Relationship between cell proliferation and transition to elongation in plant roots

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ABSTRACT Relationship between two main growth processes, cell proliferation and elongation, is reviewed. In literature, meristem and elongation zones are discriminated according to: increase in relative growth rate, change in cell shape, cessation of mitoses, and change in cell structure, vacuolation in particular. Relative growth rate is almost constant along the meristem and increases sharply in the course of cell transition to elongation. The transition of cells to elongation cannot be considered as a continuation of meristematic growth after the cessation of divisions. The most valid criterion of the cell transition to elongation is a sharp rise in relative growth rate, rather than change in cell shape (form factor). In the growing root tip, there are two regions of more active accumulation of proteins. The first is associated with the fastest cell proliferation while the second corresponds to enhanced cell elongation. The results of experiments with X-irradiation and cytostatic drugs suggest that cell transition to elongation is independent of cell proliferation and is regulated by the processes determining the life-span of cells in the meristem. The rate of cell transition to elongation is controlled by the processes determining both the life-span of cells in the meristem and the rate of cell proliferation. For most meristematic cells, the life-span of most cells in the meristem remains unchanged in treated roots. Thus, if cell proliferation and transition to elongation are regulated independently, any retardation of cell proliferation will automatically result in deceleration of the cell transition to elongation. Cell kinetics in roots is similar to that in some mammalian tissues capable of long-term proliferation.

KEY WORDS: *root, cell proliferation, cell elongation, transition to elongation, meristem, cell differentiation*

Introduction

Plants extend by both cell division and elongation. The growth by cell elongation is typical of the plants. In the course of elongation, not only the cell volume increases manifold during a short time period, but the cell morphology is rearranged. Cell elongation involves an extensive uptake of solutes coupled with the formation of a large central vacuole occupying the major part of cell volume and an essential reorganization of the metabolic machinery. A fully elongated cell is invariably rich in many various low-molecular constituents (sugars, amino acids, organic acids, amides, etc.), as compared to a meristematic cell. The activities of various enzymes employed in their transformations are also much higher. Thus, during cell elongation, the meristematic cell, whose metabolism is directed mostly to the proliferative activity, develops to the differentiated cell with tissue-specific functions.

The purpose of the present paper is to analyze relationships between the cell proliferation and subsequent cell elongation in the roots. There is an enormous body of literature on the regularities of these processes in the roots, but their relationship is still not clearly understood.

The structure of the growing part of the root

Although the root can be very long, its growing part never exceeds 1 cm, except the aerial roots. In roots, the growing part is typically shorter than the elongating region of a shoot (Ivanov, 1983a). The growing root tip consists of meristem and elongation zone, the latter being usually longer than the former. As a rule, cells divide mostly in the meristem. However, there is no agreement as to where the boundary between the meristem and elongation zone is. Recently, Baluska and coworkers (Baluska *et al.*, 1990, 1995, 1996; Kubica *et al.*, 1991) proposed the presence of a distinct transitional region between the meristem and the zone of rapid cell elongation which they defined as «the post-mitotic isodiametric

Abbreviations used in this paper: PIG, post-mitotic isodiametric growth.

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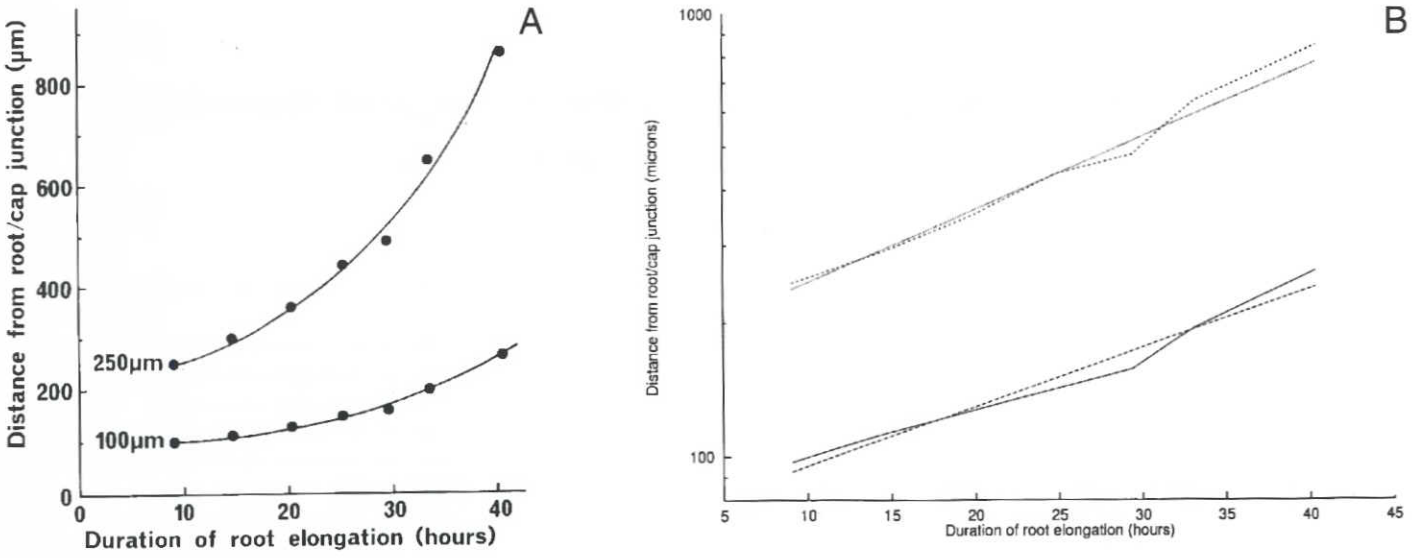

Fig. 1. Position of packets of metaxylem cells at various times during root growth. (**A**) *The two curves in the figure trace the positions held by two packets, during root growth, that were 100 microns and 250 microns from the cap/quiescent center junction at the start of root growth (Barlow, 1983).* (**B**) *The same data in semilogarithmic scale.*

growth (PIG) region». In recent years, the presence of PIG-region in root is discussed in literature (Ishikawa and Evans, 1995; Baluska *et al.*, 1996). We will return to this problem after re-examination of various criteria usually employed for distinguishing the boundary between the meristem and elongation zone.

The following criteria are used: (1) increase in relative growth rate, (2) change in the cell shape, (3) cessation of mitoses, (4) change in cell structure, especially relative enlargement of vacuolar compartment.

(1) Relative growth rate: its change along the growing part of the root

It is evident that the growth rate of elongating cells is higher than in the meristematic ones. For example, in the maize roots, the elongation period is shorter than an average cell cycle (Ivanov, 1981). During one mitotic cycle, the cell length doubles, on the average. During the elongation period, the length of cells increases 15 to 20-fold. Consequently, not only the cell growth rate, but also the relative growth rate is higher in the elongating cells.

In classical experiments of Julius Sachs, the marks made by Indian ink were applied for examining the distribution of the growth rate. The distances between the marks increased much more markedly in the elongation zone than in the meristem, but this technique cannot provide information about changes in the growth rate at the boundary between these two zones.

Application of the Indian ink marks is inadequate for estimating the growth rate along the meristem because of a slow growth rate of the meristematic cells and presence of a root cap, which partially covers the meristem, and a mucilage on the root surface. Later, some direct and indirect methods were worked out for measuring the growth rate changes along the root axis.

Wagner (1937) calculated ratios between the lengths of cell complexes (cell packets) arranged one by one along the root axis in the meristem and the apical half of the elongation zone. Each cell

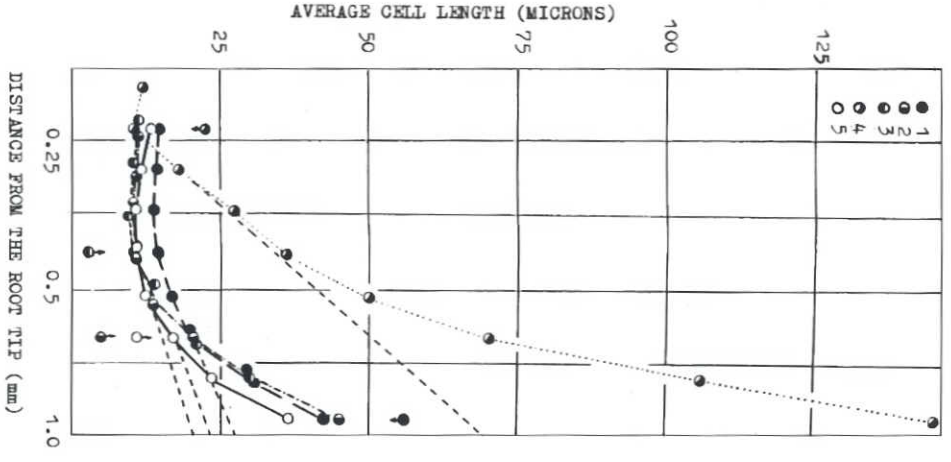

Fig. 2. Average cell lengths along wheat root axis: *1, pericycle; 2, cortex; 3, exodermis; 4, metaxylem; 5, rhizodermis. The arrows indicate position of last mitosis. Dashed line shows hypothetical cell lengths if relative growth rate remains the same as in meristem. (Data from Balodis and Ivanov, 1970).*

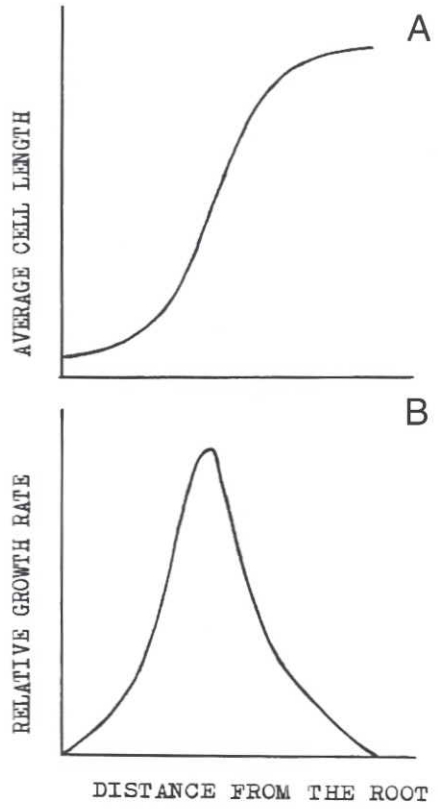

Fig. 3. Aproach used by Barlow *et al.*, (1991) for calculation of relative growth rate along the root axis. *(A) Logistic curve of the change of average cell length along root axis. (B) Derivative of A with respect to L (R-relative growth rate in units 8 micron microns $^{-1}$). Time can be introduced by multiplying R by the cell production rate Q, of the corresponding file of meristematic cells; the relative elemental rate elongation can then be estimated in units microns*microns $^{-1}$*h $^{-1}$ (or more simply, %h $^{-1}$).*

complex represents the progeny of one «maternal» meristematic cell, which was earlier formed from an initial cell of file. As the seedling roots extend at a constant rate, it is highly probable that time intervals between the formation of two successive maternal cells are equal, on the average. In this case, the ratios between successive complex lengths (i) along the root axis indicate whether the relative growth rate (k) changes with displacement of the complexes from the root tip. The values of i turned out to be similar along the meristem in different tissues, no matter how often the cells divided. At the same distance from the root tip, near the upper boundary of mitosis zone in most tissues, i sharply increases in all tissues. Thus, Wagner concluded that k was similar in different tissues, remained unchanged along the meristem and sharply increased where the elongation zone begins.

Similar conclusions were drawn by Hejnowicz and Brodzky (1960) and Ivanov (1974, 1981) who employed another approach proposed earlier by Burstrom (1941) for the analysis of growth of the elongating root cells after the cessation of mitoses. They measured the lengths of metaxylem cells along the root axis. These cells terminate their mitotic activity much closer to the root tip than the cells of most tissues. In roots, the cells grow symplastically (Sinnott and Bloch, 1939; Brumfield, 1942; Ivanov, 1983b; Kubica *et al.*, 1991), and k is similar in different tissues at the same distance from the root tip. For this reason, it is possible to measure the cells along only some but not all cell files in order to evaluate the relative growth rate in all tissues. The lengths of metaxylem cells started to increase exponentially at a certain distance from the root tip, approximately before the level of cessation of mitoses in most tissues. Above this level, the cell lengths begin to increase very sharply. A careful analysis has shown that above the region of last mitoses, k in the metaxylem is equal to the average value of k below this boundary (Ivanov, 1974,1981).

Barlow (1983) suggested another approach for studying meristematic cell growth during the first days after the germination. He has shown that the root cell walls which were in dry seeds are stained more brightly than in cells generated after seed germination. It made possible to visualize the displacement of individual successively arranged cells and their descendants (arising «cell packets») in the germinating roots. Analysis of the curves in these papers has shown that k was practically constant along the meristem (Fig. 1). The cell distance from the root tip (L) exponentially increases with time at constant k, [the logarithm L linearly increases with time (Fig. 1)]. The lengths of packets exponentially grow too (Luck *et al.*, 1994).

An upward inflection of the k curve near the proximal boundary of the meristem is evident from the analysis of curves of average cell lengths (l) along the meristem (Fig. 2). Such curves are typical of the roots of various plants (Balodis and Ivanov, 1970; Ivanov, 1981; Darbelley *et al.*, 1989; Baskin *et al.*, 1995). In the meristem, l is minimal at nearly the middle of this zone. Then it gradually increases and somewhere above the region where the last mitoses occur, begins to increase more sharply. The l reduction in the apical meristem results from accelerated cell proliferation, and the subsequent l rise results in a lower proliferative activity. In the meristem, the dividing cells are the longest; variations in the lengths of individual cells are due to their extension during the interval between two mitoses (Ivanov, 1971). But after cessation of

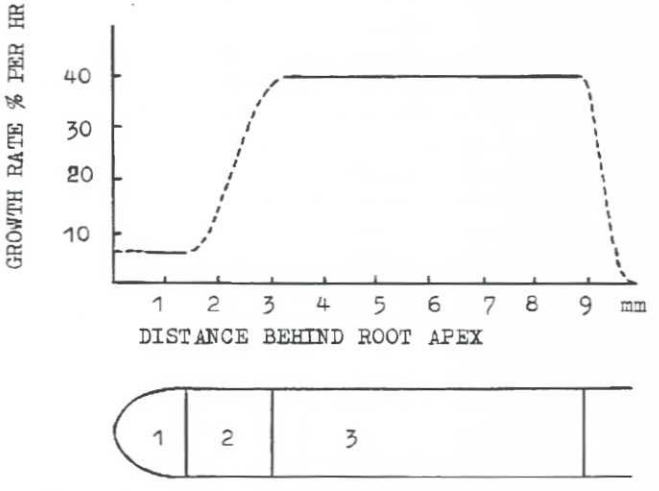

Fig. 4. Growth zones observed in a living root of Phleum pratense and growth rate of epidermal cells in each zone. *Solid lines indicate the actual rates as determined from growth curves. Dotted lines show the estimated rates in regions of the root where it was impossible to obtain an accurate determination. Below graph is a diagram of the root drawn to the same scale as the abscisssae of the graph (Brumfield, 1942).*

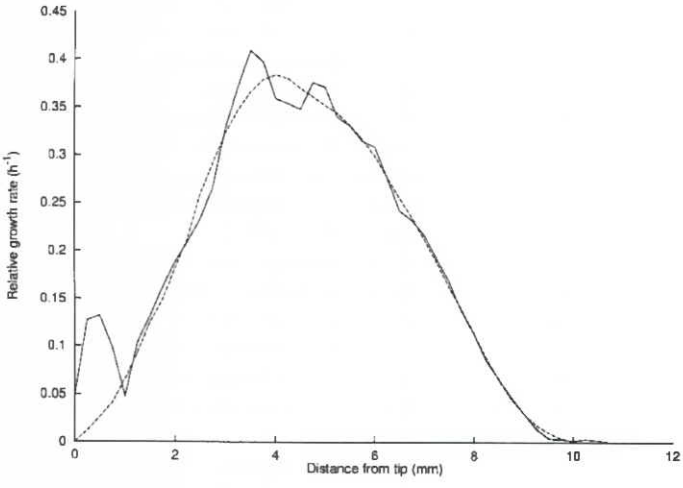

Fig. 5. Change of relative growth rate along the root axis. *1, measured values; 2, smoothed values. Plotted from Table 1 in Erickson and Sax, 1956.*

mitoses in most tissues, l increases more sharply than expected if k remained unchanged (Fig. 2). At constant k, l would be doubled at a doubled distance from the root tip. However, l doubles noticeably closer to the root tip. In some tissues, the mitoses stop more closely to the root tip than in other tissues (for example, metaxylem in roots of the monocotyledonous plants or atrichoblast of the rhizodermis in Trianea). In these tissues, l at first doubles upon doubling of the distance from the root tip (Fig. 2). However, at a certain distance from the tip, the cells start to increase more sharply (see, for example, Wagner, 1937, Figs. 9-15; Cutter and Feldman, 1970: extension of trichoblasts and atrichoblasts in Trianea root, Fig. 15.; Ivanov, 1974: metaxylem cells in root, Fig. 13). This analysis of cell length changes along the root axis suggests a sharp rise in k at the upper end of the meristem.

However, Barlow *et al.* (1991) arrived at a different conclusion concerning the k changes along the root axis. They described l changes along the growing part of root by logistic curves and determined k by differentiation of these curves with respect to L (Fig. 3). These authors concluded that k increased along the meristem and reached a maximum at the middle of the elongation zone. This approach to evaluation of k changes along the root axis is not correct, at least for the meristem, where l changes depend not only on the relative rates of cell extension (k), but also on the relative rate of their multiplication. The second process was not taken into consideration by these authors. Moreover, the logistic curve inadequately describes the changes in cell length along the meristem (Fig. 2).

Brumfield (1942) was the first to develop a more accurate method for estimating the relative growth rate. He kept the *Phleum pratense* seedlings in a specially designed moist chamber and regularly made pictures of the same groups of meristematic cells in the root tips as they developed to maturity. He plotted the growth curves of individual cells and cell packets produced by them and estimated the relative growth rate at various distances from the root tip (Fig. 4). He has shown that the growing part of the root consists of three successive regions. Cell divisions occur only in the apical region, where the cells grow at k equal to 5.4% per hour. In the second region, k increases from 5.4 to 41.2% per hour. In the third region, the cells grow exponentially with k equal to 41.2%. It is clear from these data that k is constant along the meristem and increases sharply during transition to elongation. Similar results were obtained by Heinowicz (1959) for wheat roots. However, these results were almost forgotten and most authors refer to the curves obtained by Erickson and Sax (1956). They concluded that k increases from the root tip along the meristem and reaches its maximum in the middle of the elongation zone. They made regular photographs of the marks located on the surface of maize root at intervals of 250 mcm and calculated the velocity (V) of displacement of the marks from the root tip along the axis. The dV/dL is k at the distance L from the root tip. The k changes along the root axis are shown in Figure 5. The curves were obtained by smoothing the experimental data using seven-point formulae (Milne, 1949). The portion of the curve subjected to the smoothing of experimental data was equal approximately to the length of meristem. Therefore, these smoothed values of relative growth rate for the meristem do not agree with the experimental data obtained by Erickson and Sax (1956) (Fig. 5). This was emphasized by Hejnowicz (1959) who pointed out that the conclusions were «based to a larger extent on extrapolation from the data on the region further away from the tip than on original data for this zone».

Thus, analysis of the cell length changes along the root axis and the observations made on the thin roots showed that in most tissues, near the region where the mitotic activity ceased, k sharply rises over a short region of root axis. This jump of k cannot be revealed when the data are smoothed by incorrect methods or by using the logistic function for describing the changes in cell length along the root axis, without taking into consideration the cell multiplication. Since the elongating cells grow at much higher k than the meristematic cells, it is likely that a sharp k increase indicates the transition of meristematic cells to elongation. However, this simple explanation is absent from most papers, whereas the curves of k changes along the root axis obtained by Erickson and Sax (1956) (Fig. 5) or Barlow *et al.* (1991) (Fig. 3) lacking any inflection at the boundary two zones are widely cited (for example, Erickson, 1976; Green, 1976; Gandar, 1983; Silk, 1984, 1992; Barlow *et al.*, 1992; Morris and Silk, 1992; Peters and Bernstein, 1997; Zieschang *et al.*, 1997).

TABLE 1

POSITION OF LAST MITOSIS ANDF THE ONSET OF FAST GROWTH IN VARIOUS TISSUES OR MAIZE ROOT (from Figs. 3-6 in Baluska *et al.*, 1990). ACCORDING TO THESE AUTHORS PIG REGION IS SITUATED BETWEEN THESE POINTS.

	Distance from root tip, mm	
Tissue	Last mitosis	Onset of fast growth
pericycle	1.6	2.2
endodermis	1.5	2.4
cortex	1.3	2.7
hypodermis	1.3	2.2
epidermis	1.3	2.2
xylem parenchyma	0.8	1.2
stelar parenchyma	0.9	1.5
metaxylem	0.4	1.6

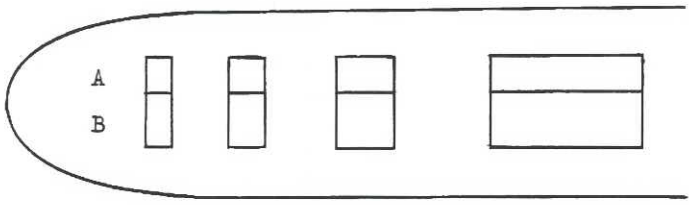

Fig. 6. Schematic representation of the change in cell shape along the root axis. *Cells A and B differ in width but have equal length because their elongation rates are similar throughout the whole growth period. However, cell A has «minimum form factor» closer to the root tip than cell B.*

(2) Change in the cell shape as a criterion of cell transition to elongation

Aluska and coworkers (Kubica *et al.*, 1991; Baluska *et al.*, 1990, 1995) assumed that «rapid cell elongation started only at the point of the lowest form-factor values where the growth in cell width ceased» (Baluska *et al.*, 1990, p.272). They suggested the presence of «a distinct transitional region between the meristem and the zone of rapid cell elongation» defined as PIG (post-mitotic isodiametric growth). Based on the above-discussed data of Barlow *et al.* (1991) concerning k changes along the root axis, they concluded that «the proximal boundary of the apical meristem does not appear to be associated with any profound change in the rate of cell elongation. Such a change occurs only later in cellular ontogeny when the slower relative rate of cell elongation, characteristic of meristematic and immediately post-mitotic cells, transforms into a more rapid one».

They consider a minimum level of the form factor in the cells as a criterion of cell transition to rapid extension. «The form factor characterizes the shape of cells, being 5.09 for a circular shape, 6 for a square, 7 for a rectangle with a side ratio of 2:1, and 12.4 for a rectangle with a side ratio of 5:1» (Baluska *et al.*, 1990). In the meristem, the length of most cells is shorter than their width. After the cessation of mitoses, the cells elongate at a high rate in the longitudinal direction. For this reason, the cells acquire an isodiametric shape at a certain distance from the tip, which is characterized by a minimum value of form factor. Later, during the longitudinal growth, the cells are lengthened and their form factor increase again. In various tissues, the cells reach a minimum level of form factor at different distances from the root tip (Table 1). Hence, these authors concluded that in various tissues, the cells begin to grow rapidly at different distances from the root tip. Kubica *et al.* (1991) opposed this conclusion to my opinion (Ivanov, 1974, 1983b). From my point of view, all cells begin to grow at a high relative rate (k) at the same distance from the root tip, irrespective of tissue specialization (Ivanov, 1983b). Such simultaneous transition to elongation is an obligatory condition for symplastic growth of root cells (Sinnot and Bloch, 1939; Brumfield, 1942), since only in this case, k remains equal in different cell files at the same distance from the root tip. This fact was confirmed by Kubica *et al.* (1991).

Baluska *et al.* (1990) suggest that the beginning of fast growth is associated with the minimum form factor. However, it is not correct because a change in the shape cannot be considered as an attribute of increasing cell growth rate, as follows from Figure 6. Cells A and B have a similar length but they differ in width. The growth rates of both cells are equal and they grow symplastically. However, they reach the isodiametric shape and a minimum value of the form factor at various distances from the root tip.

Moreover, in the meristem, the cells differ in their lengths at the same level from the root tip. You can find large premitotic cells of isodiametric shape among small cells. The former appear to have low form factor, but they do not start immediately their elongation.

The position of RIG region along the root axis is tissue-specific (Table 1). In some tissues, PIG region is located prior to the increase of the relative growth rate but in other ones after it. Therefore, k can be not constant along PIG region in some tissues and vary in different tissues. Thus, PIG region cannot be considered as a population of similar cells because they vary in k. Therefore, the form factor (i.e., cell shape) does not meet the requirement of criterion for the cell transition to elongation.

(3,4) Cessation of mitoses as a criterion for cell transition to elongation

In roots, the mitoses usually occur in the meristem. However, their cessation is not a suitable criterion for the cell transition to elongation. In various tissues, mitoses stop at different distances from the root tip (Fig. 2). In most cases, they stop before k increases, but in rare cases mitoses were observed in large elongating cells (for example, Balodis, 1968; Rost *et al.*, 1988).

The retardation of proliferation in the basal part of meristem results from exit of some cells from the mitotic cycles. The duration of mitotic cycle does not increase in this part of meristem (Balodis and Ivanov, 1970; Ivanov, 1981).

I have previously shown that even complete suppression of cell divisions by X-irradiation at high doses did not affect the time of cell transition to elongation (Ivanov, 1968, 1981, 1994). In irradiated roots, during their limited growth period after irradiation, the growing part consisted of two regions: apical, in which cells extended at k similar to that in the unirradiated cells, and basal, in which cells extended at high k similar to that in the elongating cells of unirradiated roots. Therefore, cessation of mitoses and cell transition to rapid growth are independent events.

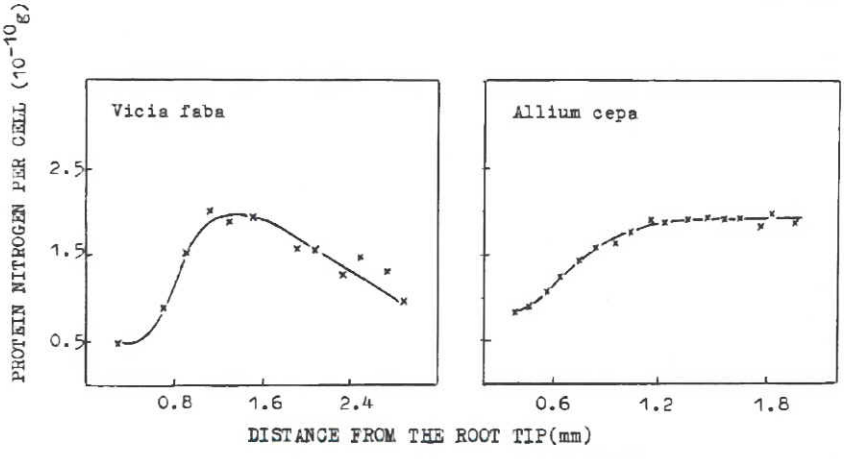

Fig. 8. Protein amount per cell along the growing region of pea root. *(Brown and Broadbent, 1950).*

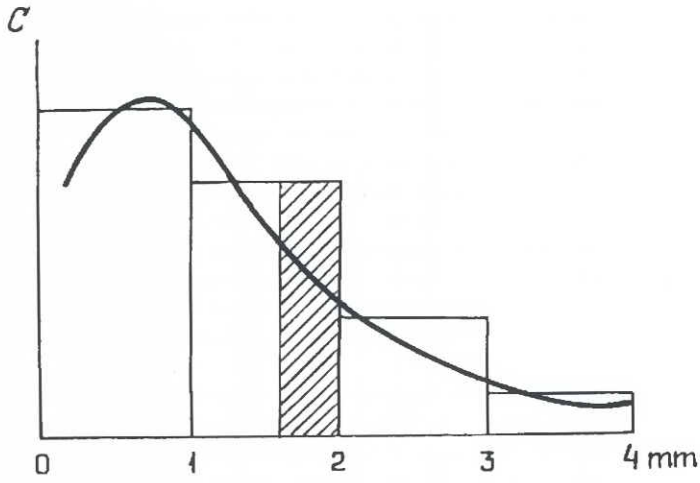

Fig. 9. Variation in protein concentration (C) along a root tip of a maize seedling. *The curve indicates the fluorescence intensity of slides stained with Procion yellow 4RS. The columns indicate biochemically determined protein concentrations on a wet weight basis. Dashed columns indicates the root region, in which the relative growth rate increases several times. (Ivanov, 1994).*

In the meristem, the cells, terminated their last mitoses, continue to grow but at low k. In these cells, the relative volume of the vacuolar system increases while the basophilia of cytoplasm declines. Possibly, their low growth rate results from the slow growth of most meristematic cells. The cells grow symplastically, and the transition to the accelerated growth takes place at the same distance from the root tip in all cell files. There can be little doubt that the mechanisms of the extension of meristematic and elongating cells are different as they essentially vary in the rate. Some chemicals and phytohormones exert various effects on the extension of meristematic and elongating cells, for example, IAA (Burstrom, 1957). These data characterize elongation as a distinct phase of cell growth, different from the extension of the meristematic cells. The transition of cells to elongation cannot be considered as the continuation of the meristematic growth after the cessation of divisions.

Rates of protein accumulation along the root axis, with respect to the cell transition to elongation

Jensen (1958) was the first to measure the number of cells and protein content in subsequent 250-μm root slices and calculate the average content of protein per cell (m) along the meristem in *Vicia faba* and *Allium cepa* roots. Some authors (Brown and Broadbent, 1950; Obroucheva, 1965; Khavkin, 1977) determined m along the elongation zone using a similar procedure. Data on the mean content of proteins per cell and mean concentration of proteins per volume (C) permit us to calculate how the rates of protein accumulation per cell (p) or per volume unit (b) change along the root if we compare them with the values characterizing the rates of cell growth and proliferation. The value p characterizes the difference between the rates of protein synthesis and breakdown. At the L distance from the root tip, p value equals to:

$$p = \frac{dm}{dL} V + xm, \quad (1)$$

where V is the rate of cell displacement from the root tip along root axis to distance L from the root tip, m is the average amount of protein per cell, and x is the relative rate of cell proliferation. In the meristem, x sharply increases above the quiescent center and gradually decreases in the basal half of the meristem (Erickson and Sax, 1956; Balodis and Ivanov, 1970). According to Jensen (1958) (Fig. 7), m increased nearly twofold from the quiescent center toward the middle of meristem. Along the basal half of meristem, m either decreased with the distance from the root tip (in *Vicia faba*) or remained unchanged (in *Allium cepa*). Hence, p increases with the distance from the root tip in the apical half of the meristem but decreases in its basal half.

In pea roots, m increases linearly along the most part of the elongation zone, but can decrease by its end (Fig. 8). From (1) it is clear that p increases with the distance from the root tip along the elongation zone up to the points where m begins to decrease. The average m value in fully elongated cells is three to five times that in the meristematic ones. However, the elongation time in these roots was shorter than the duration of mitotic cycles (Obroucheva, 1965; Ivanov, 1974). Hence the average p values in elongating cells are higher than in the meristematic ones. It follows from the m changes along the root axis that there are two regions of active protein accumulation in the growing region of root (Ivanov, 1974, 1981, 1994). The first is located in the middle of meristem and the second corresponds to the elongation zone. Between these regions, the protein accumulation is much slower.

A similar conclusion follows from the changes in protein concentration per unit volume (C) along the root axis estimated by biochemical and histochemical methods (Ivanov, 1994) (Fig. 9). C has maximum at a short distance above the quiescent center and then decreased.

The rate of protein accumulation in a unit of volume (b) can be calculated from (Ivanov, 1994):

$$b = C \frac{dv}{dL} V + \frac{dc}{dL} \quad (2)$$

where V is the rate of cell displacement from the root tip to the distance L; dv/dL is relative growth rate at the distance L from the root tip (Ivanov, 1994). A similar equation was used earlier (Erickson and Goddard, 1951; Erickson and Sax, 1956; Silk and Erickson, 1980). b sharply increased above the quiescent center to the region with maximum C because both C and V=kL increased. After C peaked, b decreased with the distance from the root tip because C decreased (dc/dL<0), k remained constant but V increased. However, at the borderline between the meristem and elongation zone, k sharply increased whereas C decreased slowly. Therefore, b rose although C declined as a result of cell expansion. If b is constant, C will decrease more markedly. Therefore, we showed the presence of two regions of active protein accumulation in the root tip and of an intermediate region between them with slow protein accumulation.

The data presented by Clowes (1958) were based on incorporation of a labeled amino acid into proteins on the sections along the root axes. He recorded the first peak near the quiescent center and the second peak in the apical part of the elongation zone, which agrees with our conclusion. However, exact interpretation of the historadiographic results is difficult because it is not known

how the concentrations of uptaken labeled amino acids and pool sizes of endogenous amino acids change along the root axes.

Erickson and Goddard (1951) and Silk and Erickson (1980) described a peak of relative elemental rate of protein accumulation located about 1.5-2.0 mm from the maize root tip. However, they suggested that k increased markedly along the meristem. This can explain the discordance with my estimation.

In the basal part of the meristem, p decreases but the accumulation of starch and lipids increases (Ivanov, 1981; Baluska *et al.*, 1990). It is more prominent in parenchyma, in which the mitotic activity stops closer to the root tip than in pericycle and rhizodermis. After the start of elongation, the amount of starch and lipids decreases sharply.

Thus, in the growing root tip there are two regions of more active accumulation of proteins. The first is associated with the fastest cell proliferation, the second corresponds to the enhanced cell elongation. The sets of synthesized proteins appear to be different in these regions as follows from dissimilar relative activities of various enzymes (Brown, 1963; Khavkin, 1977) and the experiments with transgenic plants (Masson *et al.*, 1993).

It is of interest that p and b rise after the cells started elongation. The activation of protein accumulation does not precede the transition to elongation. Therefore, it is likely that the transition of cells to elongation is not due to higher p and b. This conclusion was supported by the findings that inhibitors of protein synthesis did not affect directly cell transition to elongation (Ivanov, 1994). Apparently, this transition is related to a newly developed ability of the cell to absorb water and modify the cell wall extensibility.

In conclusion, it is necessary to discuss the metabolic pattern of PIG region. Baluska *et al.* (1995) concluded that «cell growth in both the meristem and the PIG region appears to be accompanied by an increase in cytoplasm, whereas this ceases to be prominent in the elongation zone where vacuolation predominates». This was inferred «from inspection of data on content of dry matter or protein published much earlier». However, the authors did not consider or compare the simultaneous changes in protein content (or concentration) and k. Our analysis has shown that PIG region is not uniform with respect to the rate of protein accumulation, at least in some tissues, because k increases sharply at some distance from the apical boundary of PIG region, whereas p decreases up to the level, at which k rises, then p sharply increases. The cells in a lower part of PIG accumulate the proteins more slowly than the apically located meristematic cells. The basal cells of PIG synthesize proteins at a higher rate than the meristematic cells.

There are some specific physiological properties of PIG cells differing them from meristematic and elongating cells (Baluska *et al.*, 1995; Ishikawa and Evans, 1995). However it is unknown whether this properties are typical of all PIG cells and how they differ in PIG cells, especially prior to k rise and after it.

Interrelations between proliferation and cell transition to elongation

In the roots growing at constant rate, the number of meristematic and elongating cells does not change or changes moderately. The descendants of the initial meristematic cells leave the meristem after completing several (n) mitotic cycles and begin to elongate. If the number of meristematic cells remains unchanged, p remains constant too. In these roots, the rates of cell multiplication and their transition to elongation are equal.

In any case when cell proliferation is retarded, the number of meristematic cells and the rate of their transition to elongation decrease (Ivanov, 1994). The cells begin to elongate after a lesser number of mitotic cycles than in normal roots. Hence n can vary and regulation of the cell transition to elongation is not based on «counting» the number of mitotic cycles by the cell.

A fundamental and most challenging problem in elucidation of the meristem organization is to clarify the mechanism of deceleration of cell transition to elongation as the cell proliferation slows down. In order to analyze various effects on transition of the meristematic cells to elongation, I proposed to determine the life-span of cells in the meristem (Tm) (Ivanov, 1968, 1974, 1981). In the steady-state growing roots, the duration of mitotic cycles along the meristem remains the same above the quiescent centre. In such roots, the cells in the basal half of the meristem and all cells derived from them begin to elongate during one mitotic cycle; due to cell proliferation of cells in the upper half of the remained meristem this basal half of the meristem is restored and so on. The life-span of cells in the meristem decreases exponentially along the meristem from the root cap to the boundary with the elongation zone.

In the roots treated with various inhibitors, the rate of cell transition to elongation can be altered due either to shortening (or lengthening) the life-span of cells in the meristem, or to inhibiting (or stimulating) cell division. If the life-span prolongs, a smaller portion of the meristematic cells leaves the meristem, and vice versa.

Haber and his colleagues have shown that cell differentiation and elongation may proceed without any mitoses in the roots of seedlings from the seeds irradiated by X-rays in high doses (for review see Haber, 1968). We studied the root growth after the direct X-irradiation of 2-day-old seedlings in high doses (Ivanov, 1968, 1974, 1981, 1994). Although no cell division occur, the irradiated roots continued to grow for some days due to the elongation of preexisted cells. The fully elongated cells reached almost the same length as in the unirradiated roots. Not all meristematic cells begin to elongate immediately after the irradiation, being unable to divide (Ivanov, 1968). The growing part of irradiated roots consisted of two zones until the root growth cessation: in the apical one the cell length changed slightly along the root axis while in the basal zone it sharply increased. We can define them as a «meristem lacking cell division» and as an elongation zone, in which the cells extend much faster than in the former. With time, the cells in the first zone gradually lengthened at somewhat lower relative rate than in control roots, whereas the number of cells in this zone decreased exponentially. In a time period equal to one mitotic cycle, their number halved, in a time period equal to two mitotic cycles, it diminished four-fold, etc.. Only a small, the most apical portion of meristematic cells did not begin their elongation. Thus, irradiation inhibits the rate of cell transition to elongation only by cessation of mitoses but does not affect on the life-span of cells in the meristem, at least of most part of meristematic cells. If the life-span of cells in the meristem remains unchanged after the irradiation it is possible to calculate the ratio between the length increment of control and irradiated roots. The results of such calculation were close to measured ratios. In maize root, the mitotic cycle lasts for 10h at 27°C. At constant cell life-span in the meristem, 80% of meristematic cells begin to elongate within 24 h: basal half, second quarter and part second eighth. In unirradiated root, the number of cells beginning to elongate is higher since the cells divide. During one

mitotic cycle, the number of cells beginning to elongate is equal 70% of total number of meristematic cells (Nm) (basal half and cells descending from them). Therefore, the number of cells beginning to elongate within 24 h is equal to 1.68 Nm, since 2.4 of mitotic cycle passed during 24 h. In roots irradiated at 100 Gy, only 0.8 Nm of cells begins to elongate because cell divisions ceased. The irradiation does not affect practically elongation. Therefore, the ratio of root length increment in control roots to that in irradiated (100 Gy) roots must be close to 2 (1.68/0.8). The measured value really was 1.96 (Ivanov, 1994).

The processes determining the growth duration in the irradiated roots as well as the transition to elongation commences are highly stable towards to metabolic inhibitors and X-irradiation in very high doses (to 8000 Gy), despite the fact that the fully elongated cells are shorter as a result of various metabolic damages to the cells (Ivanov, 1981, 1994) .

The results of these experiments suggest that cell the commencement of transition to elongation is independent of cell proliferation and is regulated by the processes determining the life-span of cells in the meristem. The rate of the cell transition to elongation (number of meristematic cells beginning the elongation per time unit) is under dual control: it is regulated by the processes determining the life-span of cells in the meristem (Tm) and the rate of cell proliferation (Ivanov, 1981, 1994). For most meristematic cells, Tm remains unchanged upon root treatments with various inhibitors but decreases at elevated temperature (Ivanov, 1981, 1994). The causes of such stability in terms of biochemical events regulating cell clock operation and k increase at meristem-elongation boundary are yet unknown. It is important that for periodical processes in plants and animals, duration of each period is independent of various chemicals (Bunning, 1958). The hypothesis of dual control of cell transition to elongation permits us to explain many facts observed upon diverse root treatment.

The growth-inhibiting effects of cytostatics and X-irradiation sharply enhance with time. This phenomenon is not due to slow inhibitor penetration into cells. It may be explained by a reduced relative rate of cell production, although the life-span in the meristem remained unchanged, at least most meristematic cells. The direct action of cytostatics or X-irradiation on the cell elongation is insignificant. At these concentrations or doses, they do not affect the growth of plant organs or their parts lacking mitotic divisions. Also, cytostatics do not affect the growth of X-irradiated plants, in which mitoses are prevented (Ivanov, 1994).

At the constant life-span of meristematic cells, it is possible to calculate from the data on mitotic index how the rate of cell transition to elongation changes with time. I made such calculations for roots treated with chloramphenicol (50 μg/ml). The calculated and observed data were in close agreement, thus supporting the above hypothesis (Ivanov, 1994). If cell proliferation and transition to elongation are regulated independently, any retardation of cell proliferation will automatically result in deceleration of cell transition to elongation. If no deceleration would occur, the root meristem would be soon exhausted because, during one mitotic cycle, 70% Nm are shifted to elongation zone. Deceleration of the cell transition to elongation can prevent the exhaustion of the meristem.

The rate of the cell transition to elongation could be governed by the concentration of hypothetical substance produced by meristematic cells. The decapitation of roots does not affect the rate of cell transition to elongation (for example, Van't-Hof, 1966; Ivanov, 1994). Apparently, the rate of cell transition to elongation does not depend on the number of cells in meristem.

To summarize, the patterns of meristematic cell transition to elongation in the roots have much in common with the cell kinetics in mammalian tissues characterized by prolonged cell proliferation, e.g. bone marrow or intestinal epithelium. The stem cells are capable of proliferating during the whole lifetime. Their descendants complete several cycles of cell divisions and then start to differentiate. If cell proliferation is suppressed by X-irradiation or cytostatic drugs, the cells pass to differentiation after completing fewer number of mitotic cycles. Just like in roots, the number of cells in a proliferating compartment exponentially decreases after irradiation, but their life-span remains unchanged (Bond *et al.*, 1965). Therefore, independence of cell transition to differentiation from the number of preceding mitotic cycles is inherent in many biological systems.

Restoration of mitotic activity results from the activation of proliferation of the stem cells in animals and of quiescent center in root (Clowes, 1963). The quiescent center is considered to be similar to animal stem cells (Ivanov, 1974, 1981, 1987; Barlow, 1976). However, in contrast to animal stem cells, the quiescent center can be regenerated from actively proliferating cells (Feldman, 1976), even after repeated decapitation of roots (Ivanov and Larina, 1983).

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