were not conspicuous until the cells were present in globular proembryonic units which were actually released for morphogenesis (Figs. 27-31) when they became speedily apparent and even more obvious in the later stages of development (Figs. 30, 31).

DISCUSSION

While it may be difficult to assign causal significance to the presence or absence of the special inclusions noted in the carrot cells, it is nevertheless suggestive that the microtubules appeared prominently in the cells that had the greatest morphogenetic propensity, whereas the other conspicuous inclusions were most apparent in cells in which these propensities seemed to be more restricted.

Suggestively, the conditions which bring about the occurrence of one or other of the special inclusions do so to the virtual exclusion of the others. For example, rotate inclusions were especially abundant in cultures in unorganized rapid proliferative growth; cratle inclusions were prominent in cultures exhibiting restricted growth coupled with some localized organization; and multifibrillar bundles appeared chiefly in the cells of cultures that were so induced that they could undergo morphogenesis. Three forms may, therefore, be alternative expressions of the same basic material or subunits, so that the end product is a function of the status of the cultured cells in question. The alternative is that the same strain of cells could give rise, quite independently, to 3 such distinctive but completely unrelated structures and this seems hardly credible. Moreover, various clones of carrot cells have consistently shown the same inclusions under given sets of cultural conditions, and all attempts thus far by accepted methods to demonstrate that these inclusions represent active viruses in the cells have given negative results. Therefore, the presence or absence of one or other of these inclusions indicates the status of the cells in question and may reflect the degree to which the expression of their innate totipotency may have been activated or may be limited.

The changes here noted in the form of mitochondria of proembryonic globules are reminiscent of earlier evidence obtained on other embryonic materials (Manton, 1961: Israel & Sagawa, 1964; and Schulz & Jensen, 1968) and they appear to represent characteristic phases of mitochondrial development.

Halperin & Jensen (1967) studied embryogenesis in cultured cells of wild carrot and have made observations on microtubules similar to those here reported, although they did not comment on the other inclusions here discussed. Another study of cultured cells in relation to problems of somatic embryogenesis appeared since the original completion of this paper (Konar et al. 1972; Thomas et al. 1972). The first of these
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papers essentially explores, for *Ranunculus sceleratus*, the microscopic features of the cultured cells which, for carrot, had been previously described. The second of these papers, from the same laboratory, deals more specifically with the fine-structural features of 'embryogenic callus' from the plant in question and to this extent it is relevant to the study here described. Clearly Street and his collaborators have seen, in the cells of *Ranunculus*, many cytological features that resemble closely some of those described for carrot cells. These features include the various forms of mitochondria, the development of plastids with their prethylakoidal bodies and globular centres, the occurrence of microtubules, the bodies which have been referred to here as multifibrillar bundles and the so-called myelin-like forms. However, by the use of more controlled procedures the study on carrot, as here reported, has related the cytological features described more closely to the various states (i.e. a-e of p. 60) in the sequence from the original, quiescent, freshly isolated, cells from the carrot root to the pro-embryonic globules that grow as embryos in an organized manner.

Cytoplasmic inclusions similar to the multifibrillar bundles of this study have also been seen in cells related to stages of sieve element formation and for these the descriptive term 'P-protein' has been used. Palevitz & Newcomb (1971) presented evidence that configurational changes within these P-protein particles do occur.

Like the multifibrillar bundles, the cratile inclusions have been variously reported and those found in the cytoplasm by Petzold (1967) and in the nuclei by Villiers (1968) are but selected examples. While Villiers revealed that these inclusions tended to disappear when the plants were starved, no pre-existing or subsequent structures were noted.

Having observed the range of the cytoplasmic inclusions during the induction of growth in quiescent cells and their progress along a morphogenetic route to cell clusters which function as proembryos the following may be offered as a basis of interpretation.

Plant cells which are in an arrested stage of development often store quantities of unusual compounds which may be soluble and of low molecular weight, viz. the wealth of non-protein nitrogenous compounds, and macromolecules, viz. special storage proteins some of which, like urease or papain, are enzymes. Nevertheless, when the cells are brought into active growth again, many of these substances become much less obtrusive as they are consumed in new growth, with the formation of new structure.

In a somewhat parallel fashion the cells in the storage organ or in culture, which are in various states of arrested growth and/or morphogenesis, may be characterized by a rich display of particular inclusions in their cytoplasm. These inclusions have no known role as organelles and their form is a function of the conditions that regulate the level of activity in the cells. Nevertheless, when the cells are reactivated to further growth and embark upon organized development, their cytoplasm is freed again of the more unusual inclusions noted, although, with their disappearance, microtubules seemingly reappear. The fine-structural changes that have been observed are therefore concomitant with, even if they are not causal to, definitive stages in the transition between quiescent cells in the storage organ to totipotent cells that may form embryos.

An initial aim of this investigation was to provide fine-structural evidence diagnostic of the earliest stages of development in cells along a morphogenetic route. Paradoxically,
however, the cells that are most able to express their innate totipotency in cultures, which are en route to embryos, prove to be remarkably free from visibly distinguishing features - free even of some special inclusions and mitochondrial forms which appear abundantly in similar cells at earlier stages and under other cultural conditions. Abundant microtubules typify the cells most capable of organized development; the other inclusions that have been described clearly characterize cells that have acquired specialized characteristics that need to be corrected before embryogenesis ensues.

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REFERENCES


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Figs. 1–7. Features of cytoplasmic inclusions in cells of proliferating carrot explants: the rotate inclusions. All calibration bars represent 1 μm.

Figs. 1, 2. Cultured cells on a medium (Bw) which does not support active growth, showing portions of large vacuolated adjacent cells with their thin parietal protoplasm.

Fig. 1 shows a portion (about 25 μm) of wall and cytoplasm.

Fig. 2 shows a portion of Fig. 1 at higher magnification. Effects due to the graduated application of growth-promoting substances are shown in subsequent figures.

Fig. 3. The cytoplasm of cells stimulated by inositol (Bw + inositol). Note the more abundant cytoplasm (cf. Fig. 2) with its complement of organelles which include ribosomes, plastids (p), mitochondria (m) and Golgi bodies (g). In addition there are the long, coarse, filamentous inclusions which here take the form of stalks (arrow) to which the rotate inclusions ('pin-wheels') are attached (Fig. 4).

Figs. 4–7. Show the effects of progressively increased growth induction by the further addition of IAA + zeatin (Fig. 4); by IAA + zeatin + inositol (Fig. 5) by coconut milk (Fig. 6) and by coconut milk + inositol (Fig. 7). All of these figures show the inclusions seen in Fig. 3, but they vary in form and abundance. IAA and zeatin (Fig. 4) emphasized well developed rotate inclusions (pin-wheels, arrowed) whereas added inositol (Fig. 5) again accentuated the filamentous inclusions. Under the stimulus of coconut milk (Fig. 6) there was more rapid cell multiplication, and ribosomes and rough endoplasmic reticulum (arrow), together with some of the more specialized inclusions, were emphasized. The further addition of inositol (Fig. 7) again accentuated the rotate and filamentous inclusions.
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Figs. 8–18. Localized organization in cultured explants: the cratile inclusions (Figs. 8–14); the fibrillar bundles (Figs. 15–18).

Fig. 8. Shows dense parietal cytoplasm as in large vacuolated cells on a basal culture medium (BMS). On this medium prominent lipid inclusions (lp) occur, but the distinctive feature is the presence of the grating-like, cratile inclusions (arrows).

Fig. 9. Shows the detail of a cratile inclusion as in Fig. 8.

Figs. 10–14. Show cells subjected to growth-stimulatory media containing NAA (Bw + NAA as in the free cells of Figs. 10–12, and Bw + CM, 1% + NAA as in Figs. 13 and 14), all of which contain the cratile inclusions. Fig. 10 shows a portion of a vacuolated free cell with cytoplasm that contained the inclusions (arrow) shown in detail in Figs. 11 and 12; the latter are at the same magnification and the scale lines represent 0.5 μm. Figs. 13 and 14 show the abundant cytoplasm in these cells proliferated on explants which, in addition to a developing plastid (p), mitochondria (m), endoplasmic reticulum and small vacuoles, contained numerous cratile inclusions (arrows).

Figs. 15–18. Relate to cells in the localized, nodular, growing centres of proliferated cultures attached to explants or free in the medium (Brs + NAA). Fig. 15 shows a general view of small cells which were densely cytoplasmic with developing vacuoles. Fig. 16 shows a field from cells of Fig. 15 with a range of vesiculate inclusions (Golgi, g; mitochondria, m; smooth and rough endoplasmic reticulum and uniformly distributed ribosomes) in cells that also contain, elsewhere, the fibrillar bundles. Figs. 17 and 18 show long, undulating, fibrillar bundles in longitudinal (Fig. 17) and transverse (Fig. 18) section. The scale line on Fig. 18 represents 0.5 μm.
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Fig. 19. Shows a living cluster of 4 proembryonic globules grown on the medium Bm + NAA as seen under the phase microscope.

Fig. 20. Shows cells as they occurred in a proembryonic globule. These cells had dense cytoplasm, numerous developing vacuoles, but they were free from the special inclusions that were prominent in the cells of Figs. 3–14.

Figs. 21, 22. Show cytoplasmic detail from cells similar to those of Fig. 20. Figs. 21 and 22 show other features of cells as at Fig. 20. These are long mitochondria (m) which appear as stretched, joined, 'dumbbell'-shapes in one view (Fig. 21), circular in another (Fig. 22), and both may derive from a cup-shaped structure with a 'vesicular rim'.

Fig. 23. Shows a stretched mitochondrion (m) in parallel with a fibrillar bundle.

Fig. 24. Shows a fibrillar bundle to emphasize the individual fibrils.

Figs. 25, 26. Show fibrillar bundles (arrows) within the nucleus and adjacent to the nucleolus (nuc).
Figs. 27–31. Fine-structural features in early stages of somatic embryogenesis: the ground cytoplasm. All calibration bars represent 1 μm.

Fig. 27. Shows a strip of cell wall with plasmodesmata (pd) in cross-section and microtubules (mt) adjacent to the wall.

Fig. 28. Shows a cell similar to Fig. 27, with remnants of fibrillar bundles (arrow), microtubules (mt) in the cytoplasm and adjacent to cell walls.

Fig. 29. Shows cells with nuclei (n) and dense cytoplasm, small vacuoles, a plastid with starch, and some myelin-like forms (arrow).

Figs. 30, 31. Similar cells with special reference to their abundant microtubules (mt) and some plastids (p).
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