



Development and Differentiation in Plants

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An overview of plant development has been presented. In lower plants like mosses, auxin and cytokinin regulate the creation and the stability of the differentiated state of various cell types. The differentiated state is plastic and all cell types dedifferentiate to ground state, the chloronema. Even in higher plants, embryonic cells become only roughly committed during shoot meristem formation. Their terminal destiny becomes specified during the post-germination phase when the rough outline gets refined. The lack of a firm determined state, clonally heritable through mitosis, indicates that the development in plants is unlikely to be specified by a rigid programme.

KEY WORDS: moss protonema; cell differentiation; phytohormones; corn embryo; fate mapping; plant development.

INTRODUCTION

The development of multicellular organisms involves the formation of several distinct cell types and tissues in a precise time-phased manner at specific spatial locations. A unique feature of plant cells, especially that of the apical meristems, is their permanent embryonic nature and in many cases even the fully differentiated state can also be reversed and the whole plants regenerated. An overview of plant development based on our studies on the protonema differentiation in the moss *Funaria hygrometrica* Hedw, and on the fate mapping of the corn (*Zea mays* L.) embryos shall be described. First, our aim is to establish the hard facts underlying the plant development at cellular level and subsequently focus on to the genes regulating the developmental steps or decisions.

CELL DIFFERENTIATION IN THE MOSS PROTONEMA

Development of the Protonema

The moss protonema is a relatively simple system and lends itself to a variety of investigations. In a moss, the gametophytic phase consists of the filamentous,

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highly-branched structure, the protonema and the miniature plants, the gametophores. Protonema development involves two stages, the chloronema and the caulonema [1]. Both the cell types are distinct and easily distinguishable. The caulonema are characterized by oblique septae, small and spindle-shaped chloroplasts, while the chloronema show straight septae and abundant chloroplasts (Fig. 1). The transition from chloronema to caulonema marks a major developmental pathway and is regulated by auxin [2]. The cytokinins induce the formation of a third cell type, the bud initials which arise as side branches from caulonema cells [3]. Bud initials are the progenitors of moss plants. During gametophore formation, a tetrahedral apical cell with 3-cutting faces is organized. It generates 3-rows of leaves around the stem axis and finally a gametophore is formed.

The chloronema and caulonema filaments are multicellular, branched and grow due to repeated cell division of the apical and subapical cells of the first or higher order filaments. The apical cell functions like a stem cell and every time it divides, an apical initial and a subapical cell are formed. Upon spore germination,

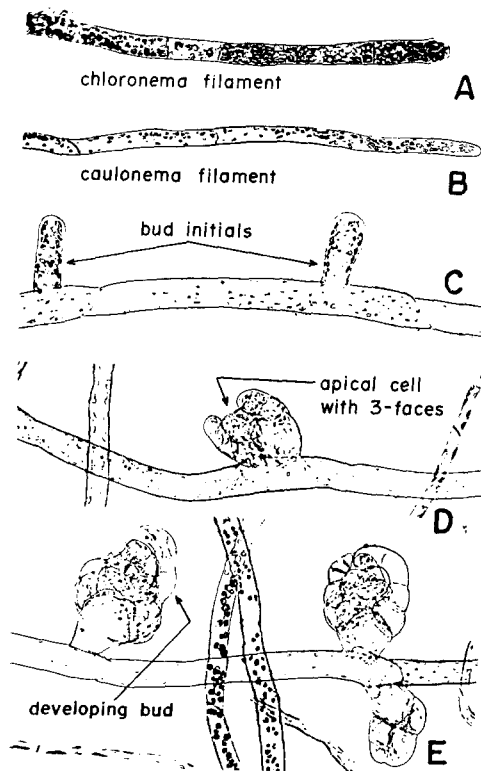


Fig. 1. Cell types in *Funaria* protonema. A, B: typical chloronema and caulonema filaments; C: bud initials 24 hr after $1 \mu\text{M}$ kinetin treatment; D, E: developing buds 50 and 76 hr after kinetin treatment.

the first order chloronema apical cell is formed and it generates a chloronema filament. After the proliferation of chloronema, the pattern changes and some of the subapical and the first order chloronema apical cells transform into caulonema initials and ultimately produce caulonema filaments. The caulonema side branch initial can differentiate into a caulonema or a chloronema apical cell or a bud initial. It is obvious that these three cell types differentiate in a precise and predictable sequence and their lineages are well-defined.

The protonema can also be regenerated from an isolated chloronema or a caulonema filament or any part of the gametophore or a sporophyte. During regeneration all cell types first dedifferentiate to chloronema stage and then redifferentiate to caulonema. The chloronema stage seems to represent the ground state as far as the differentiation of other cell types is concerned. The regenerated protonema can be haploid or diploid depending on the source of inoculum. The two phytohormones, auxin and cytokinin regulate not only the status of protonema differentiation but also the subsequent development of the gametophores. Cytokinin-over-producing mutants of the moss *Physcomitrella* form constitutively a large number of buds [4]. Light, nutrients (especially nitrate and phosphate), and 3',5',-cyclic AMP [5] also modify the differentiation of chloronema and caulonema.

Regulation of Cell Differentiation in Suspension Cultures

By manipulating the auxin level and inoculum size, the cultures of predominantly caulonema filaments (>70%) are readily obtained [6]; their formation occurs in two steps, the initiation, followed by enhancement or amplification. At low inoculum cell densities (<0.1–0.2 mg/ml) up to 10% caulonema are initiated. This response depends on the endogenous auxin content as modified by the cell density [7, 8] and can be delayed or blocked by the auxin antagonist PCIB (parachlorophenoxyisobutyrate). The amplification is also regulated by auxin, but by levels higher than that for initiation. This auxin can be supplied externally (apparent K_m for IAA 0.4 μM) or produced endogenously. For the latter situation, the chloronema need to be grown in an auxin-free medium buffered at pH 5.0–5.5 (Fig. 2). The amplification begins after *ca.* 6 days and this lag represents the time for the build-up of endogenous auxin. The lag can also be prolonged by PCIB (Fig. 3). Auxin also inhibits the growth of chloronema (apparent K_m for IAA 0.1 μM). Auxin effects on chloronema inhibition and induction of caulonema differentiation ultimately lead to the formation of cultures containing predominantly caulonema.

The phytohormone abscisic acid (ABA) leads to the formation of yet another cell type. ABA inhibits both cell division and cell elongation and cells formed in the presence of ABA develop into club-shaped resting (or dormant) structures, the propagules.

Similar to the imaginal discs of *Drosophila*, a firm state of cell determination clonally heritable through mitosis is not found in the moss system. Depending on the particular phytohormone applied, the destiny of the caulonema side branch initial can be specified. The caulonema formed in the auxin-free buffered medium

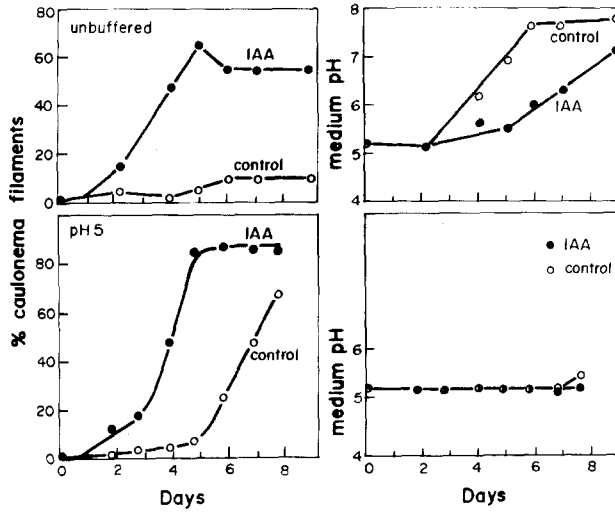


Fig. 2. Differentiation of caulonema in unbuffered (control) and buffered media (15 mM sodium succinate, pH 5.0). Left panels show the proportion of caulonema filaments, while right panels show the bulk medium pH changes. The unbuffered medium turns basic due to nitrate utilization. Note the differentiation of caulonema without auxin (IAA) in buffered medium after 5 days.

are highly responsive to various phytohormones. Upon transfer to fresh buffered medium, the side branches differentiate into chloronema, but if auxin or cytokinin or ABA is added individually, caulonema or bud initials or gemmae differentiate (Fig. 4).

For the cytokinin-induced bud formation, the caulonema cells act as the target cells. In many mosses such as *Funaria* and *Physcomitrium*, the spontaneous bud initials normally arise as side branches either from the caulonema cells or from the lowermost cell of the second order chloronema [9, 10]. In the PCIB-treated cultures, besides the lowermost cell of the chloronema, cells located higher up also formed bud initials in the presence of kinetin. These observations

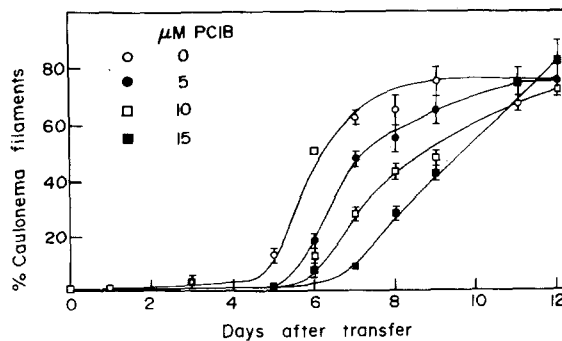


Fig. 3 Effect of auxin antagonist PCIB on the duration of lag prior to caulonema differentiation in the medium buffered at pH 5.0.

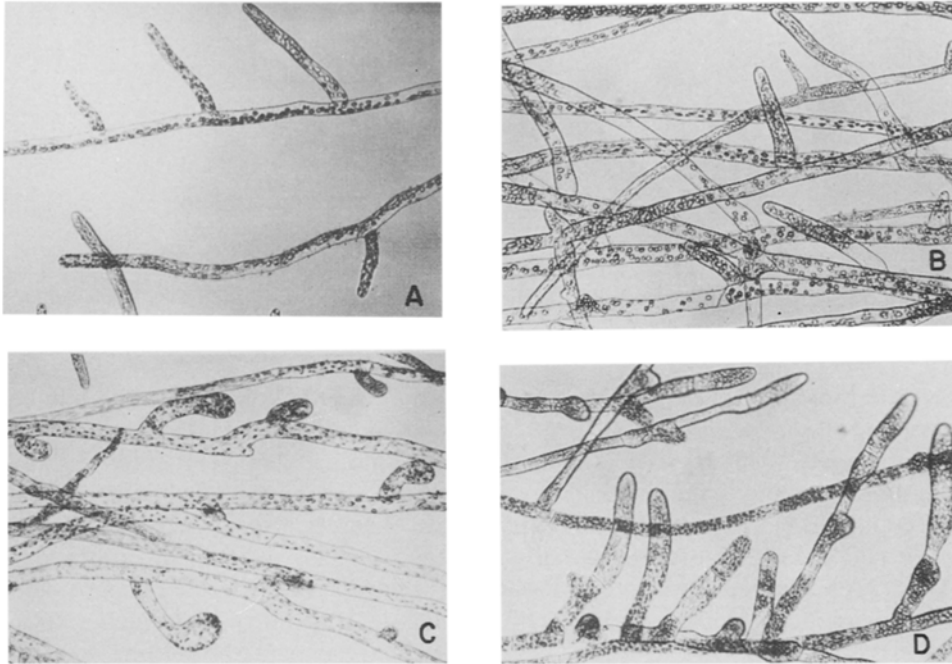


Fig. 4. Destiny of caulonema side branch initials. The caulonema produced in the auxin-free medium buffered at pH 5.0, were harvested and transferred to fresh medium (buffered with 50 mM sodium succinate, pH 5.0). A: control; B: with $1\ \mu\text{M}$ IAA; C: with $1\ \mu\text{M}$ kinetin; D: with $1\ \mu\text{g/ml}$ abscisic acid. Protonema photographed 30 hr after treatment.

raise questions about the acquisition of cytokinin-competence by target cells. The molecular basis of competence is not known at present. In the shoot apices, the ability of cells to synthesize auxin and to respond to it go side by side [11].

The growth and differentiation of each cell type is regulated by a very specific auxin concentration. The auxin level required for bud initial formation is higher than that for chloronema growth, but less than that for caulonema differentiation. Auxin is also needed for the normal growth of chloronema. The caulonema filaments formed in the auxin-free buffered medium are devoid of secondary chloronema. Normal chloronema filaments are restored in the presence of $5\ \mu\text{M}$ PCIB presumably due to a reduction of endogenous auxin to a level that does not inhibit chloronema. Upon raising PCIB to $15\ \mu\text{M}$, the size of individual chloronema cells is also reduced. A low level of auxin is thus needed to sustain the normal growth of chloronema. As the auxin requirement for chloronema growth is the lowest, during protonema regeneration the dedifferentiation of all cell types of chloronema stage, the ground state, can be explained.

Cytodifferentiation of Caulonema

In liquid cultures, the formation of caulonema apical cells is observed 24–30 hr after transfer of inoculum. Three events – partitioning of chloroplasts,

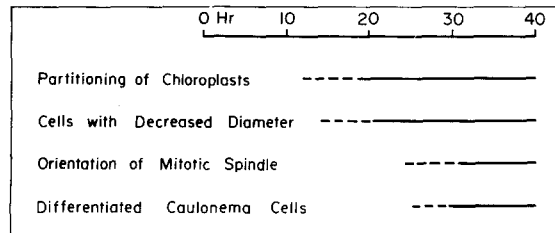


Fig. 5. Temporal sequence of processes associated with caulonema differentiation. The broken lines indicate the variation in time when the particular process is first distinguishable; the solid line indicates that in most of the cellular aggregates, the process has already occurred.

formation of cells with smaller diameter, and orientation of mitotic spindle preceding the formation of oblique septa are distinguishable (Fig. 5) before the formation of the caulonema apical initial [12]. About 12–18 hr after the transfer, the first visible change is the unequal distribution of chloroplasts either in the apical cell or in the newly-formed side branch initial. The upper-half of the cell shows fewer and smaller chloroplasts than the basal-half. This unequal distribution of chloroplasts is referred to as partitioning of chloroplasts. The diameter of cells with partitioned chloroplasts is usually smaller than those of chloronema cells. After these events, the tip cell divides in such a way that the mitotic spindle, which is initially parallel to the axis of the cell, changes its position to an oblique orientation. With the deposition of wall, an oblique septum is formed and a caulonema apical initial becomes organised. In the chloronema apical cells, the spindle does not change its orientation and straight septae are formed. The caulonema cells possess a specific mechanism which controls the plane of septae. The most important event seems to be the partitioning of chloroplasts which introduces asymmetry and generates regional diversity. As a result thereof, the cell becomes polarised.

Complexity of Cell Differentiation Process

Our studies on the moss protonema over the past several years demonstrate clearly that what was initially thought to be a simple process of cytodifferentiation, is in fact exceedingly complex, and to make further progress, an understanding of the factors that regulate the cell diameter, distribution of plastids, orientation of mitotic spindle and the deposition of wall will be required. Further, the subcellular events leading to caulonema differentiation are initiated from a group of cells. The redifferentiation of bud initials into caulonema in the mutant *pg-1* illustrates this feature (Fig. 6). This mutant produces predominantly caulonema filaments (>65%) in the absence of an exogenous auxin [5]. A microscopic examination shows that the bud initials do not develop into gametophores, but first dedifferentiate into chloronema and then redifferentiate to caulonema. The spontaneously arising bud initial elongates and then divides into two cells. The chloroplasts multiply in the tip cell leading to the organization of a typical chloronema apical cell. The dedifferentiated buds redifferentiate into

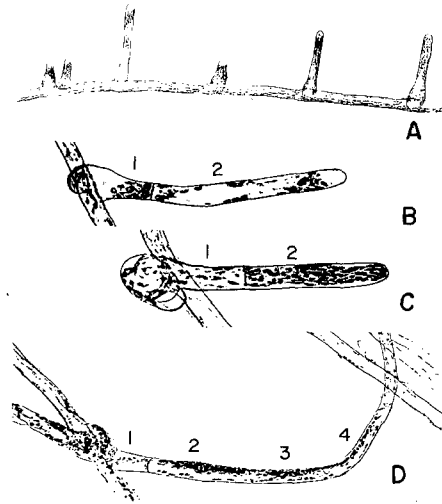


Fig. 6. Differentiation of caulonema from bud initials in the mutant *pg-1*. A: elongation of the bud initials forming the side branch initials; B, C: the initial divides and chloroplasts multiply in the tip cell leading to the organization of a chloronema apical cell; D: the apical cell divides and, after producing several chloronema cells (marked 1–4), caulonema cells are produced.

caulonema after producing a certain number of chloronema cells. In no case do buds redifferentiate into caulonema directly. Sixty per cent of dedifferentiated buds produced 4 ± 1 chloronema cells, while others produced up to 9 cells. The formation of caulonema from a group of chloronema cells, even in the presence of adequate auxin, indicates that another factor is also involved. Whether a cell counting or a distance measurement is involved, is also unknown at present.

Auxin and Cytokinin Assumed Role of Hormones in Bryophytes

The phytohormones such as auxin, cytokinin, abscisic acid, gibberellins and ethylene are also synthesized as products of secondary metabolism by microorganisms [13]; however, there is no evidence that these chemicals play any regulatory role in them. The situation in algae is not clear because of the difficulty in getting enough axenic material. ABA may be involved in the adaptation to stress in some algal species, as an increase in ABA following salt-stress has been reported [14]. The first clear evidence for the regulatory role of auxin and cytokinin is to be found in some of the bryophytes. In liverworts, auxin stimulates rhizoid production, while its role in caulonema differentiation has already been discussed. Throughout the vascular plants (ferns and seed plants) auxin shows rhizogenic (root-inducing) response. It is generally believed that whereas in animal systems, the steroid (and peptide) hormones and their receptors co-evolved, such a situation may not have occurred in plant systems. Auxin and cytokinin seem to have been incorporated in the regulatory mechanisms only after the specific receptors or hormone-interacting physical or chemical steps had evolved. The bryophytes could be among the simplest plants where such a regulation evolved

and therefore it may be easier to understand the mode of auxin action in mosses than in the higher plants. This is another reason why mosses continue to draw a lot of attention to unravel the ground rules governing the action of auxin and cytokinin.

CELL LINEAGE ANALYSIS OF CORN PLANT DEVELOPMENT

The moss protonema offers a limited opportunity to study spatial aspects of development. We have therefore been tracing the fate of genetically marked cells of the corn embryo [15, 16]. Using conventional anatomy, lineages during embryonic development have been analysed in many plants. The cleavage patterns in the zygote and proembryos are quite regular and the lineages during embryogenesis show several specific patterns. The developmental significance of these patterns is yet to be fully understood. In corn, much has been learnt about the overall development, because a large number of mutants are available. The most useful factors to mark cells are the anthocyanin or the plastid mutations, or those affecting the morphology of the tassel or the ear shoot. When kernels (or seedlings) heterozygous for one or more factors are X-rayed, the dominant allele is often lost. Plants developing from irradiated material show randomly located sectors or clones [15]. Most of the clones are derived from the outermost cell layer of the shoot apex.

Analysis of Shoot Apical Meristems in Chimeras

The study of colchicine-induced periclinal chimeras in *Datura* has demonstrated unequivocally the presence of three independent cell layers in the shoot apex [17]. As cells derived from each layer are also present in the leaves and floral parts [18], at least one cell from each fundamental layer must contribute derivatives to an organ. The existence and functioning of the layers has been further supported and confirmed in a large number of monocotyledonous and dicotyledonous plants [19, 20]. The outermost, second and third cell layers have been referred to as L-I, L-II and L-III. The biological significance of layered arrangement is yet to be known and it may reflect the preferred or the predominant orientation of cell division in the shoot apex.

These layers though distinct and independent are, however, not permanent in an absolute sense. Normally the cells of these layers divide anticlinally and the periclinal divisions occur at the time of leaf and flower initiation. However, occasionally periclinal divisions unrelated to organogenesis can occur and in such instances a cell derived from L-I could end up in L-II. The fate of this cell depends on its new location and, therefore, position rather than lineal descent is important. In tobacco upto 4-cell layers contribute lineages to the leaf, but the contribution of each layer is different [21]. Lineages of the 4th layer contribute to the central core in the lower half of the midrib, while the major part of the leaf blade is derived from L-III. At the margin only the derivatives of L-I are present. The periclinal divisions followed by cell displacements lead to variegation

patterns in the chimeric plants; since these events occur randomly in time and space, no two leaves show identical patterns of variegation.

Node as a Developmental Unit

The pattern of clonal restriction shows that the construction of a corn plant is modular, i.e. it is made up of repeating units. The sectors typically start at the base of the internode or in the ear shoot, extend up in the internode, and finally terminate in this or the next leaf. The axillary bud is thus related developmentally to the leaf above and not to the leaf in whose axil it is located [15]. Each repeating unit (node) thus consists of an axillary bud, internode and the leaf above. Because of the distichous arrangement of leaves, the successive units must be arranged 180° apart. An unexpected observation is the formation of the margins of the leaf blade and sheath from different lineages. In the sheath, the lineage in the submarginal position expands laterally and continues along the margin of the blade.

Tassel and Ear Shoot

A sector includes 25–50% of the total spikes and therefore 2–4 cells develop into the tassel [22]. When a sector includes the central spike, it divides it vertically into two halves showing that the central spike originates from at least two cell lineages. Several mutations are known to bring about phenotypic transformations similar to that of the homeotic genes. Clones have been observed in several of these [15]. The factor *ramosa* (*ra-1*) introduces an additional order of branching in the tassel and ear shoot. The *ramosa* sectors in the tassel are characterized by the presence of all spikes on one side of the central spike indicating that *ramosa* affects the developmental fate of only one of the two cells producing the central spike. The gene *tassel seed-6* (*Ts-6*) transforms all the male florets into female. Wild type sectors are observed in the tassels heterozygous for *Ts-6* at a low frequency and only one-fourth of the total spikes are included in a sector. Though *ramosa* and *tassel seed-6* bring about transformations phenotypically, they act late in tassel development and affect the developmental fate of a single clone and not of the four cells that collectively become the tassel. In this respect their domain of action seems to be different from that of the fruitfly homeotic genes.

In the cob, the sectors are typically longitudinally oriented and curved elliptically. No relationship between the clonal boundaries and the rows of kernels is found despite the fact that pairs of spikelets arranged side by side develop from single branch primordia [15]. The clonal tissue switches laterally either a complete row or often only half a row. A row of spikelets or even individual spikelets can develop from two different but adjacent lineages. The proliferation of cell lineages in parallel files as a basis of row formation appears unlikely.

Shoot Apical Meristem and Node Formation

The formation of nodes by inducing clones at the dry kernel stage has been investigated by Coe and Neuffer [22] and Johri and Coe [15]. Typically the sectors

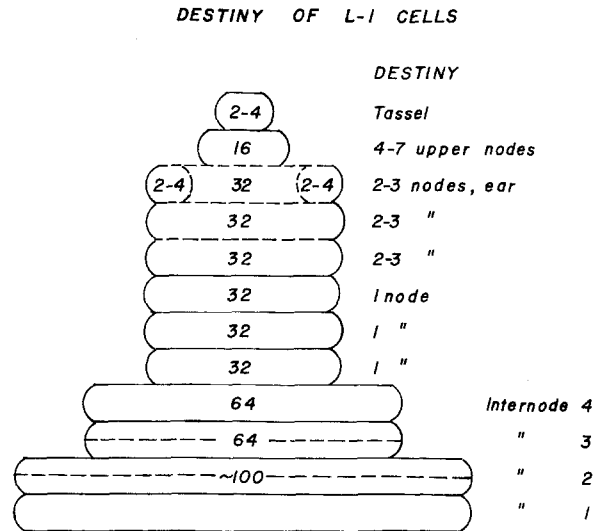


Fig. 7. Destiny of the cells derived from the outermost cell layer of the corn embryo at the dry kernel stage. The number of cells generating the first internode is not yet known.

extend about two or three nodes each in the lower six to eight nodes (nodes 7 to 15) and about four to seven nodes in the upper nodes (nodes 15 to 20). Formation of tassel from two to four cells has already been discussed. These cells are followed by 16 cells destined to become the four to seven upper nodes (above the ear) and finally three levels of 32 cells each representing the lower six to eight nodes. The 32 cells at a given level develop into two or three nodes. The ear shoot is derived from two to four cells which arise as a subset of the 32 cells at that level. Functionally the L-I layer of the shoot apex (dry kernel stage) behaves as if it is organised like a stack of rings (Fig. 7).

The sectors include only part of the second and third internode but a complete internode at nodes four through six or seven. Internodes two and three are thus represented by at least two cell layers, while internodes four through seven by a single layer of cells. Groups of cells become destined at successively higher levels and the specification progresses from the base of the plant towards ear-bearing nodes [23]. After a node is specified, each nodal initial cell no longer behaves as an independent component and collectively all the nodal initials become destined to produce a particular single node. The average sector width indicates that the lower nodes are specified when there are about 32 L-I cells. Sectors measuring about 1/64th of the culm perimeter still span the entire internode in the lower nodes. Thus widening of the axis precedes the lengthening during internode development. Subsequent development of an internode has not yet been investigated.

In the apical meristem the cells are committed in two zones [24]. The cells at the distal end become the tassel while those at the base produce the lower six to seven nodes. The cells in between the two zones are uncommitted and are in transition to become committed. The formation of nodes is like a wave of

commitment gradually progressing towards the distal end. The formation of rings also progress from the lower nodes towards the tassel. The development of corn plant thus involves three successive and interrelated events: (a) the formation of rings, (b) the commitment process to produce single nodes, and (c) an expression of this commitment leading to the development of internodes. These events are separated from each other temporally and spatially only slightly and the shoot apical meristem represents a structure where the above events have been arrested at a specific instant of time in development. The origin of the shoot meristem has also been traced. It becomes determined just prior to or during its morphological differentiation approximately 10 to 12 days morphological differentiation approximately 10 to 12 days after pollination [16].

CONCLUSIONS

The process of cell differentiation in plants is unlikely to involve a rigid specification of a cell's destiny or fate. The fate of the marked cells in corn and the multiple cell differentiation potentialities of the subapical initial cell in moss protonema provide no evidence for the presence of a firm determined state. Similarly, the overall plant development does not seem to be specified by a rigid developmental programme. There is a lot of variability. During the shoot meristem formation only a rough outline of the plant is generated and later during the post-embryonic development based on the growth conditions and inputs from the environment, the outline seems to get refined and only then the final make-up of the plant emerges.

What regulates the acquisition of competence to respond to a particular phytohormone is not clearly understood. As mentioned earlier, in the shoot apical meristem the ability to respond to auxin may be related to its production. It is quite possible that during this process, the cells acquire specific labels that confer on them the target character for a second hormone. The cells must be very finely tuned to detect and respond to different levels of auxin and conceivably there could be either multiple receptors or steps responding to subtle changes of auxin level. A major progress should occur in the area to fill up the gap between the regulation of gene expression and the expression of morphogenetic patterns.

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