PROPERTIES OF CELLS IN THE ROOT APEX [1]

BY PETER W. BARLOW

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SUMMARY. — This article discusses properties of cells root apices, especially those of *Zea mays*.

1) Rates of cell division throughout the meristem of *Zea* roots were determined from a pulse labelling experiment, the results of which were analysed according to the method of Macdonald (1970). The durations of the mitotic cycle in epidermis, cortex and stele regions of the meristem have a similar value of about 18 hrs, but towards the tip of the root the duration of the cycle is slower (25-35 hrs). Cells of the quiescent centre have the slowest average rate of division (162 hrs), though not all the cells here may divide.

2) Rates of RNA synthesis in the meristem are discussed together with some of the difficulties in interpreting experiments where radioactive precursors of nucleic acids are fed to a dividing cell population. The quiescent centre has the lowest rate of synthesis of any of the regions studied. Some other properties, such as size and RNA content, of nucleoli within the root are also consistent with the low metabolic activity of the quiescent centre.

3) Cells of the quiescent centre divide infrequently, grow little in size and have low rates of nucleic acid and protein synthesis. The theories that could account for the quiescence of these cells, such as starvation of nutrients, or retention of the dormant state that they possessed in the seed, are critically examined and found to be unsatisfactory. A new theory is put forward which proposes that the quiescence of these cells is the result of antagonistic directions of cell growth. Cells at the pole of the epidermal lineage are locked into a state of non-growth by the cells of the cap columella initials whose direction of growth is at right angles to the former cells. These non-growing epidermal cells in turn prevent growth of cells at the pole of the cortex which, in their turn, lock cells at the stelar pole into a non-growing state. Thus, a hemisphere (at least in *Zea*) of non-growing cells — the quiescent centre — arises. Implicit in this theory is the idea that cell growth controls division.

4) In the meristem the rate of cell growth is greater in more basally located cells than in those more proximal while the rates of cell division and metabo-

ïism remain constant in both regions. This disharmony of growth and division means that synthesis of factors required for division cannot reach the critical concentration neccessary for mitotic functions in the cell. This provides an explanation for the cessation of mitotic division and the occurence of polyploid cells and other departures from normal mitosis found in maturing cells of the apex.

5) One neglected aspect of differentiation is briefly mentioned : it is the role of cell-to-cell communication in maintaining a particular state of differentiation. It is proposed that if the degree of cell communication changes during the life of a cell so the properties of the cell will change. An example is given of the changes that occur in the cells of the root cap with respect to intercellular communication and changes in cellular physiology.

RESUMEN. — **Propiedades de las células en el ápice de la raíz**, por Peter W. Barlow. — En este artículo se discuten las propiedades de las células en los ápices radicales, con especial referencia a los de *Zea mays*.

1) Por medio de experimentos de «pulsos marcados» se determinó, en raíces de *Zea*, la velocidad de división a través del meristema, analizando los resultados según el método de MacDonald (1970). Se encontró que la duración de los ciclos mitóticos en la epidermis, la corteza y la estela poseen un valor similar, de aproximadamente 18 horas, mientras que su duración es mayor hacia el extremo del ápice (25-35 hs.). Las divisiones más lentas (162 horas) corresponden a las células del centro quiescente, si bien puede que no todas ellas se dividan.

2) Se discute la velocidad de síntesis de RNA en los meristemas con relación a algunas de las dificultades que se encuentran al interpretar los resultados de experimentos realizados con precursores de ácidos nucleicos radioactivos, los cuales se suministran a poblaciones de células en estado de división. De todas las regiones estudiadas el centro quiescente es el que posee la velocidad de síntesis más baja. Otras características, tales como el tamaño y contenido de RNA de los nucleolos, concuerdan con la baja actividad metabólica de los centros quiescentes.

3) Las células de los centros quiescentes se dividen con poca frecuencia, aumentan poco de tamaño y poseen una lenta síntesis de ácidos nucleicos y proteínas. Se examinan críticamente las teorías que podrían explicar la quiescencia de sus células, tales como el agotamiento de nutrientes o la retención del estado de dormición que poseen en las semillas, llegándose a la conclusión que no son satisfactorias. Se propone una teoría que considera que la quiescencia de las células es el resultado de la dirección antagónica de su crecimiento. Las células en el polo del «linaje» epidérmico están constreñidas a un estado de reposo por las células iniciales de la columela de la caliptra que crecen en ángulo recto con respecto a las primeras. Estas células epidérmicas en reposo impiden a la vez el crecimiento en el polo de la corteza, la cual bloquea de igual manera las células del polo estelar induciéndolas a un estado de reposo. Así, se produce una hemisfera con células que no crecen — el centro quiescente — por lo menos en *Zea*. Está implícito en esta teoría la idea de que el crecimiento celular controla la división.

4) En los meristemas, la velocidad de crecimiento celular es mayor en los elementos ubicados en la base que en aquellos más proximales, mientras que la velocidad de división y el metabolismo permanecen constantes en ambas regiones. Esta desarmonía entre el crecimiento y la división significa que la síntesis de factores requeridos para la última no pueden alcanzar la concentración crítica necesaria para las funciones mitóticas de las células. Esto provee una explicación para el cese de la división mitótica, la presencia de células poliploides y otras desviaciones de la mitosis normal de las células del ápice que se encuentran en estado de maduración.

5) Se examina brevemente un aspecto al que no se le dió la debida importancia respecto a la diferenciación : es el papel que juegan la comunicación de célula a célula en mantener un estado particular de la diferenciación. Se propone que si cambia el grado de comunicación celular durante la vida de una célula, también cambian las propiedades de dicha célula. Se da un ejemplo de los cambios que se producen en la caliptra de la raíz con respecto a la comunicación intercelular y los cambios en la fisiología de éstas.

Roots grow by cell division and cell enlargement. The meristem of the root is the region where cell division and cell growth both occur, in the more mature region of the root divisional activity ceases and cell growth alone takes place. This latter region is the so-called zone of elongation. Cells in still older parts of the root contribute nothing to the growth in length of the root, though it is here that new meristematic zones appear when lateral root primordia and cambium activity are initiated. I wish to discuss some of the properties of the meristem and apex of roots with particular emphasis on those of *Zea mays*.

Zea mays is a favourable plant in which to study the activities of the root meristem as it has a well defined histological organization. In a median longitudinal section of a *Zea* root files of cells are obvious in the main body of the root; stele, cortex and epidermal tissues can be recognized as lineages of cells of similar size and staining properties. These cells would be considered to make up the "Körper" in the terminology of Schuepp (1917). The lineages converge to a central point at the pole of the Körper. In the cap there is a central cylinder of cells which divide predominantly transversely, with respect to the axis of the root; this is the columella. Around the columella are cells which divide more frequently in a longitudinal plane. These cells Schuepp would consider to be "Kappe". There is a thick cells wall which creates a permanent boundary between Körper and cap in *Zea* and other Gramineae, with their so-called 'closed' type of apical construction. But in

some other species, such as *Vicia* or *Fagus*, with roots of an 'open' construction, this boundary is not well defined. Nevertheless, in *Vicia* it is possible, with careful observation, to distinguish a thicker wall between what may be presumed to be cap columella cells and cells of the cortex — stele complex (Clowes, 1967 a). The cause of the difference between the open and closed type of root construction lies in the way in which cell divisions are orientated early in the development of the root apex, and this difference may be genetically determined.

By studying the geometry of cell patterns in normal roots and in roots recovering from excision of pieces from the apex, Clowes (1954) arrived at the conclusion that in *Zea* there was a hemispherical group of cells at the summit of the Körper, where the epidermis, cortex and stele cell lineages converged, that was not normally active in cell division. Clowes later (1956 a) reasoned that if this interpretation was correct then these cells should rarely, or not at all, synthesise DNA or undergo mitosis. In fact, Clowes was able to show that after allowing roots to incorporate radioactive precursors of DNA and then tracing the location of the radioactivity by autoradiography, there was indeed a group of cells, in exactly the position predicted, that have a much lower level of DNA synthesis than other cells in the meristem. This region of non-dividing cells Clowes called the "quiescent centre". A striking feature of the organization of the meristem is that cells surrounding the quiescent centre are actively dividing. One may ask how neighbouring populations of cells can behave so differently. I shall try to provide an answer to this question, but before doing so I want to describe mitotic activities in the meristem.

Cell Division: Clowes followed up his finding of a low rate of DNA synthesis in the quiescent centre by defining the rates of cell division there and elsewhere in the meristem. He used (Clowes, 1961 a & 1965 a) three techniques: the rate of accumulation of metaphases by colchicine, the rate of labelling of nuclei with tritiated thymidine (H^3-TdR, thymidine labelled with tritium is a specific radioactive precursor of DNA) and the kinetics of nuclei labelled with a pulse of H^3-TdR. All techniques confirmed that the cells of the quiescent centre had a low rate of division while cells in regions elsewhere in the meristem divided much faster. Only the pulse labelling tecnique gives any precise information about the duration of the component phases of the cell cycle: these

phases are called G_1, S, G_2 and Mitosis. G_1 is the period of interphase that follows mitosis (when the chromosomes and the cell divide) and preceeds the period when DNA is synthesised, this latter period is the S phase; G_2 follows the S phase and is terminated by mitosis (the original work defining these phases is by Howard and Pelc, 1953). The pulse labelling technique was devised by Quastler and Sherman (1959) to study the cell cycle in cells of mouse and was first used with plant material by Wimber (1960). The procedure is to place growing tissue (e.g. roots) into a medium with H^3-TdR for a short period, or pulse, and then return it to a medium free of H^3-TdR. During the pulse H^3-TdR is taken up into the nuclear DNA of cells in the S phase only, the cells that have incorporated the isotope can then be detected by autoradiography. If roots are fixed at different times following the pulse and mitoses examined for the presence or absence of isotope labelling, it is found that immediately, and for a short time after the pulse, no mitoses are radioactive. These mitoses are derived from cells that were in G_2 at the time of the pulse and subsequently entered mitosis. Later, the proportion of labelled mitoses rises as cells in S at the time of the pulse now enter mitosis, later still this proportion falls as their place is taken by cells that have come from G_1. The proportion of labelled mitoses subsequently rises for a second time as the originally labelled S phase cells come into mitosis for a second time. By following the sequential appearance and disappearence of labelled mitoses we can estimate the duration of the G_2, S, and G_1 phases, as well as the total cycle time, T. The duration of mitosis, M, can be estimated from a knowledge of the mitotic index and the duration of T. But the analysis of pulse labelling data is not easy. Wimber (1960 & 1963) describes a method of analysis which is based on a theoretical expectation of the way in which the proportion of the labelled cells population in mitoses should change with time. However, not all populations behave in the way expected because of the great variability that exists between cells in the duration of their cell cycle and its component phases. As a consequence many workers have been lead to adopt arbitrary methods to analyse their data (see Mendelsohn and Takahashi, 1971 for a critique). To overcome this arbitrary approach, my colleague Peter Macdonald of the Biomathematics Department in Oxford, devised a theoretical model to accomodate all the known variabilities in cell cycles together with an accompanying computer programme that automatically analyses

TABLE 1

The lenght of the mitotic cycle and its component phases in different regions of the meristem. Standard errors are included, although in the case of the values for M the error is standard deviation.

Region	T	G₁	S	G₂	M
Cap Columella initials	14.1±0.6	0.8±0.6	7.2±0.7	4.6±0.6	1.4±0.1
Cap periphery.......	∼20±32	∼6.2	6.6±1.0	5.8±1.6	∼1.4±0.4
Quiescent Centre....	162.0	126.1	20.7	11.0	4.2
Stele just above Q.C.	15.2	2.5	6.3	5.1	1.3
Stele 200 μm *	19.6±0.6	7.7±0.6	6.0±0.3	2.4±0.2	3.5±0.8
Stele 400 μm........	18.3±0.8	6.4±0.7	5.5±0.3	3.0±0.2	3.4±0.7
Stele 700 μm........	19.4±1.1	9.6±1.1	3.7±0.3	3.3±0.3	2.8±0.5
Central Stele 900-1000 μm	17.1±0.7	6.9±0.9	4.7±0.4	4.0±0.3	1.5±0.1
Peripheral Stele 900-1000 μm..........	16.6±0.5	6.7±0.5	5.4±0.2	2.7±0.2	1.8±0.2
Cortex just above QC	∼35	∼17.4	10.2	5.4±0.7	∼ 2
Cortex 200 μm	∼26	∼15.9	4.2	4.1	∼1.8
Cortex 400 μm	23.8±1.3	12.6±1.3	4.3±0.4	4.2±0.5	2.8±0.5
Cortex 700 μm	18.9±0.8	9.1±0.9	3.5±0.3	3.9±0.4	2.4±0.4
Cortex 1000 μm	17.8	8.9	3.6	3.6	1.7
Epidermis 200 μm...	∼26	∼16	∼4.2	∼5.0	
Epidermis 700 μm...	∼19	∼9	∼3.5	∼4	
Epidermis 1000 μm..	∼19.8	∼10.9	∼3.6	∼3.6	

* Refers to distance from Cap-Quiescent Centre boundary.

pulse labelling data. All that is required for the analysis is the number of labelled and unlabelled mitoses at various times after the start of the pulse, the labelling period and the mitotic index. This theoretical work is now published (Macdonald, 1970) and has been used to estimate parameters of the cells cycle in various regions of the meristem of *Zea* using data I obtained from a pulse labelling experiment in 1967. Table 1 shows these estimates. The results corroborate Clowes' (1965 a) findings concerning the relative rates of division and the durations of the periods of interphase. The table also gives estimates of cell cycles in more or less the whole meristematic region of the root.

Certain assumptions have been made in handling these data. These are: 1) The Growth Fraction (i.e. the proportion of the total cell population engaged in the mitotic cycle is equal to 1.

2) The distribution of phase durations is normal.

3) The labelling period is equal to the time the roots were allowed to grow in H^3-TdR.

In practise small deviations from these assumptions fortunately make little difference to the final computed result. In fact, data exists for the growth fraction for four regions of the maize root. The data are that of Clowes (1971) and are shown in Table 2. A feature of interest is that, at least in the cap initials and stele just above the quiescent centre, not all the cells are taking part in the mitotic cycle. In the quiescent centre itself just under half the cells are cycling, but the fastest dividing cells are doing so about six times faster than the average cell. No doubt if a growth fraction could be obtained in more basal regions of the meristem the growth fraction would be found to decrease as the limit of meristematic activity is approached.

TABLE 2

Duration of division and Grow Fraction in four regions of the meristem
(taken from Clowes. 1971)

Region	Cycle Time (hrs)		Growth Fraction
	Average	Minimum	
Cap Initials...............	16	10	0.86
Quiescent Centre....................	231	40	0.44
Stele just above Q. C........	25	14	0.82
Stele 200 μm from Cap-Q. C. boundary.....	18	14	0.99

The different choices opened to us concerning the distribution of the phase durations, are that they are normally distributed, or have a gamma distribution, or a log-normal distribution. Table 3 shows the differences that such a choice can make, the differences are expressed more in the variation of phase length than in the mean phase length itself. In plant cells there is no evidence that allows us to prefer any one type of distribution; however, in animal cells, yeast and bacteria there is some evidence that speaks against the duration of the cell cycle, and some of its component phases, having a normal distribution.

TABLE 3

Durations of phases of the cell cycle in the Cortex 700 µm from the Cap-Quiescent Centre boundary computed using different distributions of phase lengths.

Distribution	Duration (hours)					
	G_2	$\sigma\ G_2$	$S + G_2$	$\sigma\ (S + G_2)$	T	σ T
Normal.......	3.90	1.79	7.39	10.4	18.9	19.5
Gamma.......	4.62	3.15	8.03	7.40	19.6	23.9
Log-Normal...	5.48	5.86	8.98	7.63	19.4	30.3

The third assumption concerning the time that label is really available to the nucleus may be important in some situations, such as developing root primordia (see MacLeod and Davidson, 1970). If there is an endogenous pool of precursors into which the isotope can enter before being utilized in S phase, then the S phase will appear to be longer than it actually is. The true length of S will be the apparent length minus the actual duration of labelling. I have found some evidence that may suggest that such a pool exists in maize roots. The reasoning is this: Table 1 shows that within the more basal regions of the meristem the length of the S phase seems to be fairly constant until a more apical position is reached about 200 µm from the quiescent centre; here and in the quiescent centre the duration of S increases. In the cap initials S is shorter than in the quiescent centre. We would expect that if H^3-TdR goes directly into the nuclear DNA there would be a higher number of silver grains in autoradiographs of a nucleus with a high rate of DNA synthesis than over a nucleus with a low rate of DNA synthesis. In other words, we would expect more grains over a nucleus at, for instance, 700 µm in the stele, than over a nucleus in the quiescent centre. But rather the reverse is found (Table 4) : in roots fixed immediately after a half-hour labelling period there are more grains over the quiescent centre nuclei than over nuclei in the stele at 700 µm. This suggests that in the more basal cells of the stele there may be a larger pool of endogenous DNA precursors that dilutes the exogenously supplied H^3-TdR so accounting for the lower grain count here than in other regions. However, other interpreta-

TABLE 4

The number of silver grains over labelled nuclei in different regions of the meristem. A nucleus was considered labelled if it had three or more grains. Counts were made on sections of roots cut a 6 μm. The labelling period was 0.5 hr with 0.5 μCi / ml H³-TdR, sp. act. 5 Ci / mM.

Region	N° Silver Grains
Cap Columella Initials	27.3
Quiescent Centre	16.9
Stele just above Q. C	19.5
Stele 400 μm from Cap-Q. C. boundary	11.3
Stele 700 μm from Cap-Q. C. boundary	10.5
Cortex just above Q. C	15.0
Cortex 700 μm from Cap-Q. C. boundary	12.6

tions could be advanced such as a differential penetration of thymidine during the half-hour labelling period. Clearly, more data is required to resolve this problem. But data about pools of metabolites is difficult to obtain experimentally (see Oaks and Bidwell, 1970, for a discussion), although Woodard, Rasch and Swift (1961) have presented evidence that suggests DNA precursor pools may exist in primary roots of *Vicia faba*.

Cells of the root cap meristem show an interesting feature that may have a bearing on their fast rate of division and their extreme sensitivity to radiation. I show in Table 1 that the G_1 phase in the cap columella initials is very short —less than one hour. Clowes (1965 a) gives an even shorter value, indeed so short that the value is a negative one of minus one hour. Although Clowes and I differ in our handling of our pulse labelling data, I believe that negative values for G_1 are real. The reason for a negative value is that G_1 does not exist; rather, DNA synthesis commences before cell division is complete. Both Clowes (1967 b) and I find that after a short labelling period with H^3-TdR, telophase chromatids are labelled, and a close examination of these chromatids shows that label is confined to regions where the chromatin has become locally more disperse. DNA synthesis in telophase is known in other organisms, especially where cell division is very rapid, such as the neuroblast in the embryo of the grasshopper, *Chortophaga* (Gaulden, 1956), or in the early divisions of sea-urchin embryos (Hinegardner, Rao and Feld-

man, 1964). Clowes (1965 a, b) believes that the lack of a G_1 phase accounts for the great sensitivity to radiation of *Zea*, of the cap initials; on the other hand, quiescent centre cells with a long G_1 phase, are relatively much more resistent. However, the duration of G_1 may not be the only reason for differences in radiation sensitivity, as, in roots of *Allium sativum*, the cap initials are also very sensitive to X-rays though they possess a G_1 phase of appreciable duration (Thompson and Clowes, 1968).

In the columella of the cap, divisions are confined to the four or five tiers of cells distal to the thick wall separating the cap from the root proper. When division of these cells ceases as they more into the centre of the cap, their nuclei are held at the 2C value of DNA (characteristic of a diploid G_1 cell) as shown by microdensitometry by Clowes (1968) and myself (Barlow, 1969 a). Continuous mitotic activity of the initials pushes these cells forward to the outside of the cap where they are sloughed off. Meanwhile, the nuclei undergo further DNA synthesis to reach a 4C or 8C level of DNA. It is known that the life span of a cap cell is about 36 hours (Juniper, et al. 1966), so to reach an 8C level of DNA in this time these cells must be undergoing an endoreduplication cycle of about 12 hours; in other words, all cell cycle activities, except mitosis, go on at much the same rate as in the mitotic initial cell.

Other Metabolic Activities of the Root Apex. Besides differences in the rates of cell division, the cells of the root apex show differences in the rate of uptake of precursors into protein and RNA (Clowes 1958, Barlow 1970 a). The quiescent centre shows the lowest rate while cells in surrounding regions have higher rates. Probably the rate of cell division is related to, or even depends on, the general rate of cellular metabolism. For this reason studies of cellular structure and metabolism could lead to an explanation of differences in meristematic activity within the apex.

Clowes (1956 b) showed that after staining sections of *Zea* roots with pyronin, an RNA specific stain, the quiescent centre region was evident as a region of low staining intensity. I have extended this observation and I have translated the subjective impression of differential staining into a semi-quantitative estimate of RNA content (Barlow 1969 b). To do this root sections were stained with Azure B, another RNA specific stain. The amount of stain bound to cell structures bears a quantitative relation to the amount of RNA present. The staining intensity of the Azure B-RNA complex

TABLE 5

Measurements of relative RNA content in nucleolus and cells in different regions of the apex. The values are arbitrary units and the values given for nucleolar and cytoplasmic staining must not be compared ; they are independent measurements and only show the relative amount of stain in cytoplasm or nucleolus when compared with the amount of stain in these structures in quiescent centre cells. Cytoplasmic staining values were made on 5 μm sections using a measuring area of 10 μm diameter and are averages of one measurement made at the basal end of a cell and one made at the apical end. The figures of nucleolar volume were computed from measurements of diameters and have been corrected for errors introduced by making such measurements on sectioned material (see Barlow, 1969 b).

Region	Nucleolus			Cytoplasm (absorption/μm³)
	Volume μm³	RNA content	Density (absorption/μm³)	
Mature Outer cap..	6.31±0.41	1.08±0.05	0.171±0.014	0.95
Cap Columella Initials	8.49±0.38	1.22±0.04	0.156±0.009	1.08
Quiescent Centre.....	5.78±0.30	1.03±0.04	0.178±0.011	1.00
Stele just above Q.C..	9.87±0.37	1.70±0.04	0.172±0.008	1.04
Stele 100 μm from Cap- Q.C. boundary.....				1.28
Stele 150 μm				1.50
Stele 200 μm	15.1 ±0.63	2.35±0.07	0.152±0.008	
Stele 250 μm				1.83
Stele 400 μm	16.54±0.56	2.38±0.06	0.144±0.006	1.66
Stele 1000 μm	18.30±0.94	2.58±0.08	0.141±0.008	

in the nucleolus of mononucleolate nuclei was measured by micro-densitometry in different regions of the stele and cap of *Zea*. The results are shown in Table 5. It is clear that the quiescent centre cells have smaller and less intensely stained nucleoli than are found in nuclei of cells elsewhere. When the amount of RNA per unit volume of nucleolus is calculated quiescent centre nucleoli seem to be the most dense with respect to RNA. I believe that density could be a measure of RNA synthetic activity as well as being an indicator of nucleolar structure. Regarding this latter point, Hyde (1967) has found that in *Pisum sativum*, nucleoli of the quiescent centre have a more tightly aggregated structure than nucleoli elsewhere. If all the nucleolar material takes up Azure B stain, then this

finding could account for the high RNA density found in nucleoli of the quiescent centre. Further, in cells of tubers of *Helianthus tuberosus* Fowke and Setterfield (1968) have shown that as cells pass from a state of metabolic inactivity to one of activity, so nucleolar ultrastructure changes from a state where the fibrillar component is densely packed to one where the fibrils are more loosely dispersed and acquire an accompanying granular component. At the same time nucleolar vacuoles make their appearance; I shall discuss these structures later. The size of the nucleolus is thought to be an indicator of nucleolar activity (Stich, 1959). In general this may be true, although within the maize root the nucleolus of the mature cap cell is about as small as a nucleolus in the quiescent centre, but the former is far more active in RNA synthesis than the latter. I find rather a better correlation between nucleolar density and RNA synthesis than between density and either RNA content or volume (Barlow, 1970 a). The size of a nucleolus probably depends on the state of the DNA template for RNA synthesis within the nucleolus, and also the relation between the rate of RNA synthesis and its release from the nucleolus. That changes in these relations occur in the meristem is indicated by the fact that as cells pass from apical to more basal regions of the meristem during growth so nucleolar volume increases (Table 5).

Measurements of cytoplasmic staining shows that quiescent centre cells have less RNA per unit volume of cytoplasm than other cells in the meristem (Table 5). Pilet and Lance-Nougarède (1965) have shown that in roots of *Lens culinaris* this is due to the fewer number of ribosomes in the cytoplasm of the quiescent centre than elsewhere. *Zea* and *Pisum* meristematic cells show a difference in staining intensity between the basal and apical ends of the cell, the basal end being more strongly stained. The reason is that cell organelles, such as plastids, tend to lie at the apical end of the cell leaving more cytoplasmic ground substance at the other end. Because of the stoichiometric relation between RNA and Azure B it would be possible to obtain an absolute estimate of the quantity of RNA nucleolus and elsewhere. It would be interesting to do this, especially as the intensity of staining in cells changes with age and some experimental treatments such as X-irradiation (Barlow, unpublished).

To follow up these measurements of RNA content in cells of the root apex, I studied the rate of RNA synthesis in different regions

of the apex using H^3-5-uridine (H^3-UdR) as a specific marker of RNA, together with autoradiography (Barlow, 1970 a). As expected, the quiescent centre has a lower rate of synthesis than elsewhere in the meristem. The study also shows that cells of the cap periphery are active in RNA synthesis, a fact that had been denied by Himes (1966) and Fischer (1968). Indeed, it would have been surprising to find that these cells really are inactive in RNA synthesis as they are very active in polysaccharide synthesis, secreting the slime that covers the cap (Juniper and Roberts, 1966; Northcote, 1966 and Pickett-Heaps, 1966) and they are also growing rapidly in size. Although I made quantitative estimates of the degree of RNA synthetic activity in a number of different regions of the apex it is difficult to know exactly how to interpret the results of experiments of this nature. I allowed roots to grow in H^3-UdR and measured, by autoradiography, the amount of isotope uptake at different times over a total labelling period of 8 hours. The degree of RNA synthesis in the nucleolus can be measured by the rate of uptake and also the level of saturation obtained with the isotope. The difficulty in estimating these values is that the cells are in a dynamic state: during the labelling period some cells divide; at this time their nucleoli breakdown and release RNA into the cytoplasm and then, when the nucleoli reform at telophase, they resume RNA synthesis. Not all cells in the meristem go through this process with equal frequency, and then a further difficulty is that as cells go from G_1 to G_2 they probably acquire different RNA-synthetic capacities related to the amount of DNA template available. Furthermore, if endogenous precursor pools exist these could seriously influence the observed degree of labelling. Nevertheless, taking into account all these factors it is possible to conclude that the level of RNA synthesis in the quiescent centre is relatively much lower than would be expected for a cell in a G_1 state. It is possible that its RNA activity may be repressed in some way. Another point that should be considered is that although quiescent centre cells do show an appreciable rate of RNA synthesis their cytoplasmic RNA content is low. As the cells grow little in size and spend a long time in G_1 one may ask why RNA does not accumulate in the cytoplasm. May be the cells contain an RN'ase that is continually breaking down a constant fraction of the newly made RNA. Partial estimates of RN'ase activity have been made in roots of *Lens* by Pilet and Braun (1970) but a more detailed analysis is required to decide whether RN'ase is present in the quiescent centre.

TABLE 6

Characteristics of nucleolar vacuoles

Region	% nucleoli with vacuoles	Max. n° vacuoles	Av. n° vacuoles	Av. volume of a vacuole μm^3	% nucleolar vol. occupied by vacuoles
Mature Outer Cap Cells....	92	8	3.2	0.08	3.1
Cap Columella Initials.....	98	10	3.9	0.09	3.7
Quiescent Centre.........	63	5	1.1	0.05	1.7
Stele just above Q. C.......	88	7	2.6	0.10	3.2
Stele 200 μm from Cap-Q. C. boundary..............	100	12	6.1	0.08	2.9
Stele 400 μm from Cap-Q. C. boundary	100	13	6.6	0.12	3.2
Stele 1000 μm from Cap-Q. C. boundary..............	100	12	6.6	0.10	—

Within the nucleoli of *Zea* there are prominent vacuoles. Many properties of the vacuole are compatible with it being an indicator of nucleolar activity (reviewed by Barlow 1970 b). Infortunately there are few studies on the behaviour of vacuoles that definitely substantiate this idea. However, there is no doubt that the vacuole is a dynamic nucleolar organelle. In tobacco cells in culture Johnson and Jones (1967) have shown that the nucleolar vacuole has an expansion and contraction cycle with a period of about 1 to 2 hours, and that nucleoli lacking a vacuole incorporate less H^3-TdR than nucleoli that possess a vacuole. I have shown (Barlow, 1970 b) that in the root of *Zea* each nucleolus contains a variable number of vacuoles that is related to the size of the nucleolus: but the percentage volume of nucleolus occupied by vacuoles is always constant at about 3 percent, regardless of the rate of RNA synthesis in the nucleolus. In the quiescent centre the volume occupied by the vacuole is a little lower at 1.7 percent and further, 37 percent of the nucleoli here do not contain a vacuole at all. Table 6 summarises this data. It may be that the nucleoli without vacuoles are completely repressed in their metabolic activity and they could account for the disproportionately low average rate of RNA synthesis in the quiescent centre. Also, growth fraction data tell us that a similar percentage of cells, 56 percent, do not divide. Although the

TABLE 7

Percentage of cells with one or two nucleoli in different regions of the meristem. Where there are two nucleoli these may be either separate or appear to be fusing

Region	Percentage		
	Mononucleolate	Binucleolate	
		Separate	Fusing
Outer Cap	95	5	0
Central Cap	86	14	0
Cap Columella Initials	89	9	2
Quiescent Centre	85	15	0
Stele just above Q. C	83	15	2
Stele 400 μm from Cap-Q. C. boundary	85	11	4
Stele 1000 »	90	5	5
Cortex just above Q. C	91	6	3
Cortex 400 μm from Cap-Q. C. boundary	90	6	4
Cortex 1000 μm »	89	4	7
Epidermis 400 μm »	67	21	12
Epidermis 1000 μm »	66	29	5

The above figures refer to roots of 2-3 cm growing in destilled water. The percentage of binucleolate nuclei varies with age of the root. The figures below refer to roots growing in Sphagnum from the time the rootlet emerges from the seedling (0 hrs) to three day later.

Region	Percentage binucleolate nuclei at the following times from root emergence			
	0	24	48	72
Cap Columella Initials	6.9	9.7	11.0	11.0 hrs.
Quiescent Centre	7.8	11.3	11.4	13.2 hrs.

evidence is circumstancial, I think that the nucleolar vacuoles do tell us something about nucleolar activity. If it were possible to look directly into the living cells of the root we should probably find that in the different regions of the meristem the nucleolar vacuoles to be pulsating at different *rates* and this could be correlat-

ed with nucleolar RNA synthetic activity. Possibly the pulsation of vacuoles is the means whereby RNA in the nucleolus is moved into the nucleus and cytoplasm. An active discharge from the nucleolus would seem to be an easier way of liberating nucleolar products than a passive diffusion process. Further, I have shown (Barlow, 1970 a) that most, if not all, the RNA accumulating in the cytoplasm can be accounted for by synthesis in the nucleolus and this may be ribosomal RNA.

Nuclei of maize contain two nucleolar organizer chromosomes but usually only one nucleolus, but often two nucleoli can be seen. Table 7 shows the frequency of mono-and bi-nucleolate nuclei in different regions of the meristem and also how the frequencies of the two types of nuclei change with age. The difference in frequencies of the two types reflects changes in the spatial relations of the nucleolar organisers within the nucleus according to the age and position of the cells. In passing, it is interesting to note that the number of nucleoli in epidermal cells has been found to be different within the two taxonomic groups of the Gramineae (Lewis and Rothwell, 1964). In the Festucoid species nuclei have many nucleoli, by contrast, the Panicoid species often have only one nucleolus in each nucleus although occasionally two are found, as in *Andropogon hallii* which has 9 percent binucleolate nuclei. *Zea mays* is a Panicoid species and shows a greater percentage of binucleolate nuclei in the epidermis than any of the Panicoid species studied by Lewis and Rothwell.

Why is the Quiescent Centre Quiescent?

Clearly, cells of the quiescent centre have properties very different from the surrounding cells. How can we explain this? Two explanations have been put forward: the first is that the quiescent centre is starved of metabolites neccessary for cell growth and division (Clowes, 1961 a; Barlow 1969 c), the second is that the quiescent centre is in some way isolated from the surrounding cells (Juniper and Barlow 1969) and it is held in the dormant state it possessed in the seed.

The starvation theory is attractive. It is known that roots growing in water culture need materials from the endosperm for growth; so it may be that the quiescent centre is at the end of a supply line of these materials, most or all of which have been drained off into the more basal meristematic cells leaving too little for the cells of

the quiescent centre to use for cell division and growth. When other cells of the meristem are stopped from dividing, such as after exposure to X-irradiation, cells of the quiescent centre commence division because metabolites are no longer used by the damaged cells and so are now available for use by the quiescent centre. The weakness of the starvation theory is how to explain the meristematic activity in the cap. To fit into the theory the cap must have an independent nutrient supply perhaps provided by a recycling of breakdown products from senescent cells at the outside of the cap. Experiments by Rabideau and Mericle (1953) and by Clowes (1970) show that the cap has adequate access to materials supplied from the shoot or endosperm, and so the quiescent centre, lying directly in the path of supply, should have access to nutrients too. Thus, the starvation theory cannot be true unless the quiescent centre is isolated in some way from surrounding cells.

Cells could be isolated from one another only if there is no channel of communication between them. We know that cells are interconnected by submicroscopic channels called plasmodesmata, and there is evidence that both small ions and large macromolecules, can pass through them (see Clowes and Juniper, 1968; Juniper and Barlow, 1969). Could it be that there are no plasmodesmata between cells of the quiescent centre and their active neighbouring cells in the cap, cortex and stele? Juniper and I examined the frequency of plasmodesmata from electron micrographs of the cells walls in the meristem of *Zea* and we found plenty of plasmodesmata between the quiescent centre and stele, between the thick wall separating quiescent centre from cap initials, and between cells within the quiescent centre itself. If we assume that the plasmodesmata are all open and can act as cytoplasmic channels of intercellular communication then it is impossible for their distribution to be the cause of quiescence.

Perhaps a clue to the cause of quiescence can be found in the way in which the quiescent centre develops in the embryonic root. There is evidence that suggests that a quiescent centre develops early in embryogeny (Sterling, 1945, 1955; Rondet, 1965). Rondet (1961, 1965) has shown that in *Myosurus minimus*, when the embryo is only 50 μm in diameter, a group of cells with a relatively much lower RNA content than their neighbours is visible in the presumptive root apex; these cells are probably the future quiescent centre. In *Lens culinaris* and *Phaseolus vulgaris* the quiescent centre deve-

lops later in embryogeny. Rondet (1965) believes that the time of development of quiescence is in some way connected with the relation between the embryo and its suspensor, although what this relation is he does not say.

It may be that cells that were the quiescent centre in the embryo remain quiescent when the embryo reassumes growth upon seed germination. There is circumstancial evidence suggesting that this is not so. Clowes (1956 b) showed that in *Sinapis alba* the newly emerging root does not have a quiescent centre and this only develops as the root grows. A similar development of quiescence occurs in the emerging lateral roots of *Zea* (Clowes 1969), *Pistia stratiotes* and *Eichhornia crassipes* (Clowes 1956 b). However, in *Sinapis* it is not known if a quiescent centre develops during embryonic growth so it is not absolutely certain that the quiescent centre in the embryonic root resumes division upon germination. Another related finding is that of Byrne and Heimsch (1970) who show that in the course of germination of roots of *Malva sylvestris* the number of quiescent centre cells increases as the diameter of the root increases. Therefore it seems that quiescence is a state that is imposed upon some cells in the meristem as the root grows.

A Theory to Explain Quiescence

It is possible to devise purely mechanical explanations for quiescence. One such arises from a consideration of cells patterns. In *Zea*, cells of the cap columella divide transversely and grow in a longitudinal direction; cells of the epidermis file also divide transversely with respect to their lineage (i.e. anticlinally). At the apex of the Körper the direction of cells growth in the epidermis is at right angles to the direction of cells growth in the columella. This does not influence the growth of the columella cells, as little, if any, growth takes place over its transverse wall. But it is this wall the one that would grow in the epidermis. Because cells do not slip over one another but are stuck fast together, cell growth is prevented in the epidermis everywhere that these cells are in contact with columella cells. If the epidermal cells cannot grow in length then adherent cortical cells cannot grow either, and likewise stele cells that are in contact with cortex. Thus a group of nongrowing cells is located at the pole of the Körper and they cannot grow solely because of the antagonism between the directions of growth and division at the columella- epidermis boundary.

As these cells are in the quiescent centre it must be that the lack of cell growth prevents cell division. This mechanical explanation can be extended to explain a quiescent region in all roots showing a boundary between cells with their direction of cell growth at right angles to each other. Also we can explain why in *Zea* the quiescent centre divides when the cap is removed: it is because the cells antagonistic to epidermal cell elongation have been removed, so giving the cells of the quiescent centre their freedom. And perhaps a similar explanation could be devised to explain why quiescent centre cells divide when the meristem is exposed to X - rays, but here we would have to invoke either the weakening of cell contacts, or a change in the direction of growth of cells in the columella and epidermis.

It seems much more difficult to apply this mechanistic hypothesis of quiescence to roots with an "open" type of meristem, such as in *Vicia* and *Pisum*. There is no obvious boundary between root proper and cap, as in Gramineae, but there is a boundary between cells of different growth directions located where the epidermal Kappe complex meets the columella of the root cap. However, the most proximal cells in association with this boundary are not quiescent - they are the cap initials, and the quiescent centre is located proximally to them (Clowes, 1959). But it would only require one cell to be connected with another of opposing growth direction for that cell to lock its neighbours into a non - growing state. I think with a careful analysis of cell patterns, together with autoradiographic techniques, it would be possible to accurately define the position and shape of the quiescent centre and provide evidence for this hypothesis. Developing lateral roots would be particularly suitable for this analysis.

If the antagonism between directions of growth and division of different groups of cells is the basis for quiescence we must then explain how the limitation to cell growth prevents cell division. Finally, it may be possible from analysis of the direction of cell division and growth, to predict the presence of other quiescent groups of cells. For instance, in some shoot apices illustrated in the works of von Guttenberg (1960 & 1961), (Clowes, 1961 b) and Dermen (1969) there is a group of cells at, or just under the apex with staining properties different from surrounding meristematic cells. Subjectively, these cells "look" quiescent. Further, these groups of cells appear to lie in a reg'on where lineages of cells with different

directions of growth converge, and cells within the "quiescent" group itself seem to have no particular orientation of either growth or division.

Cell Growth in the Meristem. As cells divide in the meristem they must also grow, otherwise the meristematic cells would become ever smaller. When cell division is inhibited, such as when roots are grown in hydroxyurea, a drug that selectively stops DNA synthesis, it becomes clear that cells in the meristem grow at different rates (Barlow, 1969 c). Cells also grow with a definite polarity which is predominantly in the direction of the root axis, although cells do exhibit some latitudinal growth in some regions of the apex. Even cells of the epidermal lineage located at the cap columella - quiescent centre boundary enlarge slightly in a longitudinal direction. As already mentioned, the lineage of these cells indicates that their division is anticlinal and so cells should elongate at right angles to the plane of division, but this cannot take place because of the resistance of the columella cells. When roots are treated with indole acetic acid all cells show a transient increase in the amount of transverse growth except cap columella cells. This is further evidence for the rigidity of the wall between epidermis and columella. It is not known what controls the polarity of growth and division, or why cells should occasionally divide at right angles to the predominant plane of division giving the 'T' — patterns we see in Schuepp's method of Körper — Kappe analysis.

In the course of studying mitotic spindles in the root of *Zea* I measured the volumes of mitotic cells in different regions of the root (Barlow, 1970 c). I found the volume of these cells to get larger with increasing d'stance from the apex. The same is true for cells in S phase followed over a greater distance in the root (Table 8). The measurement, taken together with the knowledge that the rate of division is constant in the regions considered, indicate that the rate of cell growth must be increasing the further the cell is from the apex. If this were not so, all the mitotic cells, or all the S phase cells, would be the same size whatever their location. The data in Table 8 also show that nuclear volumes increase with distance from the apex. This increase is probably due to an increased water content of the nucleus (Lyndon, 1967), but whatever the cause, it can have little direct effect on the rate of cell division or DNA synthesis as none of the three parameters can be correlated.

I think the observation of changes in cell size may have an

TABLE 8

Dimensions of cells and nuclei in different regions of the meristem. Cells in S phase are those in which there is a labelled nucleus. The dimension given here is area because the measurements were made on sections prepared for autoradiography and were too thin (6 μm) to make an accurate determination of cell diameter. The size of mitotic cells is given as volume, as thicker (10 μm) section were used so allowing a more reasonable measure of cell diameter to be made owing to the increased chance of the section containing the maximum cell diameter.

Region	Area (μm²) of S phase		Volume (μm³) of mitotic cell
	Cell	Nucleus	
Cap Columella Initials.	125	53	1327
Quiescent Centre.............	135	60	1150
Stele just above Q. C................			1165
Stele 200 μm from Cap-Q. C. boundary.	168	66	1680
Stele 400 μm »			1781
Stele 700 μm »	262	74	
Stele 1000 μm »	337	83	

important bearing on the regulation of cell division and differentiation. Within the meristem the rate of cell growth outpaces the rate of cell division. It is reasonable to suppose that the processes of cell division are controlled by specific factors: one is required for the initiation and completion of DNA synthesis, others are required for the initiation and completion of mitotic processes such as chromosome coiling, spindle formation and cytokinesis. These factors are synthesised in interphase cells and must reach a particular concentration in the cell before they can function. The factors controlling mitotic events function in the cytoplasm and the one for DNA synthesis functions in the nucleus. We have seen that the rate of synthesis of DNA and RNA is constant in regions basal to about 200 μm from the cap - quiescent centre boundary and so these factors are probably also synthesised at constant rates in these regions. Superimposed upon the meristematic cell is a gradient of a substance that promotes cell enlargement. This substance may be auxin produced by the senescing cells in the basal regions of the root (cf. Sheldrake and Northcote, 1968). Towards the basal end of the meristem cells are promoted to a faster rate of growth than those at the apical end. As a consequence of the constant rate

of synthesis of factors for division, and the increasing rate of cell growth in the more basally located cells in the meristem, a certain point will be reached in the root where division factors cannot reach the critical concentration in the cytoplasm required for mitosis to take place. This position in the root, where an imbalance between growth rate and synthetic rate occurs, is the limit of meristematic activity. Although meristematic activity may cease DNA synthesis can still take place because the growth of the nucleus is not sufficiently great to dilute the factor required for DNA synthesis below the limiting concentration. Thus, endoreduplication of DNA occurs, the cell does not divide, and so the cell becomes polyploid. By slightly extending the number of specific factors involved in division we can explain the origin of binucleate cells and endomitosis. In fact, during the differentiation of certain tissues, such as xylem and tapetum, the cells show a binucleate and/or endomitotic development (List, 1963; Tschermak-Woess, 1967). The following scheme clarifies the relation between the different factors and nuclear behaviour:

Circumstance	Result
$\dfrac{\Delta G}{\Delta T} \triangleright \left(\dfrac{\Delta [D] + \Delta [K] + \Delta [S] + \Delta [C]}{\Delta T} \right)$	Mitosis
$\dfrac{\Delta G}{\Delta T} > \dfrac{\Delta [K]}{\Delta T} \triangleright \left(\dfrac{\Delta [D] + \Delta [S] + \Delta [C]}{\Delta T} \right)$	Binucleate or multinucleate cell
$\dfrac{\Delta G}{\Delta T} > \left(\dfrac{\Delta [K] + \Delta [S]}{\Delta T} \right) \triangleright \left(\dfrac{\Delta [D] + \Delta [C]}{\Delta T} \right)$	Endomitosis
$\dfrac{\Delta G}{\Delta T} > \left(\dfrac{\Delta [K] + \Delta [S] + \Delta [C]}{\Delta T} \right) \triangleright \dfrac{\Delta D}{\Delta T}$	Endoreduplication

G = cell growth, D = DNA synthesis factor, K = cytokinesis factor, S = spindle factor, C = chromosome coiling factor, = critical concentration, T = time.

Using this scheme we would predict that the number of meristematic cells should decrease the older the root becomes. As cell growth rate outpaces division rate, cells progressively closer to the apex will eventually reach the critical volume that does not allow cell division. An indeed the meristem does become smaller with age. So here we have a basis on which to explain aging and the

TABLE 9

Distribution of plasmodesmata over the walls of cells in the root cap

Region	Plasmodesmata		
	per μm^2 of		per μm^3 of cell volume
	Transverse wall	Longitudinal wall	
Cap Columella Initials.............	1487 ± 208	530 ± 108	3.38
Cap Centre 200-300 μm from Cap Q. C. boundary....................	772 ± 288	75 ± 14	0.51
Mature cells at periphery of cap.....	513 ± 123	45 ± 6	0.25

cessation of division in the meristem, as well as a basis for describing the relation between mitosis, polyploidy and growth.

Cell growth and intercommunication via plasmodesmata may play and important role in determining differentiation of cells. For instance, the changing propierties of cap cells (see Northcote and Pickett - Heaps, 1966) may be accounted for, in part, by changes in the degree of communication between cells as they grow. Mitotic cells of the cap initials have the greatest number of plasmodesmata per μm^2 of cell wall, but these cells eventually cease dividing but continue to grow in size causing the number of plasmodesmata per unit wall area to fall (Table 9). The degree of communication per unit volume of cell falls and this could affect the intracellular milieu allowing the cells to drift into another biochemical pathway. The idea here is that differentiation is not simply a specific switch in genetic action but can be brought about by what might be called a drift of "cellular entropy". A high degree of communication keeps cells in a particular biochemical state: using the root cap as an example this is mitosis; as communication becomes less so cellular activity becomes organized in a more chaotic way which is expressed in another biochemical pathway, in this instance in the formation of starch and slime.

A second example of differentiation in the root, where intercellular communication may play an influential role, is the xylem cell which arises in the heart of the meristematic region. Differentiation of a lineage of xylem cells could be maintained by three

things: by the plasmodesmatal connections across their transverse walls, their synthesis of cell enlargement factors e.g. auxin (Seldrake and Northcote, 1968) and their fast rate of cell growth which prevents mitosis. I have shown that these cells have a faster growth rate than their surrounding cells of the meristem (Barlow, 1969 a) and, as one might predict, they are the first to become polyploid in the apex (List, 1963). They are unable to influence their lateral neighbours to a similar pathway of differentiation since the number of lateral plasmodesmatal connections are rapidly being diluted by the fast rate of cell growth and by the accompanying growth and division of their neighbours. So the developing xylem cells are isolated and can follow a course of development different to that of their neighbours. But this course of development is directly affected by the properties of the more basal xylem cells in the same lineage, as it is these more mature cells that are providing the trigger (auxin) for the initial change towards xylem differentiation in the uncommitted cell at the distal end of the lineage. Thus, differentiation is self propagating, both by cell growth and by the influence that growth has on cell to cell communication.

I have tried to outline some of the problems of cell growth and differentiation that a study of root meristems poses. I have kept the discussion to the more basic facets of root meristem organization, such as cell division and a descriptions of differences in the behaviour of cells in different regions of the meristem. But the apex is also a region where not simply cells, but also tissues, differentiate and they differentiate in particular places and with definite patterns. Their pattern of organization presents many questions of general biological interest, aspects of which have been discussed by Torrey (1963) and Wardlaw (1968).

But I believe a proper understanding of the more complex levels of organization, such as tissue patterns, can only come from a thorough understanding, of the past history of the cells that contribute to the pattern. And meristems are the differentiated cells of the future.

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