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THE ISOLATION AND CULTURE IN VITRO OF THE QUIESCENT CENTER OF ZEA MAYS[1]

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ABSTRACT

Using 3-day-old seedling roots of *Zea mays* L., cv. Kelvedon 33, it was possible to remove the root cap by a simple surgical manipulation without damage to the root proper. By a further small cut, the quiescent center (QC) itself was isolated. This double-convex lens-shaped tissue piece 100 × 250 μm is composed of 1000–1500 cells representing only 0.25 mm^3 in volume. The explant was demonstrated unequivocally by ^{3}H-thymidine incorporation before excision and then by autoradiography to be composed of the specific cells usually designated the quiescent center. Using sterile techniques, the QC's were placed on nutrient agar slants and allowed to grow in culture. Of a number of nutrient media tested, only a medium supplemented with organic nitrogen components, indoleacetic acid, kinetin and inorganic nutrients plus sucrose (S2M + K –2,4-D) was effective in eliciting development. Thirty to 40 percent of the 150 isolated QC's grown on this medium formed elongated roots, up to 2 cm in length in 3–4 weeks. Roots developing on agar medium showed in their proximal portion a vascular pattern with 5–6 metaxylem elements or variations of this pattern, but as the root elongated, the vascular pattern was progressively reduced in complexity at the more distal end to a small central group of metaxylem elements. When agar-grown roots were transferred after one week in culture to a liquid nutrient medium of the same composition, the initially reduced vascular pattern evident in the proximal tissues became progressively more complex in the distal portion of the root and after 2 cm of elongation, showed an essentially normal primary vascular tissue pattern characteristic of the seedling root.

THE QUIESCENT CENTER (QC) consists of a population of cells differing physiologically (Clowes, 1961; Jensen and Kavaljian, 1956), biochemically (Clowes, 1958a; Fisher, 1968; Jensen, 1956) and anatomically (Clowes and Juniper, 1964; Phillips and Torrey, 1974a), from the surrounding cells in the apical meristem of elongating roots. From the time of the original demonstration of the QC by Clowes (1956a, b; 1958b) until the present, workers visualized and, indeed in the main, characterized the cells comprising the QC by demonstrating their reduced capacity to incorporate labelled precursors for DNA synthesis and labelled precursors of other organic compounds (Barlow, 1974; Clowes, 1958a). In addition, cells of the QC were often described on the basis of the duration of their mitotic cycles (Barlow, 1969; Clowes, 1961; Phillips and Torrey, 1972). Numerous workers (Barlow, 1974; Clowes and Juniper, 1964; Juniper and Barlow, 1969; Phillips and Torrey, 1974a, b) have examined the QC at both the light and electron microscope levels, and have described and compared the contents of these quiescent cells with the surrounding non-quiescent cells.

Until relatively recently, investigators considered that the QC consisted of a group of metabolically uniform cells. Such a bias was illustrated in investigations in which as many as 17 sub-populations of cells within the root apical meristem were described, of which the QC was counted as one homogeneous population (Barlow, 1971). More recently investigators have come to realize that the QC is not composed of cells uniform in every characteristic (Avanzi, Bruni and Tagliasacchi, 1974; Phillips and Torrey, 1972, 1974a, b; Webster and Langenauer, 1973). Gradients in activity as well as in ultrastructural features can be associated with cells of the QC (Phillips and Torrey, 1972; 1974a, b). Clowes (1971) stated that the QC itself can be divided into sub-populations of cells and estimated that 50–60 % of these cells do not divide; of the remaining cells of the QC which do divide, differences in cycle times are found. These investigations, as well as more recent work of our own (Feldman 1975b), suggested that although the QC usually appears as a metabolically inactive, homogeneous population of cells, such a characterization represents an incomplete description of the potentialities of these cells.

Earlier, Clowes (1959) showed that cells of

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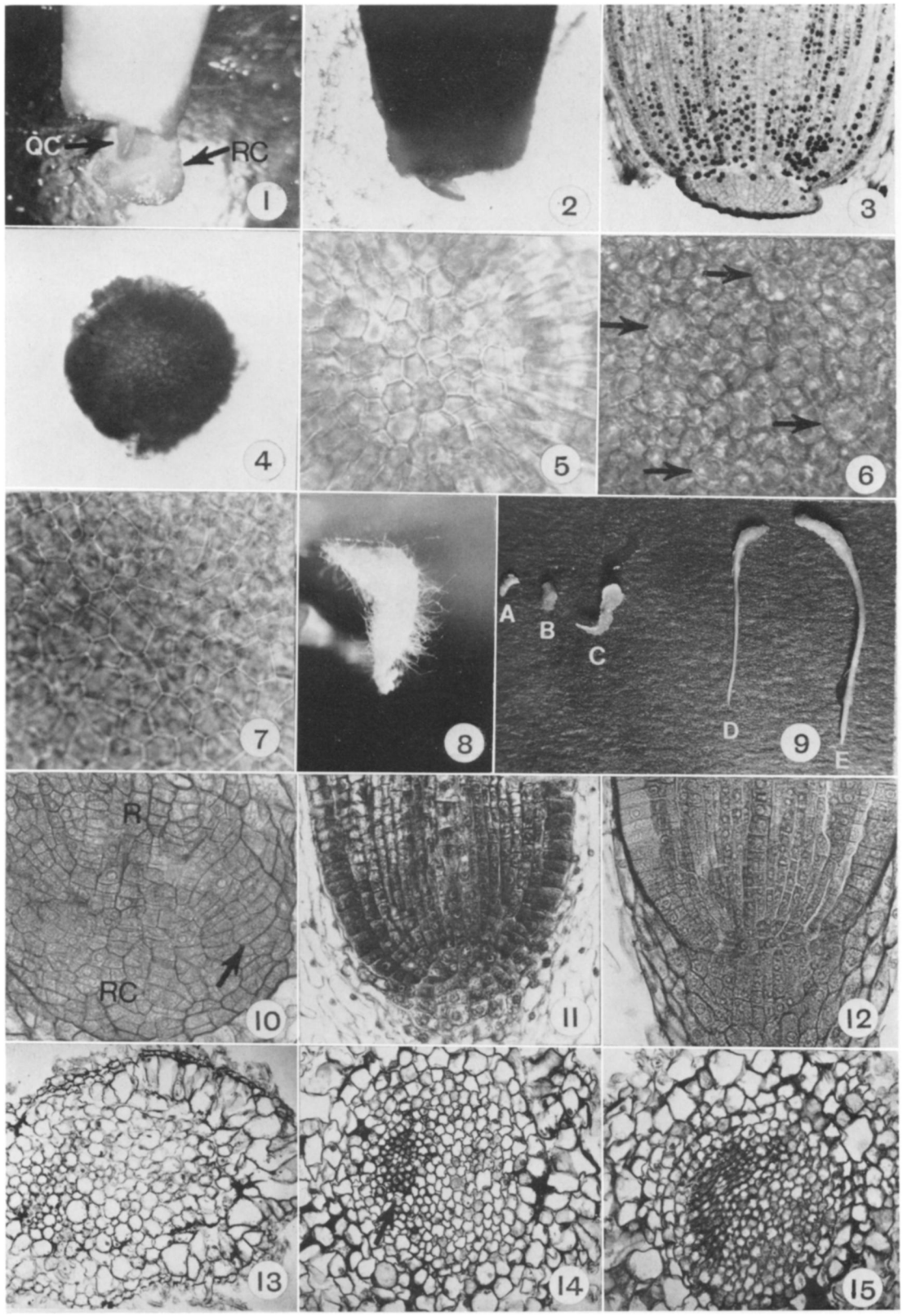
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the QC were activated and entered cell division following X irradiation which destroyed the apical initials as the source of new cells and thus the QC served for regeneration of a new root apex. From experiments in which the root cap is removed and subsequently regenerated by cells of the QC (Barlow, 1974; Bednara, 1974; Feldman, 1975a), one may observe the capacity of the QC to alter its inactivity and to express a developmental pathway not normally seen in mature, rapidly elongating roots. With the exception of a few unsuccessful attempts at examining isolated QC's (Clowes, 1968), almost all forms of examination previously noted have attempted to characterize the QC from an analysis of studies conducted in situ. Little understanding exists as to the regulatory effects of the QC on normal root growth and tissue differentiation and of the effects of other root tissues on the QC itself. The paucity of these types of observations clearly has limited and in part accounts for our admittedly superficial understanding of the morphogenetic roles of the QC. The aim of the work presented here was to examine the structures and developmental potentialities of isolated QC's placed in culture and to relate these findings to ongoing developmental processes in intact roots.

MATERIALS AND METHODS—Corn seed (*Zea mays*, cv. Kelvedon 33) was obtained from Hurst, Ltd., Witham, Essex, England. The seed was surface sterilized in one-half strength commercial chlorox, rinsed three times with distilled water and aseptically germinated in the dark at 23 C. After three days, seedlings with primary roots 25–35 mm in length were selected and used in all experiments.

Isolation and characterization of the quiescent center.—The cultivar Kelvedon was originally selected because of the ease with which the root cap could be removed by a break in the cells of the root-cap-initial layer (Feldman, 1975a). Typically, the surgical removal of the cap left all tissues proximal to the root-cap junction intact and uninjured. Occasionally, it was observed that a small portion of the tissue located just proximal to the cap was also removed during surgery (Fig. 1, 2). This lens-shaped tissue piece was readily separable from the cap. To define this tissue precisely, intact 72-hour-old seedling roots were immersed in one-half strength Knop's solution (Cutter and Feldman, 1970) containing methyl-^{3}H-thymidine (specific activity 6.0c/mM) at a concentration of 0.3 μc/ml for 12 hr. Immediately thereafter, roots were decapped so that the piece of tissue just proximal to the root-cap junction was almost completely severed from the main body of the root, as well as being completely separated from the root cap. The cap was discarded, but the severed tissue, plus a small portion of the apex to which it remained attached was excised, fixed in fresh FAA and prepared for autoradiography as described elsewhere (Feldman, 1975a).

The autoradiograph in Fig. 3, prepared from a root similar to that in Fig. 2, convincingly demonstrates that the lens-shaped tissue observed at the time of root-cap removal was the QC. Note that the break or tear occurred at or just below the first uniformly labelled layer of cells, previously designated the proximal meristem (Feldman, 1975b). Longitudinal sections on either side of the median revealed that the separation layer continued at or just distal to the proximal meristem around the entire proximal circumference of the QC. A few scattered cells located within the QC were also labelled. With the cultivar Kelvedon it was a simple surgical operation to isolate the QC precisely and cleanly with little or no damage to cells of the QC itself since the separation at both the distal and proximal surfaces occurred in the easily torn, thin-walled cells of the root-cap initial layer and proximal meristem, respectively. Thus, isolated QC's could be obtained aseptically and in good numbers from seedling roots.

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Fig. 1–15. Isolation, culture and structure of cultured quiescent centers of *Zea mays*, cv. Kelvedon 33. **1.** Seedling root tip showing root cap (RC) and the quiescent center (QC) both partially dissected free from the root. × 30. **2.** Root tip after root cap removal with QC still partially attached. × 35. **3.** Autoradiograph of a median longitudinal section of a decapped root similar to that shown in Fig. 2. Note the almost complete lack of nuclear labelling in the QC except on the proximal edge. × 100. **4.** Isolated intact QC, dissected from the root and photographed in face view. × 100. **5.** Enlarged view of distal surface of isolated QC. × 300. **6.** Surface view of proximal surface of isolated QC. Arrows denote enlarged cells in the position of future metaxylem elements. × 300. **7.** Proximal surface showing no cell pattern. × 300. **8.** Seven-day-old root from cultured QC. The original, isolated QC remains in contact with the agar surface. Same as in Fig. 9a. × 12. **9.** Roots developed from cultured QC's. a–c after 5–10 days in culture; d–e after 14–28 days in culture. × 0.8. **10.** Longitudinal sections of partially reorganized root apex in 8–10-day-old cultured QC. Root cap (RC), main body of root (R). Arrow denotes root cap junction. × 240. **11.** Median longitudinal section of root apex from 3-week-old cultured QC. × 240. **12.** Median longitudinal section of root apex from 4-week-old root from QC cultured in liquid nutrient medium. × 240.—Fig. 13–15. Transections of root from 3-week-old cultured QC on agar medium, cut at different distances from proximal end. **13.** Section through original cultured QC, 100 μm from base. × 200. **14.** Section, 950 μm from base. Arrow indicates asymmetric differentiation of vascular tissue. × 200. **15.** Section, 1110 μm from base. × 225.

TABLE 1. *The development of isolated QC's from seedling roots of Zea mays, cv. Kelvedon 33 on different nutrient media after 4 wk. In all cases, the pH of the medium was adjusted to pH 5.5 prior to autoclaving. In those cases where no growth or only slight growth was recorded, observations were extended to 2 months after initial sub-culturing.*

Modifications of medium	Observations and results
White's Medium (White, 1954)	
Increased sucrose from 2% to 4%	Very slight growth
Replaced sucrose with 4% glucose	No growth
Soybean callus medium (Fosket and Torrey, 1969)	
Unmodified	Slight growth, much callusing
Increased sucrose from 3 to 4%	Slight growth, mostly callus
Increased kinetin from 0.1 to 1.0 mg/l	Slight growth, mostly callus
Omitted kinetin	No growth
Reduced NAA from 2.0 to 0.2 mg/l	Good growth of callus and primordium formation, but very slow (greater than 2 months)
Added 10^{-7} M GA_3	No growth
Reduced NAA from 2.0 to 0.2 mg/l and added 10^{-7} M GA_3	Very little growth
S2M medium (Torrey and Fosket, 1970)	
Unmodified	Slow growth but much callusing
Replaced 4% with 4% glucose	No growth
Omitted 5×10^{-6} M 2,4-D and added 0.001 mg/l zeatin	Growth occurred but less effective than with kinetin
Omitted 2,4-D and added 0.1 mg/l kinetin (S2M + K −2,4-D)	Organized root development in 30–40% of the cultured QC's in 3–4 weeks

Methods of QC culture—QC's were initially explanted and cultured in three basic media (Table 1). All the media were prepared so that the *p*H of the final solutions was approximately 5.5. In addition, each of the media was supplemented with 1% agar. After autoclaving (constituents of some media, as per instructions, were millipore-filtered instead of autoclaving), 20 ml of the still hot medium were apportioned to sterile 25 × 150-ml culture tubes, which were capped with cotton plugs and the agar was allowed to harden in a slanted position.

QC's were excised from 72-hour-old seedling roots and aseptically transferred to the agar slants. An effort was made to place the proximal surface of each QC on the agar surface of the nutrient medium. The tubes were then recapped, sealed with parafilm and returned to the dark at 23 C. In some instances, QC's were grown for a period on the agar slants and then transferred to 50 ml of liquid medium of the same constitution in 125-ml erlenmeyer flasks. Tissue pieces in liquid culture were placed on a horizontal rotary shaker (approximately 50 cycles/min) in the dark at 23 C.

Tissues were fixed in either freshly prepared FAA or in chromium-acetic acid solution (Sass, 1958), dehydrated, embedded in paraplast, and sectioned at 10 μm. For general observation sections were stained with either fast green and safranin or safranin, orange G, tannic acid and iron alum (Sharman, 1943).

RESULTS—*The isolated QC*—Complete removal of the QC permitted direct observation of this tissue at both its proximal and distal surfaces. The tissue formed an almost perfect sphere, 200–300 μm in diam (Fig. 4). The distal surface (Fig. 5) was composed of more or less isodiametric cells at its center, not arranged in any apparent pattern except for a tendency toward radial files. These cells, which bordered directly on the root cap junction, showed no particular thickenings on their walls. In contrast, a surface view of the proximal end of the QC showed enlarged cells, generally three or four in number, arranged symmetrically around a central point (Fig. 6). Frequently, the cells surrounding the enlarged elements were arranged in a layer or concentric ring of more or less equal-sized cells whose radial axes, if extended, would intersect in the center of these enlarged elements. Sometimes no enlarged elements were observed in the surface view of the proximal surface of a QC (Fig. 7).

QC culture on nutrient agar—The results of culturing the QC on various agar media are summarized in Table 1. Generally, the simpler the medium with regard to hormones and organic nitrogen compounds the poorer the response of the cultured QC in both the amount of new tissue produced and in the development of an organized structure. Media were evaluated as effective on the basis of their ability to promote development of the QC's within 2–3 wk. The S2M medium

supplemented with 0.1 mg/l kinetin lacking 2,4-D (designated S2M + K –2,4-D) proved the best medium for development of those tested. Of the 150 QC's sub-cultured on this medium approximately 30–40 % survived and grew into organized structures.

Since the S2M + K –2,4-D medium proved optimal for culturing QC's, the following results, except when noted otherwise, were of QC's cultured on this medium. From 0–5 days after subculture the QC enlarged somewhat, but showed little marked morphological development. Five to 10 days after sub-culturing, a structure morphologically similar to a root and sometimes profusely covered with root hairs (Fig. 8) formed. The long axis of the 5 to 10-day-old regenerated structure coincided with the direction of the longitudinal axis of the root from which the QC was originally dissected. During the succeeding five days the tissue continued to enlarge, gradually assuming the morphology of intact, cultured roots (Fig. 9a, b, c). Between 14 and 28 days of culture, structures with the form of an excised cultured root were produced (Fig. 9d, e). In a period of 3–4 wk the QC, barely 100 μm in length initially, developed a root approximately 2 cm long, an increase in length of about 200 times.

Histological examination of root-like structures derived from cultured QC's at different periods of culture showed that in those structures which later formed roots an ontogenic development of an organized root apex occurred. From the apparently unorganized proliferation which occurred initially there developed a partially organized meristem seen in cultured QC's at day 8–10 (Fig. 10). In a later stage, the elongating cylindrical root at 21 days showed a clearly organized root apex (Fig. 11) and roots elongating in liquid medium for four weeks showed a well defined apex with root cap, protoderm, and ground meristem and procambium (Fig. 12). Such an apex was found in 14–28-day-old elongated roots such as are seen in Fig. 9d and e.

Vascular patterns derived from QC's sub-cultured on agar—The arrangement of the primary vascular tissues and the structures produced were investigated in detail in 15 sub-cultured QC's. Transections of the tissues differentiated in five 28-day-old sub-cultured QC's were made at 20-μm intervals along the entire length of the roots. Fig. 13–22 are of transections taken at various levels in an acropetal direction along one root and represent the vascular patterns obtained from QC's cultured on solid nutrient medium. Fig. 13 is representative of a cut through proximal tissue closest to the agar surface, while Fig. 22 was within the root apex. Fig. 13 is a section through the original sub-cultured QC. Note that the QC more or less retained its circular shape. Cells in the interior were isodiametric and remained undifferentiated. Proceeding in an acropetal direction, an asymmetric differentiation often was observed (Fig. 14, 15). At a distance of several hundred micrometers from the original QC and in the differentiated central portion, the first metaxylem elements were observed. Generally five or six discrete metaxylem poles were evident (Fig. 16), each pole composed of several vessel elements. However, the number of individual vessels was reduced closer to the apex (Fig. 17). At still more distal levels the vessel elements became enlarged and the distance between the individual xylem poles was reduced, but the poles remained discrete and separate from each other (Fig. 18, 19). Eventually, the separate elements came into direct contact with each other, reducing the remaining xylem to a single central group of elements, usually 3 or 4 in number (Fig. 20, 21). This arrangement of vascular tissues continued into the apex (Fig. 22) where it could be seen in the blocked-out pattern in the procambium.

The arrangement and successive changes of the metaxylem elements illustrated in the root in Fig. 13–22 were more or less representative of the patterns of vascular tissues seen in the other sub-cultured QC's grown on agar nutrient medium.

In all roots examined that showed good development on agar nutrient medium the proximal portion of the root showed a primary vascular tissue pattern in which the metaxylem elements were arranged symmetrically as discrete poles. Transections from two other roots at a level approximately equivalent to that in Fig. 15 of the first root described are shown in Fig. 23, 24. Five or six clearly separated metaxylem poles were evident in a well-delineated central cylinder. More distal in such roots, the individual metaxylem poles became less distinct. This change is evident in Fig. 16 in the first root and a similar reduction in vascular pattern size and complexity is seen in Fig. 25 in another root. In still other roots, the individual metaxylem poles were joined, forming an almost complete peripheral ring of xylem (Fig. 26, 27, 28). However, in all roots derived from QC's sub-cultured for a sufficiently long time on the S2M + K –2,4-D agar nutrient medium, the primary xylem pattern was reduced to a small central group of elements such as is seen in Fig. 20 in the first root and in other roots (e.g., Fig. 29).

Roots transferred from solid to liquid medium—In some instances QC's were transferred 5–7 days after sub-culturing from agar medium to an identical liquid medium (minus agar), as previously noted in Materials and Methods. Two to three weeks after sub-culturing in liquid medium, these QC's had developed as normal roots, which grew at a somewhat more rapid rate than identically aged roots on solid medium. Apices from QC's cultured in liquid media for 21 days were larger than those produced by comparably aged

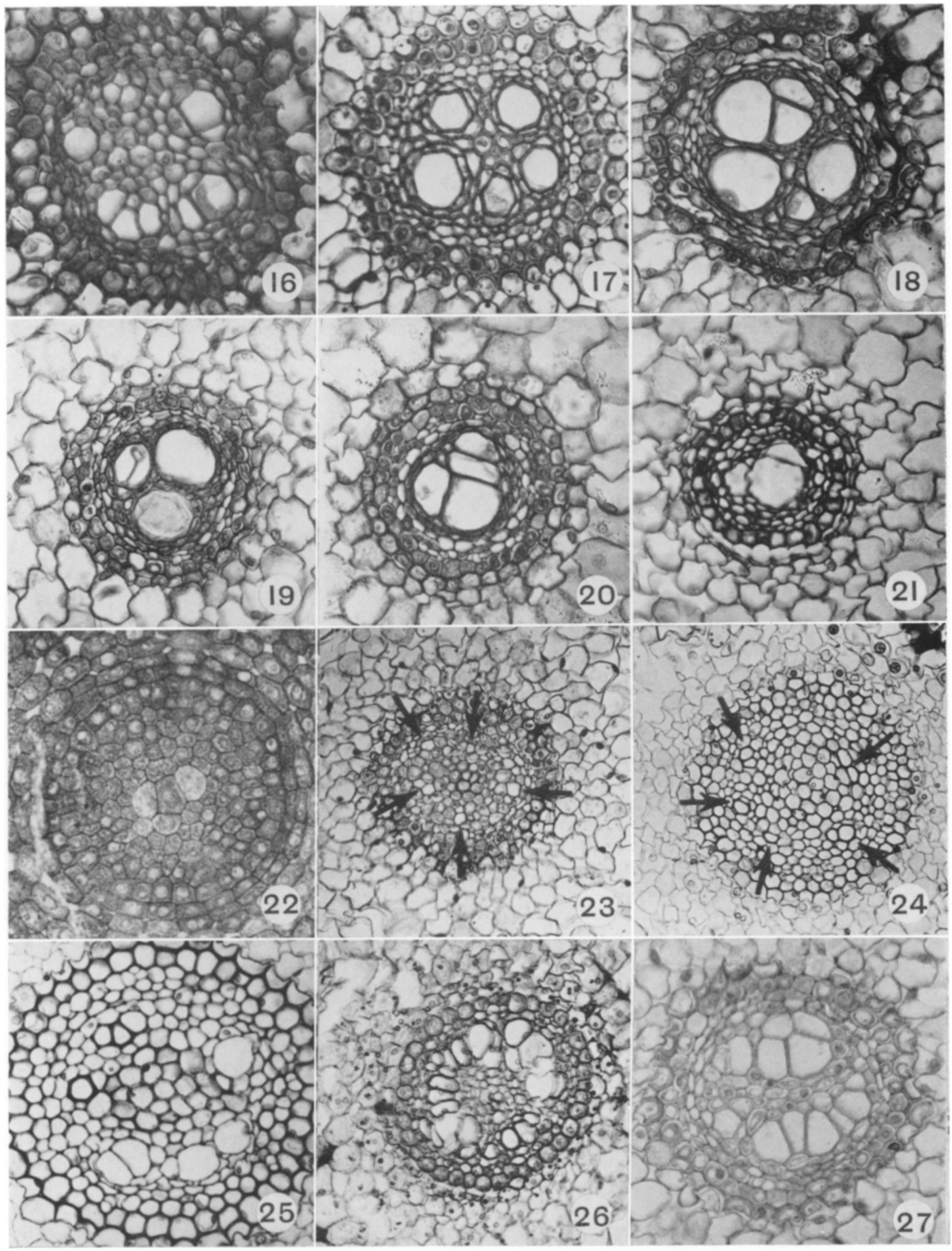

Fig. 16–27. Transections of roots derived from cultured QC's. 16–22. Additional sections of root from 3-week-old cultured QC grown on nutrient agar medium seen in Fig. 13–15. **16.** Section, 1930 μm from base. $\times$ 225. **17.** Section, 2900 μm from base. $\times$ 225. **18.** Section, 5820 μm from base. $\times$ 225. **19.** Section, 9220 μm from base. Note reduction to three metaxylem groups. $\times$ 225. **20.** Section, 17,640 μm from base. $\times$ 225. **21.** Section, 19,000 μm from base. Note grouping of metaxylem elements. $\times$ 225. **22.** Section, 22,480 μm from base of the root through the apical meristem. $\times$ 325. **23.** Transection of another cultured QC at 970 μm from root base.

roots on solid medium (cf., Fig. 11 and 12). It would be difficult to distinguish the apex in Fig. 12 from those of older, intact primary roots.

Figures 30–38 are transections from the proximal end to the distal or root-tip-end of a root derived from a cultured QC started on agar S2M + K –2,4-D medium for 5–7 days then transferred to liquid S2M + K –2,4-D medium for the subsequent 21-day period. The sections show the dramatic changes in primary vascular pattern which occurred during root development in the same axis. The central cylinder of the nutrient agar-grown root is illustrated in Fig. 30, 31. Fig. 32–37 are sections of the same root after transfer to liquid culture medium. Fig. 38 is representative of the pattern evident in the procambial tissues in the root apex. It is evident that in the root cultured on nutrient agar medium, reduction in the metaxylem tissue from five poles to a central group had already occurred as described earlier (cf. Fig. 31 with Fig. 20 of the first root). After transfer to liquid medium, the vascular pattern returned to the more typical pattern of 5–6 strands seen in intact primary roots of corn. This change was progressive, occurring over several millimeters, with occasional addition of a new metaxylem pole (Fig. 32 already shows five metaxylem poles; Fig. 36 shows the addition of a sixth pole which can be traced into the apex). The organization of the vascular tissues in this root is remarkably similar to that of the intact corn seedling root sectioned at the same level (Fig. 39).

DISCUSSION—The physical isolation of the QC of the root is possible in cultivar Kelvedon 33 because of the peculiarly fragile nature of newly formed cell walls in the meristematic cells of the root cap initial layer and of the proximal initial layer. In the course of the isolation, these meristematic cell layers are destroyed and the very slowly dividing cells of the disc-shaped QC remain. That the separation occurs in the manner that it does suggests the comparable nature of these two discrete layers of initials.

The QC, thus isolated, comprises about 1000–1500 cells in the shape of a double convex lens approximately 100 × 250 μm or 0.25 mm^3 in volume. On the distal or root cap face, the cells appear to be more or less isodiametric; on the proximal face one frequently observes a cell pattern of four or five enlarged cells arranged in a circle among the otherwise isodiametric cell population. Thus the QC is manifestly polarized, having a root-cap face and a proximal face; additionally, there is an inherent pattern already present in the group of cells of the proximal face.

The routine isolation and successful culture of isolated QC's of the root apex, reported here for the first time, open a new approach to the study of the organization of the root apex and in particular of the potentialities and inherent properties of the specialized sub-population of cells which comprise the QC. A detailed analysis of the nutritional and hormonal requirements of this isolated tissue piece for direct and rapid development into a normal root has not yet been performed. It has been shown that the QC of *Zea* cultured on a defined medium (S2M+ modifications listed in Table 1) is capable of forming a well organized root of normal morphology. From anatomical studies, based on serial cross sections of developed roots, it is apparent that development occurred directly from the polarized tissue piece rather than from callus. This result contrasts with the work of Reinhard (1954) who, having cultured the QC plus some additional tissues cut from the pea root apex (his explant 2), observed that such cultured tissues initially grew "without any favored direction, producing a more or less round lump of tissue," from which a root was subsequently organized and developed.

The autonomy exhibited by the QC indicates that even when isolated from the root apex, this sub-population of cells retained its inherent root-forming capacity and regenerated a new root, independent of influences derived from other portions of the root. Because of its own inherent properties, rather than from conditions imposed on it by surrounding populations of cells, the isolated QC acts as a center for root organogenesis.

Certainly no other comparable-sized packet of cells could be isolated from its context anywhere within the whole organism and act in such a direct way to form an organized root. That the isolated QC should have this innate regenerative capacity is suggested by examining this tissue in intact roots. It is a population of cells from which, and around which, the architecture of the root is established and perpetuated. The imposition of a state of quiescence, by what ever constraint, does not of itself modify the capacity of the QC to reassume a meristematic condition. Exactly which cells in the isolated QC participate in the polarized regeneration of a new root are not yet known. In decapped roots, in which the QC was otherwise intact, it was shown that only the most dis-

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Arrows mark 5 newly differentiated metaxylem poles. × 130. **24.** Transection of another cultured QC, at 1050 μm from root base. Arrows mark metaxylem poles. × 130.—Fig. 25–27. Transections from basal regions of 3-week-old cultured QC's grown on nutrient agar. **25.** Section, 2010 μm from base. Note 6 metaxylem poles. × 225. **26.** Section of another root at 1535 μm from root base, showing ring of immature metaxylem. × 130. **27.** Section, 2620 μm from root base. × 225.

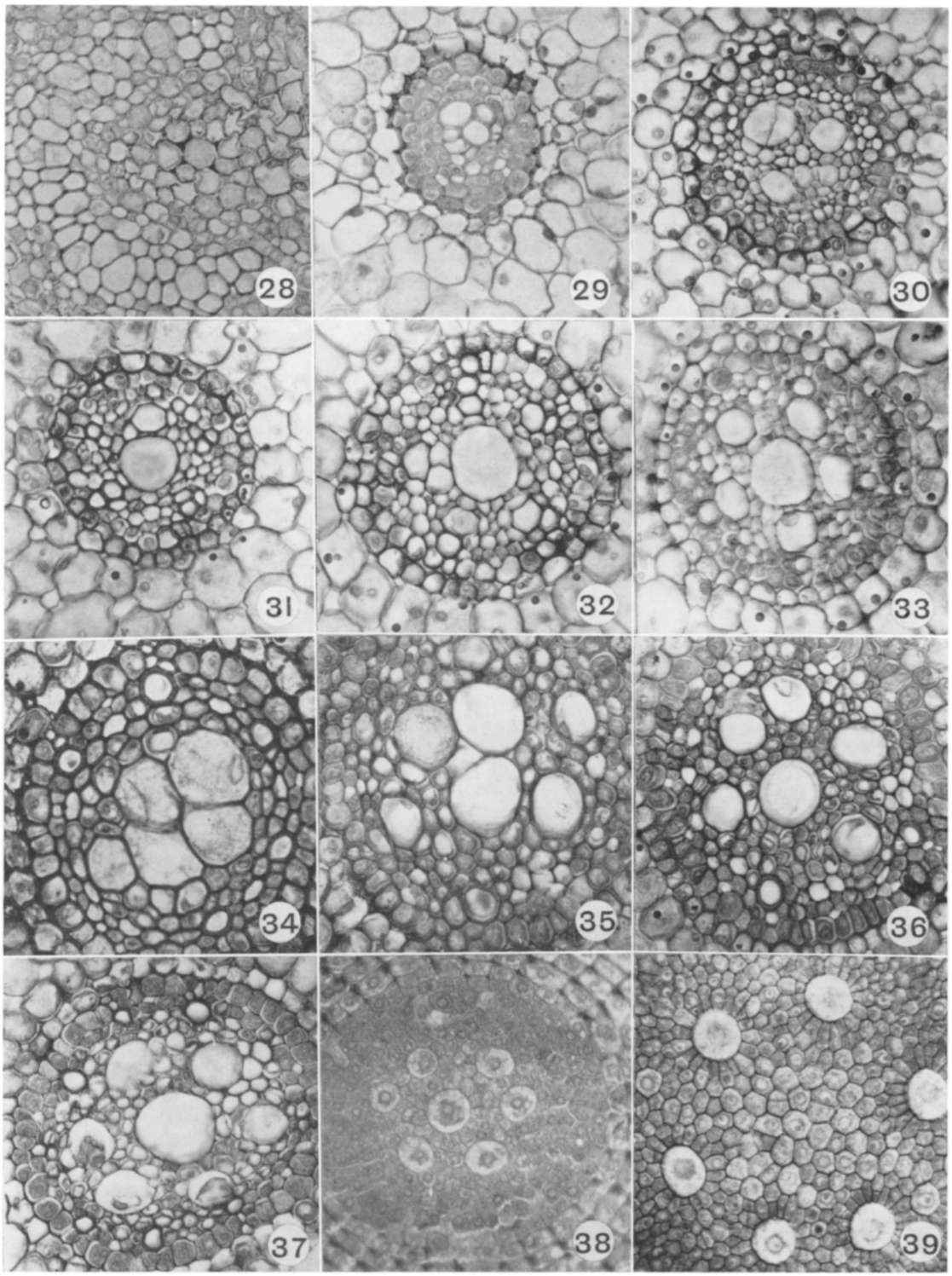

Fig. 28–39. Transections of roots derived from cultured QC's. **28.** Section, 2430 μm from root base. Note incomplete ring of metaxylem. × 225. **29.** Section, 20,100 μm from root base, showing reduction in vascular tissues in nutrient agar-grown roots (cf. Fig. 21). × 225.—Fig. 30–38. Transections all from root developed from QC culture for 4 weeks, first on nutrient agar medium, then in liquid medium. All × 225. **30.** 1368 μm from base. **31.** 2574 μm from base. **32.** 6074 μm from base. **33.** 7254 μm from base. **34.** 8478 μm from base. **35.** 10,064 μm from base. **36.** 11,414 μm from base. **37.** 13,682 μm from base. **38.** 15,644 μm from

tal QC cells participated in the regeneration of a new root cap (Barlow, 1974; Feldman, 1975a), and that this regeneration occurred from cells in which there was a marked re-orientation in the number of transverse divisions with respect to the long axis of the root (Barlow, 1974). The observation that isolated QC's from the outset, exhibited polarized growth is a clear expression of the unique intrinsic nature of this tissue. The various gradients already known to exist within the cells of the QC (Barlow, 1974; Clowes, 1971; Phillips and Torrey, 1972) provide a basis for this polarized growth. Although we lack data on the orientation of mitotic figures in isolated QC's, certainly such a re-orientation could account for the very early polarity indicated in the differentiation of new roots.

The behavior of isolated QC's grown in culture was similar to that of QC's in intact roots exposed to large doses of X irradiation in that in both instances an entirely new root was regenerated (Clowes, 1961, 1963; Clowes and Hall, 1961). In both irradiated roots and in isolated QC's the proximal and distal layers of initials cease functioning. In each case the regenerative capacity of the QC is brought into action and the QC serves as the source of new cells for the reestablishment of a new organized meristem.

Isolation and culture of the QC in vitro should provide information to resolve some of the controversy as to the forces creating the quiescent state in the intact root. Unfortunately, the isolation process itself changes so many features of the integrated tissue system of the root apex that the questions concerning cellular interactions remain unanswered. In so far as the limited data of Table 1 provide information on hormonal requirements, it would appear that isolated QC's of *Zea* require an exogenous supply of cytokinin to become active in root development. This fact argues against the idea that the QC itself rather than the meristematic initial cell layers is a site of cytokinin synthesis. Even this conclusion must be tentative since isolation could have changed endogenous synthetic activities drastically.

A closely related question is why isolating and sub-culturing should activate the cells of the QC and in such a way that it produces an organized root structure directly. Clowes (1971) pointed out that the triggering mechanism for the QC as a whole—and indeed this could be extended to specific subpopulations within the QC—must differ from that in other parts of the root. From previous work it was suggested that in intact roots substances produced in the proximal and distal initial layers surrounding the QC accounted for the reduced metabolic activities in the quiescent population of cells (Feldman, 1975a; Webster and Langenauer, 1973). An impairment in function of these initials, leading to a reduction in the amounts of inhibitory substances produced by them, was thought to result in the partial or complete activation of the cells comprising the QC. In surgically removing the QC, one has done so at the expense of the proximal and distal layers of initials which were destroyed when the QC was dissected out for sub-culturing. If these initial layers were indeed the sites of production of some substance leading to reduced QC activity, then their destruction or absence, at least initially, in isolated QC's could account for the activation of organized growth as observed in these sub-cultured tissues. Destruction of these initials need not affect the autonomy or internal integrity of the QC itself nor change its intrinsic character. By isolating QC's one has removed a constraint on this tissue, a constraint proposed to have originated, at least in part, in the layers of initials surrounding the intact QC.

In the processes involved in the differentiation of a root from cultured QC's, two particular aspects in the development of this tissue merit special attention: 1) the reestablishment of a root apex, and 2) the differentiation and maturation of a vascular system. At the time of the initial subculturing, the cells which composed the QC represented but a small number of those in an intact apical meristem. During the early period of culture in vitro, cell division activity within the QC led to the reestablishment of a newly organized apex including a root cap and the formation of the root cap initial layer and the proximal initial layer which characterizes the normal corn root apex. Although smaller in actual dimensions and in total number of cells involved than in a seedling root apex, the structural characteristics of the regenerated root apex were normal and once established and active led to the formation of typical, if reduced, mature root structures. The ontogenetic development of the regenerating root apex warrants further detailed study.

The development of the mature primary tissues of the root from cultured QC's can be studied in serial sections by starting at the proximal end or root base and following the changing pattern in the direction toward the root apex or distal end of the root. Such a series from a QC grown on nutrient agar is illustrated in Fig. 13–22. Figures 13 and 14 represent the immediate product of QC activity in forming a new root. Although no in-

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base, showing pattern of blocked-out metaxylem in region of apical meristem. **39.** Transection, 200 μm proximal to the root cap junction in an intact seedling root, showing the enlarged future metaxylem elements in the procambium. × 250.

ternal vascular pattern is seen, the pattern of a central cylinder, surrounded by radially elongate "cortical"-like cells is evident. The central cells are lengthened in the direction of the root axis. Internal differentiation of vascular tissues is soon evident (Fig. 16) and reflects a pattern reminiscent of that seen on the proximal face of the QC at the time of its isolation. Such a pattern presumably results from activities of an organized root meristem. Thereafter, in nutrient agar-grown roots, the vascular cylinder in cross section becomes smaller and the vascular tissue pattern simpler, reflecting a smaller root apex (such as seen in Fig. 11). The basal portions of this root reflect the residual size and pattern of the original isolated QC and the distal portions reflect the activity of a reduced QC. Culture of the QC on nutrient agar, even in a medium allowing development of a root structure, must reduce the available nutrients or provide them in limiting amounts. The QC of the regenerated root and the complexity of the vascular pattern are reduced in parallel fashion on agar medium.

Transfer of the agar-grown isolated QC's to liquid nutrient medium after the root had begun its development produced better root development and a more normal vascular pattern formation. The series of sections from a root cultured on agar and then transferred to liquid medium (Fig. 30–38) illustrates a reduced vascular tissue pattern (Fig. 30) and then a restitution of the complex pattern more typical of the normal root. At the level of blocking-out of the vascular pattern near the apex, the liquid-cultured root derived from an isolated QC (Fig. 38) is very similar to that of a seedling root (Fig. 39).

Changes in mature vascular pattern along the root reflect changes in the activity of the root apex at the time the pattern is determined. In these developed roots one can observe in the mature root tissues the changed activity of the apex. The liquid medium is more conducive to normal development than the same nutrient medium solidified with agar. The effect of a liquid medium may be due to any of the following causes: improved uptake or availability of medium components, increased leaching of root-produced metabolites, or some other change related to the physical state of the substrate. The simplest explanation would be the availability of nutrients. On solid medium, as roots continued to grow, their apices frequently were elevated above the agar surface, and thus no longer made direct contact with the nutrient medium. It is suggested that some substance in the medium (perhaps simply sucrose) becomes limiting at the absorbing basal end of the regenerating root, thus reducing the amounts of nutrients available for the differentiation of more acropetally located vascular tissues forming at the apex. Webster and Langenauer (1973) have shown that starving a root may indeed alter the activity of the QC's in corn. One might therefore suggest that the changes in pattern observed in regenerated roots may actually be a reflection of the activity of the QC. Though we have been unable to relate a particular developmental stage of the apex to certain vascular patterns, it is clear that during the ontogeny of the regenerated root, both the complexity of the apex and vascular patterns changed. The developing root responds to the local environment and the effects are evident in the formation of the vascular pattern. Further analysis of the relationship between the activity of the root apex and of the QC in particular, and the formation of the vascular tissue pattern, may be possible by an in vitro system.

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