Regeneration of the root pole in surgically transected carrot embryos occurs by position-dependent, proximodistal replacement of missing tissues

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Summary
Torpedo-stage carrot embryos were surgically transected at various locations along the shoot-root axis and explants of the cotyledon-bearing shoot pole were sectioned and examined in order to provide a more detailed description of root pole regeneration. When excisions occurred at the sites of the future hypocotyl, the future radicle or the future root apical meristem, the regenerating axial tissues exhibited patterns of cellular organization that were nearly identical to those seen in unsevered controls. To accomplish this restoration, new cells, of the type normally found at each cutting site, were produced behind a regeneration dome that formed over the original surgical site. The regeneration dome was displaced by division and expansion-driven extension of the longitudinal axis, and cells in the renewed region quickly acquired individual anatomical traits and collective tissue morphologies that corresponded to those of cells in the analogous locations in intact embryos. Although no clear mechanism is implied, the results of these experiments suggest that interactions between cells near the surgical margin permit them to retain their sense of location within the original structure, and apprise them of the removal of their basipetally positioned neighbors. With varying-length remnants of the shoot serving as the only vestige of the original size and shape of the embryo, cells close to the site of excision were apparently reconfigured to commence ordered divisions that ultimately reconstituted the embryonic axis.

Key words: pattern formation, positional information, regeneration, somatic embryogenesis, Daucus carota.

Introduction
Observing regeneration following the surgical removal of body parts is one of the oldest strategies for learning about the developmental capabilities of organisms. Studies with primitive animals such as planaria (Brøndsted, 1969), hydra (Bode and Bode, 1984) and sea urchin (Hörstadius, 1939); arthropods (reviewed in Goss, 1969) and certain vertebrates (Thornton, 1968; Muneoka and Bryant, 1982) and lower plants (e.g. algae, mosses, ferns [Stange, 1965]) have yielded important insights into the mechanisms for position-sensing and reacquisition of form following experimental disruption of the normal body plan. However, many organisms have not proven to be particularly amenable to this approach and the common theme that may be seen to run through all this work is that the quality of information is ultimately determined by the largely unknown attributes of each system to endure surgical trauma and to sustain recognizable patterns of growth.

Recently, we discovered that the embryos of higher plants (carrots), produced in liquid culture, were capable of regenerating amputated portions of the embryonic axis. When torpedo-stage embryos were transected into variously proportioned explants of root and shoot pole, we found that, depending on the length and polarity of the axial segment, an area beneath the surgical margin became an active zone directing the replacement of missing structures (Schiavone and Racusen, 1990). Besides showing that higher plant embryos are amenable to study by dissection and subsequent regeneration, these findings are especially significant because they occur without the apparent involvement of two key features of growth that have historically tended to complicate the analysis of pattern formation in plants, well-defined meristems and unrestrained cellular dedifferentiation.

Meristems in higher plants, particularly in the root and shoot apices, are the site of most cell divisions and are thus responsible for the basic arrangement of specialized tissues. Surgical experiments on mature plants have shown that apical meristems possess a fair degree of autonomy; portions of shoot meristems can restore missing collateral tissues (Smith and Murashige, 1970; Bull, 1980) and the excised quiescent center of roots will regenerate the root body and the root cap
were performed under a laminar flow hood. In brief, torpedo-stage cultures were treated, and embryos initiated, according to previously published methods (Schiavone and Cooke, 1985). All surgical procedures used sterile instruments and were performed under a laminar flow hood. In brief, torpedo-stage embryos (ca. 1000 μm in axial length) were transected at points corresponding to 10, 20, 25, 40, 50, 60, 75, 80 or 90% of the way along the shoot-root axis. Following the nomenclature we established previously (Schiavone and Racusen, 1990), surgical explants were designated according to the percentage each piece occupied in the intact embryo. Thus, for example, a cut that divided the embryo into 10% shoot pole and 90% root pole was termed a 10S:90R cut. Excised shoot poles were transferred to MS media without 2,4-D (MSE) solidified with 1.2% agar. Shoot poles in the process of regenerating the missing root pole were fixed in formalin-glacial acetic acid (FAA) at various time points between 6 and 12 days post-surgery.

Materials and methods

Cultural conditions and surgical technique

Suspension cultures of Daucus carota L. cv. Danvers were continually maintained by transferring radicle explants from steriley germinated seedlings to 100 ml volumes of MS media (Sigma Chemical, St Louis MO, USA) supplemented with 5 μM 2,4-dichlorophenoxyacetic acid (2,4-D). Liquid suspension cultures were treated, and embryos initiated, according to previously published methods (Schiavone and Cooke, 1985). All surgical procedures used sterile instruments and were performed under a laminar flow hood. In brief, torpedo-stage embryos show little inclination towards rampant division, the observed restoration of missing portions of the embryonic axis affords an opportunity to examine how the orchestration of regional pattern-forming events gives rise to the characteristic body plan of a terrestrial plant. This paper presents results of our efforts to ascertain how the restoration of excised parts takes place. Because the development of mature plants is guided by cell divisions within meristematic regions, we were interested in determining if there was obvious structural reorganization near the surgical margin, perhaps serving as the functional equivalent of an apical meristem, that coordinated the emergence of differentiating root pole tissues. The present analysis of cell types and positions within the regeneration zone revealed no such remodelling; instead, the missing portions of an excised shoot pole were replaced in a proximodistal fashion, with the appropriate, differentiated zones of the shoot–root axial tissues (hypocotyl, radicle) appearing first, and the root apical meristem (RAM) appearing last. In this respect, the progression of root regeneration in carrot embryos bears a rather surprising similarity to the proximodistal regeneration of structures seen during limb regeneration in vertebrate embryos.

Microtechnique and data collection

Fixed material was dehydrated and paraffin-embedded following standard procedures. Shoot poles were mounted on a rotary microtome, sectioned at 8 or 10 μm and sections were stained with safranin O. Median longitudinal sections were selected for further analysis and photography. The lengths of each tissue zone (measured along the mid-line in median-longitudinal section) as well as the overall embryo length (referred to as the axial length) were obtained by use of a camera lucida, a digitizing tablet and digitizing software (Sigma Scan, Jandel Scientific, Corte Madera, CA, USA). The cross-sectional area of cells was determined by hand using a calibrated ocular eyepiece. Only cells that were regular polygons (hexagons or rectangles) were selected and, of these, no cell was measured if it was within 3 cell diameters of either the epidermis or provascular cylinder. The latter rule was necessary because we have consistently observed that average cell size decreases near these boundaries.

Terminology employed for plant embryo histology

Because the anatomy of late-stage embryogenesis (i.e. embryos past the torpedo stage) has not been particularly well-characterized, especially with regard to vascular tissue development (Esau, 1965a), it was necessary to modify some existing botanical terminology and to propose some original subcategories to denote embryo stages and tissue zones. Several years experience with somatic embryos in our laboratory has led us to the conclusion that their development in liquid culture is not very synchronous, and we have found axial length (specifically the distance between the cotyledonary notch and the root tip) to be a more reliable indicator of embryo stage than cultural age. The terms for the basic embryonic ground tissues, such as: procambium, protoxylem, provascular tissue, ground meristem and protoderm, were employed according to definitions set forth in Esau (1965b). Somatically derived, torpedo-stage embryos, which are developmentally similar to the dormant zygotic embryos in seeds, were defined as a slightly tapered axial structure of less than 1500 μm, bearing two cotyledons. As viewed in paraffin thin section, embryos at this stage of development displayed a rather simple histology, consisting of an epidermis, provascular strand and a ground meristem of densely cytoplasmic cells, which extended from the lower axis tip to the cotyledons (Fig. 1).

During germination zygotic embryos organize root and shoot apical meristems, and it is from focal cytokinines in these meristems that the seedling is produced. The establishment of the apical meristems and their subsequent activity represents the fundamental transition between the embryonic and mature portion of the plant life cycle. The situation is somewhat different in somatic embryogenesis in that no
Proximodistal regeneration in carrot embryos

Fig. 1. Longitudinal views through torpedo- (A) and pre-plantlet-stage (B) control embryos. Provascular tissue occurs as a darkly staining strand running along the midline of the organisms; curvature of the axis in specimen (B) permitted only portions of the vasculature to be captured in individual sections. The zone of small cells between the lower end of the vascular strand and the intensely stained root cap is the nascent RAM, while the tissue between the RAM and hypocotyl (H) is the radicle. Bars, 200 μm.

A period of dormancy is introduced at the torpedo stage, and that the development of the two apical meristematic regions appears less well synchronized. Thus, we defined a pre-plantlet stage for somatic embryos that were in the process of organizing the RAM but still lacked an obvious shoot apical meristem (SAM). From a series of hand- and paraffin-embedded thin sections, we determined that most embryos having axial lengths between 1500 and 4000 μm could be classified as pre-plantlets. Viewed in section, pre-plantlets underwent progressive differentiation of the vascular tissue as protoxylem elements could be found anywhere from the cotyledons to within 200 μm of the extreme root pole. Somatic plantlets, containing both SAM and RAM, were usually longer than 4000 μm and corresponded in appearance and growth potential to zygotic plantlets.

In terms of tissue zones within embryos and pre-plantlets, we made the following distinctions. First, in accordance with its position below the notch formed by the cotyledons, the region occupying the upper 10 to 20% of the axial length was termed the future hypocotyl. We consistently observed that the cells of this region did not increase markedly in number or appearance during the progression from embryo to pre-plantlet. In contrast, the tissue beneath the future hypocotyl was arranged into a hollow cylinder around the core of provascular tissue, and consisted of highly vacuolated cells which were up to 4-fold larger in cross-sectional area than cells in the hypocotyl. The presence of bounding protoderm and the arrangement of cells near the conical tip into radial files, distinguished this region as the future radicle. As explained in the results, continued expansion of cells in this region accounted for most of the overall increase in length of embryos. At the pre-plantlet stage, the extreme distal portion of the radicle (lower 10% of the axis) exhibited the features of a root apical meristem, i.e. radially arranged files of small, densely cytoplasmic cells radiating from a central focus and a terminal root cap.

In transected embryos, the location of the cut, as measured along the axis from the cotyledonary notch, was termed the surgical site. Cells injured during excision were not immediately sloughed during recovery and regeneration, and served as a readily identifiable marker that was termed the surgical margin. Immediately after cutting, the surgical site and the surgical margin were synonymous; however, as regeneration proceeded, the margin was displaced from the original site of surgery. In presenting our observations of complete axis restoration, we may localize the remnants of the surgical margin with some precision, but owing to the events of regeneration and normal embryo maturation, the location of the surgical site becomes an approximation. Finally, in evaluating regeneration phenomena in excised shoot poles, it is worth noting that surgical manipulation of carrot embryos temporarily interrupted, but did not permanently suspend, the normal procession of morphogenetic events leading to a plantlet. Therefore regeneration of the root portion of the embryonic axis occurred in conjunction with the transition to the first stage of post-embryonic development.
Results

Torpedo embryo to pre-plantlet stage transition

Of 30 sectioned control embryos between 900 and 4400 µm in axial length, 25 produced clear median-longitudinal thin sections and were selected for analysis. As shown in Fig. 1, the progression from torpedo to pre-plantlet stage involved extensive vacuolation of cells in the nascent radicle as well as the formation of the nascent RAM. During the process of vacuolation, the cells within this region also underwent an increase in cross-sectional area. As embryo length increased from ca. 1000 µm to over 4000 µm, cells in this region enlarged up to 3-fold in cross-sectional area compared to cells in either the future hypocotyl or future RAM (Fig. 2). This increase in radicle cell size also appeared to be responsible for much of the total increase in embryo length, since the lengths of the future hypocotyl and future RAM zones appeared to change little while the length of the future radicle increased sharply (Table 1). Portions of the vascular system began to mature during the torpedo to pre-plantlet stage transition as protoxylem elements were observed to extend from 200 µm of the root-tip into the cotyledons of organisms larger than 3000 µm. In smaller pre-plantlets vascular tissue maturation was less advanced, with protoxylem elements being found in rather discontinuous arrays emanating from either pole.

General features of root pole regeneration

Root pole regeneration usually began 4–6 days post surgery. The first outward sign was the formation of a shallow dome of tissue, typically beginning at the center of the surgical margin. As a result of apparent cell proliferation within the dome, the face of the surgical margin first began to bulge (Fig. 3), and then flatten as the region of dividing cells gradually extended outward to encompass the entire face of the surgical margin. Subsequent extension of the longitudinal axis was obvious from daily monitoring of axial length in living embryos and from the displacement of the dome and portions of the surgical margin seen in thin sections. The diameter of the regenerating root pole was nearly identical to that of the shoot pole explant, and in the final stages of axis restoration, the shallow dome gave way to the tapered form of a typical root apex. As considered in the discussion, it is not clear how dome morphology is related to cell divisions and the generation of internal forces within the tissues, nor have we yet performed any type of analyses that would allow us to discern which of the cell layers undergo the requisite increases in division frequency. The region near the dome certainly lacked the obvious organization of a root apical meristem (c.f. Figs 3, 4A and 4C), and there were no diagnostic, linear arrays of younger and maturing cells that might have allowed us to infer shared origins or lineage. Instead, during the augmentation of axial length, cells beneath the epidermis of the dome began to acquire the physical traits of cells expected for that position along the shoot–root axis.

Shoot poles from more than 100 dissected embryos were monitored for daily changes in external features (lengths, gross tissue differentiation) during the complete course of root pole regeneration, and 39 shoot poles with various stages of regenerating root poles were embedded and serially sectioned for this study. Of the embedded embryos, 31 produced clear median-longitudinal sections and were used for further analysis. The axial length of embryos in the surgically manipulated group ranged from 440 to over 11 100 µm while the control embryo axial length ranged from 950 to 4400 µm. All experimental embryos were considered while formulating a general impression of the anatomical trends during root-pole regeneration, while only those manipulated embryos with axial lengths <4000 µm were evaluated in making morphometric comparisons with control embryos. Although this study dealt with 9 classes of cuts along the shoot–root axis, qualitative similarities in observed patterns of regener-

| Table 1. Selected morphometric parameters from control and regenerated embryos |
|-----------------|-----------------|-----------------|-----------------|
| Experimental condition | Hypocotyl length | Radicle/axial length | RAM length |
| Control | 25 | 662±33 | 0.61 | 370±13 |
| Hypocotyl cut | 6 | 755±56 | 0.55 | 455±25 |
| Radicle cut | 7 | 762±46 | 0.58 | 388±23 |

Experimentally treated embryos which had entered the pre-plantlet stage and were less than 4000 µm axial length were utilized for this analysis. Tissue lengths (µm) were obtained from median longitudinal sections of whole embryos. Data are average±s.e.

![Fig. 2. Plot of the ratio of radicle cell to hypocotyl cell cross-sectional areas vs. axial length in control and surgically treated embryos. Data are composite averages (±s.e.) utilizing dimensions taken from representative cells in several embryos. Embryos were grouped into three size classes corresponding to torpedo (<2000 µm), pre-plantlet (2000–4000 µm) and plantlet (>4000 µm) stages. Note that surgically treated embryos in the pre-plantlet stage undergo precocious enlargement of radicle cells relative to similar-sized control embryos.](image-url)
Fig. 3. Development of the regeneration dome in transected embryos. Panels A, C and E are external photomicrographs of the shoot-pole end of torpedo-stage embryos at 0, 4 and 6 d post-surgery. Panels B, D and F are corresponding median longitudinal sections of the same embryo explants. Note the formation of the regeneration dome across the surface of the surgical margin in C and E and the presence of a cluster of densely cytoplasmic cells directly underneath the dome in D and F. Arrows indicate the location of the original surgical site. Bars, 200 μm.
Fig. 4. Median-longitudinal sections of torpedo-stage embryos which had undergone surgery at one of three locations along the longitudinal axis. (A) An embryo that had been transected through the future hypocotyl and was in the early stages of axis-reestablishment. Cells in the region directly beneath the regeneration dome and below the surgical site are similar in size and appearance to the orginal hypocotyl cells above the surgical site. (B) Later-stage regeneration from a hypocotyl transection, similar to that shown in panel (A). The pre-plantlet shown here had restored the remainder of the hypocotyl (H), and went on to form the radicle (with larger diameter cells) and the RAM (not visible in this plane of section owing to curvature of the specimen axis). (C) Early regeneration of the radicle following transection of the embryo in the zone of the future radicle. Note that cells on both sides of the surgical site are relatively large and highly vacuolated and that no meristematic region has yet appeared under the regeneration dome. (D) A later view of regeneration following surgery through the future radicle. Both the radicle (with larger diameter cells) and the RAM (densely stained area at the root tip) are visible in this plane of section. The curved tissue fragments extending from the ends of the cotyledons are the remnants of thin veins of tissue that occasionally subtend cotyledons in culture-grown embryos. (E and F) Regeneration of a new RAM following prior removal of the future RAM site in a torpedo embryo. Arrows indicate the approximate location of the orginal excision. Note the radially arranged files of cells and the presence of a well-defined root cap in the view at higher magnification. Bars, 200 μm.

Regeneration from the future hypocotyl

Treatments of this type involved cuts classified as 10S:90R and 20S:80R. A total of 12 shoot poles were analyzed in this group. Within a 3–4 days post-surgery, cells immediately underneath the regeneration dome appeared densely cytoplasmic and were similar in overall size to cells above the surgical margin (Fig. 4A) (n=5). When explants were examined 8–10 days post-surgery, the shoot poles had reestablished the future radicle and the distal zone of cells scheduled to form a RAM (n=7). The cells of the regenerated radicle were highly vacuolated and had cross-sectional areas 2- to 4-fold larger than those of either the future hypocotyl or future RAM (Fig. 2). Fig. 4B represents an embryo 9 days post-surgery that had restored all missing tissues and organized a RAM and root cap. The surgical margin in shoot poles in this group migrated slightly down the shoot–root axis as cells above it underwent normal increases in cell size and number.

The embryos that had fully regenerated the root pole, and could thus be classified as pre-plantlets, underwent morphometric analysis to determine the lengths of the hypocotyl, radicle and RAM. In control pre-plantlets, the hypocotyl and RAM lengths were observed to remain nearly constant while elongation of the radicle was the primary determinant of overall increases in embryo length (Table 1). Thus, the hypocotyl–radicle boundary existed as a relatively fixed point relative to the base of the cotyledons. Shoot pole explants, which were derived from transects of the future hypocotyl, regenerated the missing hypocotyl, radicle and RAM tissues, and went on to mature into pre-plantlets displaying hypocotyl lengths which were only slightly greater than those of control pre-plantlets (Table 1). This suggests that as new cells were laid down in or near the regeneration dome, the resulting cylinder of augmented tissue was capable of establishing some sort of distal boundary which, in turn, resulted in a hypocotyl of the correct length. Once the regeneration dome had proceeded beyond this boundary, the morphology of newly produced cells changed to a larger, more vacuolate type, indicating the transition to radicle formation (c.f. Fig. 4B). The length of the regenerated RAM was also similar to RAM lengths in control embryos (Table 1), and the timing of its appearance corresponded with that seen in undisturbed organisms. The RAM appeared in control embryos which were between 1100 and 1900 μm in axial length, while pre-plantlets in this experimental group displayed features of the RAM when they were between 1100 and 2300 μm in axial length (data not shown). It is not clear if this somewhat larger size range reflects a significant difference between the onset of the RAM in surgically manipulated versus control embryos, or was simply a result of a smaller sample size.

Regeneration from the future radicle

Data from 16 embryos with cuts between 25S:75R and 80S:20R were pooled into this class. The embryos that developed regeneration domes and which were examined within ca. 6 days post-surgery had restored a portion of the future radicle (Fig. 4C) (n=4). Cells beneath the regeneration dome were highly vacuolated and larger in cross-sectional area than the cells of the future hypocotyl. The exception was with cells immediately below the dome surface which were of a size and shape similar to cells associated with the protoderm. Embryos selected 3–6 days later (about 10 days post surgery) and subjected to thin-sectioning characteristicly had restored all missing cell types including the RAM and occasionally a root cap (Fig. 4D) (n=12). Because cells of the future radicle were included in these shoot pole explants, the surgical margin in this group tended to migrate away from the surgical site during the normal, rapid enlargement of cells in the regenerating radicle segment.

Since the future hypocotyl in this group was undisturbed by surgery, it was not surprising that the hypocotyl length, and thus the location of the hypocotyl–radicle boundary was similar to pre-plantlets in the control group. Cells of the existing and regenerated portion of the radicle were larger in cross-sectional area than those of the control embryos when considering embryos less than 4000 μm in axial length (Fig. 2). This size discrepancy was apparently due to a precocious enlargement of radicle cells in transected embryos since the cross-sectional areas of radicle cells were within a
similar size range once both cut and control embryos were greater than ca. 4000 μm in axial length. As the embryo enlarged to axial lengths between ca. 2000 and 2400 μm, cells at the basal end of the embryo formed a RAM. The axial length and the lengths of the hypocotyl, radicle and RAM regions of these regenerated embryos were similar to those transected through the future hypocotyl, above.

Regeneration from the future RAM

Regeneration following removal of only the extreme tip of torpedo embryos (90S:10R; n=3) was much more rapid than that seen in hypocotyl and radicle transections, and we were unable to capture an early stage of regeneration that did not include reestablishment of the RAM. In addition, embryos in this group elongated rapidly, 2 of the 3 embryos achieving axial lengths twice that of the largest control embryo within the time frame of these experiments. Fig. 4E is a micrograph of an embryo which had been subjected to removal of a portion of the future RAM. Cells within the restored RAM site resembled cells in the hypocotyl in that they maintained a dense cytoplasm and had relatively small cross-sectional areas (Fig. 4F). Each of the embryos in this group shed the surgical margin completely during regeneration of the RAM area. The average length of the RAM in these embryos was 467±68 μm, which included regenerated tissue. Despite their greater axial length, this value is similar to the RAM lengths of more typically sized embryos in the other experimental groups (Table 1).

Discussion

Position-sensitive regeneration, following incremental reductions of the embryonic axis, provides a window through which one may observe and evaluate the contribution of regional subroutines to the overall scheme of embryo pattern formation. The selection of nine, proportionately spaced surgical sites purposely ignored our knowledge of evolving tissue boundaries in order to ascertain if the system for position-sensing within the embryo possessed a high degree of spatial resolution, and to what extent it might be corrupted by surgical insult. The results showed remarkably consistent patterns of tissue replacement, suggesting that the somatic carrot embryo is irrevocably committed to organizing the longitudinal axis into three strata that differ in cellular morphology, density and their contribution to overall length. The region of the future hypocotyl consisted of small, densely packed cells and was restricted to the upper 20% of the axis. Likewise, the architecture of the nascent root apical meristem was based on small cells and a relatively fixed, short length. The intervening zone of the future radicle, however, developed with large diameter cells, whose continued expansion effectively determined the rate and extent of axis elongation.

Two features of root pole regeneration were particularly noteworthy, especially as they are not typically observed in plant systems. First, the replacement of missing tissues proceeded, with no appreciable discontinuity of structure, directly from cells at or near the site of surgery. The lack of scar tissue or a transition zone between existing and regenerated tissues, suggests that, despite the notable plasticity of many plant developmental processes (reviewed in Jennings and Trewavas, 1986), somatic carrot embryos are capable of replacing tissues with a high degree of spatial precision. Second, regardless of the location of surgery, the characteristic regions of the embryo were sequentially restored in the same order and polarity, and at a similar stage of maturation to intact controls. This finding implies that cells within the shoot pole remnant, most of which have neither the structural nor positional characteristics of those in the developing meristem, retain access to relevant portions of the program which directs the patterning and timing of late-stage embryogenesis.

The earliest visible indication that the system was responding to axis severance was the remodeling of tissues in the region of the provascular strand, within a few cell layers of the surgical margin. The apparent initiation of renewed cell divisions in this region gave rise to a narrow band of cells, similar in size and degree of vacuolation to those seen in plant meristems; however, it is not certain if proliferation of these cells is directly responsible for shaping the dome, or if they become the cellular initials that support axis replacement. We cannot, for example, rule out the possibility that the release of physical stresses within the cell wall lattice of cut embryos simply favors the distension of the tissues in the central core, in turn promoting local dedifferentiation of cells near the wound site. It is likely that resolution of issues pertaining to the roles that particular cell layers play in root pole restoration will require cell lineage analysis (cf. Poethig, 1987) during regeneration in chimeric plants. Whatever the ultimate function of these cells, however, their temporal and spatial proximity to processes that govern the systematic restoration of the plant axis provides us with an inducible morphologic marker with which to explore further how positional values are monitored within the embryo.

The formation of a regeneration dome and the position-dependent sequence of tissue replacement in carrot embryos supplies a rather surprising parallel to the early processes of limb regeneration in amphibians and certain other vertebrates. Severance of a limb from a salamander, for example, leads to the production of a rounded blastema on the end of the limb stump. Coordinated events of cellular dedifferentiation, renewed cell division and cell migration within this region direct the organization of new tissues, and, following still-mysterious cues for guidance, produce a functional replica of the missing portion of the limb. Accumulated observations from a variety of limb-regeneration experiments have been successfully interpreted in terms of Wolpert's (1969) original model for positional information, in which cells in a polarized structure such as a limb monitor their relative position according to an internal system of circumferential and proximodistal
coordinates (Kauffman, 1984). The physiological basis for this acquisition of positional identity is thought to be a combination of local cell–cell interactions and more global diffusion of a chemical morphogen (French et al. 1976; Bryant et al. 1981). In axolotls and newts the topical application of trans-retinoic acid to the sites of amputated limbs resulted in the production of duplicated portions of the forelimb (Maden, 1982; Thoms and Stocum, 1984), suggesting that this substance is involved in establishing proximodistal values along the limb axis. More recent work has shown that natural gradients of retinoic acid exist in limb buds of chick embryos (Thaller and Eichele, 1987).

The possibility that the passive or active transport of analogous growth-active substances in plant embryos might play a role in regeneration phenomena is an open question. The known plant growth regulators are relatively small molecules that can be synthesized by most growing cells, and, thus, theoretically at least, fit the requirements for a diffusible morphogen. In addition, there is indirect evidence that the best characterized of these, auxin, is basipetally transported along the axis of somatic embryos of carrot (Schiavone, 1988). There is at present, however, no evidence for natural gradients of auxin in embryos, nor is it known what, if any, effects a localized application of a plant growth regulator might have on embryogenesis.

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References


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