THE CELLULAR ORGANIZATION OF ROOTS AND ITS RESPONSE TO THE PHYSICAL ENVIRONMENT

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INTRODUCTION

All higher plants have roots, though the fraction of the plant's mass that is root varies widely. For example, Spanish moss, *Tillandsia usneoides*, is very nearly rootless in the adult state, whereas members of the Podostemaceae and some orchids (e.g. *Taeniophyllum*) are almost all root when in the vegetative phase. These examples illustrate not only the wide range of forms adopted by roots and root systems (for a review see Barlow, 1986) but also the wide range of environments in which roots exist: *Tillandsia* and *Taeniophyllum* both occupy arid aerial environments, the Podostemaceae live only in the turbulent waters of cataracts.

Although roots thrive in such diverse environments, they are all (with few exceptions) cylindrical and arise in a similar way from an apical meristem. Moreover, the meristem of each species has its own characteristic and, within certain limits, invariant pattern of cellular organization.

Roots may be subjected to great changes in the external environment during their growth. In the soil they encounter variations in temperature, chemical composition and compaction, while aerial and aquatic roots may also experience fluctuations in light quality and quantity. Although many of these environmental variables may severely affect processes in meristematic cells, the root as a whole can accommodate and survive them. Roots are thus strongly homeostatic. This chapter surveys the effects of environmental variables on the cellular processes of root development and asks how perturbations in these processes may be compensated for or corrected.

PROCESSES OF CELL DIVISION

Cell division is cyclical: a cell grows, divides into two, and then both of the two new cells in their turn grow and divide. Within the cell, the nucleus and cytoplasmic organelles also reproduce and divide. Details of cell and nuclear cycles are discussed below, together with effects

that environmental factors have upon them. It should be noted that the environmental changes mentioned in this chapter are often imposed by experimentation in the laboratory and are usually applied abruptly. Gradual changes (e.g. in temperature) are much more likely to occur in natural conditions and in these circumstances there is the possibility of some concomitant adaptation.

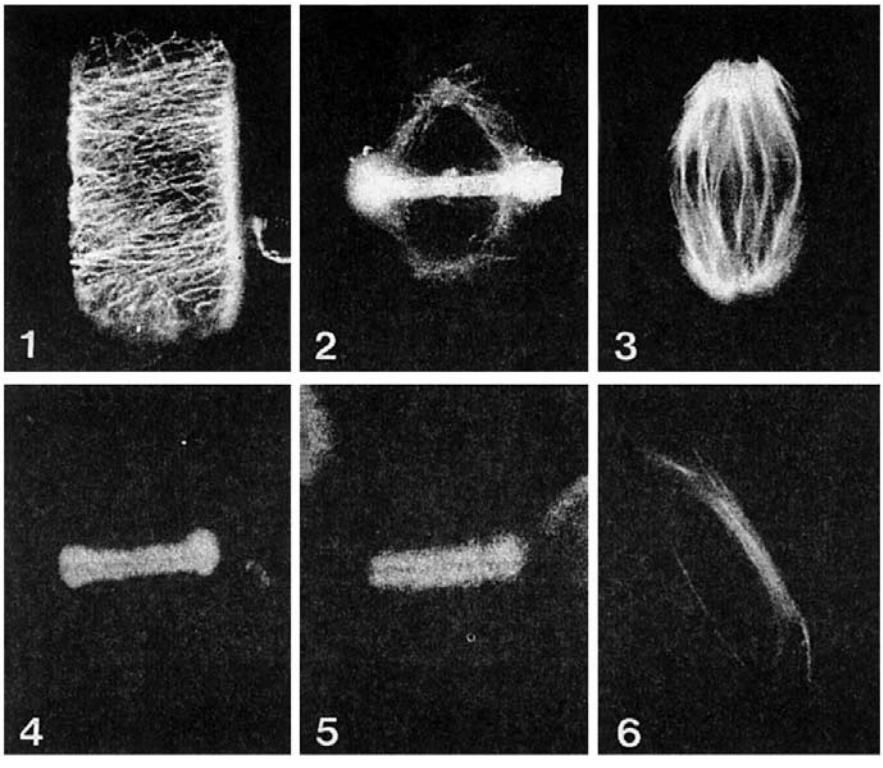
The cytoskeleton of meristematic cells

For many years cytologists have considered that the interior of the cell is ordered, but only recently have the elements conferring order been identified. The term 'cytoskeleton' is used to denote the macromolecular framework that underpins cell structure and upon which the processes of division, growth and differentiation rely. Many of the responses of cells to environmental effects may ultimately trace to some change in the cytoskeleton.

An important component of the cytoskeleton is the system of microtubules (MTs), the major structural unit of which is the protein tubulin. MTs are readily seen by electron microscopy, but recently they have been seen with the light microscope (which views whole cells rather than the ultra-thin sections of electron microscopy) after conjugating them with fluorescently labelled antibodies (anti-tubulin)(Wick *et al.*, 1981; Wick & Duniec, 1983, 1984).

In interphase cells, MTs labelled with anti-tubulin are seen under the plasmalemma lying at right angles to the principal direction of cell growth (Fig. 1). These cortical MTs are believed to regulate the orientation of the cellulose microfibrils in growing primary cell walls (Heath & Seagull, 1982). As the nucleus approaches mitosis, the cortical MTs disappear and give place to more localized hoops of MTs known as the pre-prophase band (PPB)(Fig. 2). In roots of onion (*Allium cepa*) the earliest PPB is about 5.6-8.3 μm wide, but later narrows to 1.2-3.5 μm (ca. 10% of the cell length)(Wick & Duniec, 1983). Prophase follows with the disappearance of the PPB and the concomitant construction, perhaps from perinuclear MTs, of the mitotic spindle (Fig. 3) upon which the sister chromatids separate. At anaphase-telophase, when the spindle is disassembling, yet another MT array, the phragmoplast, makes its appearance (Fig. 4). At first it is a set of short MTs lying mid-way between the two groups of separated chromosomes (Wick *et al.*, 1981). Later, the phragmoplast spreads centrifugally as an expanding annulus of MTs in whose wake the new cell wall

(or cell plate) is formed. Eventually the phragmoplast reaches the sides of the cell and the plate attaches to its walls. A remarkable feature is that the site of attachment closely corresponds to the zone occupied by the PPB earlier in mitosis (for a review see Gunning, 1982a). Each of these three



Figs. 1-4. Microtubules (MTs) in meristematic cells of *Allium cepa* revealed by indirect immunofluorescence using anti-tubulin antibody. Fig. 1. Cortical MTs in an interphase cell. Fig. 2. A pre-prophase cell showing a strongly fluorescent hoop of MTs (the PPB) and perinuclear MTs. Fig. 3. MTs of the mitotic spindle of a metaphase cell. Fig. 4. MTs of the phragmoplast of a telophase cell. Figs. 5 and 6. F-actin filaments in cells of *A. cepa* revealed by staining with a fluorescent, rhodamine-labelled derivative of phalloidin. Fig. 5. Actin in the phragmoplast. This is the same cell whose MTs are shown in Fig. 4. Fig. 6. An interphase cell with arrays of actin. Figs. 1 and 2 previously appeared in Clayton & Lloyd (1984) and Figs. 4, 5 and 6 in Clayton & Lloyd (1985); they are reproduced with the permission of the authors, Wissenschaftliche Verlagsgesellschaft and Academic Press.

arrays of MTs - PPB, spindle and phragmoplast - and perhaps the groups of cortical MTs also, are assembled at MT organizing centres (MTOCs); the sequential appearance of these arrays during mitosis suggests a sequential activation of the MTOCs (Lloyd & Barlow, 1982).

Another macromolecule, F-actin, has also recently been recognized as a cytoskeletal element of meristematic cells. It can be located by light microscopy through the use of fluorescently labelled phalloidin, a drug thought to stain actin specifically. Actin is a contractile molecule and has therefore been sought in the mitotic spindle. However, in onion roots both spindle and PPB are free of actin (Clayton & Lloyd, 1985 ; Gunning & Wick, 1985); the only place where MTs and F-actin coexist is the phragmoplast (Fig. 5). Here, both macromolecules appear at the same position and spread together in advance of the cell plate towards the side walls. In some interphase cells F-actin is found running as long fibrils to the inside of, and perpendicular to, the cortical MTs (Fig. 6).

There may be a third component of the cytoskeleton, the so-called microtrabeculae, in meristematic and other cells, but these are not yet well characterised. Microtrabeculae have been revealed in the electron microscope using special preparative techniques (Hawes, Juniper & Horne, 1983; Tiwari *et al.*, 1984). They consist of a lattice of proteins to which are attached the cytoplasmic organelles and perhaps the nucleus also. Such a microtrabecular lattice could serve as a scaffold supporting the MTs and actin.

Any environmental effect upon cell division might be explained by changes in the structure or function of the above-mentioned cytoskeletal elements. One such effect is that of low temperature. MTs depolymerize at temperatures near $0^{\circ}C$, the mitotic spindle disintegrates and the chromosomes, which cannot separate, revert to interphase to give a tetraploid cell; even if the temperature then rises, such cells may be unable to divide.

Many chemicals, including herbicides and fungicides, influence divisions through effects on the MTs; spindle function may be inhibited and cells consequently arrested at metaphase. However, defects of mitosis can also be caused by agents that impair the other cytoskeletal elements involved in division. For example, drugs such as caffeine and deoxyguanosine induce binucleate cells by preventing cell plate formation, apparently by interfering with different stages in the cytoskeletal transformations (Lasselain, 1979). Caffeine is noteworthy because its inhibition of cytokinesis can be mimicked by calcium deficiency (Paul & Goff, 1973) and overcome by addition of Ca^{2+} or Mg^{2+} (Becerra & López-Sáez, 1978). Its effect

can also be counteracted by adenosine, but enhanced by dinitrophenol (an uncoupler or oxidative phosphorylation) and low oxygen concentration (4% O_2 in the gas equilibrium phase) (González-Fernández & López-Sáez, 1980; López-Sáez, Mingo & González-Fernández, 1982). These findings suggest that ATP and a Ca^{2+}- or Mg^{2+}-activated ATPase are required for cytokinesis and, together with evidence from ultrastructural studies (López-Sáez, Risueño & Giménez-Martín, 1966; Paul & Goff, 1973), that they operate at the site of the phragmoplast.

Calcium regulates many intracellular processes including polymerization. The protein calmodulin binds Ca^{2+} and by so doing may regulate aspects of both MT and actin activity (Schleicher *et al.*, 1982). Observation of the intracellular location of fluorescently labelled antibodies to calmodulin in relation to the newly forming cortical MTs at early interphase and to the MTs of the PPB in onion and pea (*Pisum sativum*) root meristems gives no indication that calmodulin affects the deployment of MTs at these sites (Wick, Muto & Duniec, 1985). Calmodulin is, however, associated with the spindle and phragmoplast (Wick *et al.*, 1985). Thus, the deployment of calcium-sequestering membranes in the spindle and phragmoplast may influence chromosome separation and cytokinesis. Clearly, any environmental factor that influences the availability of Ca^{2+} and energy sources, or disturbs the integrity of the cytoskeleton, is potentially capable of upsetting mitosis and cell division.

The nuclear cycle

The nuclear cycle is intimately connected with cell growth and division. The two major compartments to the nucleus, the chromosomes and the nucleolus, will be dealt with separately.

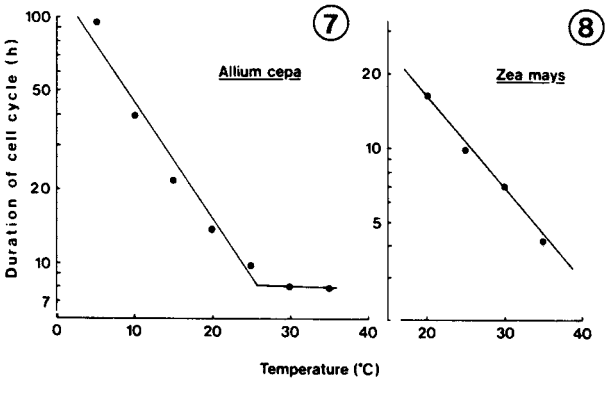
Chromosome replication and mitosis

The nuclear cycle provides for the replication of chromosomes, new copies of which are distributed to the daughter cells arising from cytokinesis. The most important environmental variables to which the cycle is susceptible are temperature, oxygen availability and moisture level. Some of the effects of these factors on the replication cycle have been reviewed in detail by Rost (1977).

Nuclei can reproduce and divide over a wide range of temperatures and the duration of the nuclear cycle decreases with increasing temperature (López-Sáez, Giménez-Martín & González-Fernández, 1966; González-Fernández,

Giménez-Martín & De la Torre, 1971). The lower temperature limit for the cycle, e.g. in barley, is about 0.5°C (Grif & Valovich, 1973) and the upper limit is a little above 35°C. Whether mitosis occurs at these limiting temperatures depends on whether the spindle and cytokinetic apparatus are also functional. For instance, nuclei may synthesise DNA and reach mitosis at a low temperature (Barlow & Rathfelder, 1985) but then progress no further because of the failure of tubulin to polymerize and construct a spindle. The optimal temperature for the nuclear cycle varies from species to species. In onion roots the cycle is fastest at 27°C and remains constant above this temperature (Fig. 7); the nuclei can still divide at 35°C but divisions cease at 40°C. Maize (*Zea mays*) shows an inverse relationship between division rate and temperature up to 35°C (Verma, 1980)(Fig. 8). Most observations have been made on crop species of temperate climates; species adapted to grow in other climatic zones (desert, tundra) may have different optimal and limiting temperatures.

Temperature-related changes in division cycle rate are accompanied by proportional changes in the duration of the component phases G_1, S_1, G_2 and mitosis (González-Fernández *et al.*, 1971). There are probably no functions absolutely specific to G_1 and G_2 since these phases are dispensed with under certain circumstances (Cooper, 1979; Navarrete, Cuadrado & Cánovas, 1983); but what regulates the duration of these phases



Figs. 7 and 8. Effect of temperature on the duration of the cell cycle in root meristems of *Allium cepa* and *Zea mays*. Data derived from López-Sáez, Giménez-Martín & González-Fernández, (1966) (1966) and Verma, (1980).

when they are present is unknown. Some of the molecular control points regulating the cycle have been reviewed by Giménez-Martín, De la Torre & López-Sáez, 1977): these control points almost certainly correspond to a specific, temporal sequence of gene action, but in plant cells these genes remain unexplored. At the molecular level, events of the S phase are the best characterized. During S, discrete lengths of the DNA double helix (replicons) are replicated in a particular sequence. Temperature influences the rate of movement of the DNA replication fork along the replicon (Van't Hof, Bjerknes & Clinton, 1978). In *Helianthus annuus* the average fork rate is 6 μm/h at 10°C, 8 μm/h at 20°C and 11.5 μm/h at 35°C. Below 15°C the S phase becomes protracted because the initiation of replication is less efficient.

Another temperature-dependent feature of DNA replication is the frequency of occurrence of sister chromatid exchanges (SCEs). In onion roots, exchanges are fewest at 25°C but become more frequent with either warming or cooling (Gutiérrez, Schvartzman & López-Sáez, 1981; Hernández & Gutiérrez, 1983). Gutiérrez *et al.* (1981) and Gutiérrez & López-Sáez (1982) have suggested that the frequency of SCEs may be influenced by intracellular and intranuclear oxygen concentration because in water-grown roots the frequencies are highest at lower temperatures which favour a greater solubility of oxygen. The significance of SCEs is obscure, but they are probably an expression of disturbances in the progress of the DNA replication fork. Because SCEs are the result of chromatid breakage and reunion they are potential sites of mutation, though in a root this is of little direct significance for their function.

Oxygen influences the duration of the nuclear cycle. Interphase in onion roots growing in water bubbled with air (20% oxygen in the gas equilibrium phase) lasts 9.8 h, whereas with 2% oxygen it lasts 41 h (López-Sáez *et al.*, 1969). The effect of oxygen does not become marked until its concentration in the medium falls to below 10%. Of the mitotic phases, metaphase and anaphase are the most sensitive to oxygen deficiency, probably because of the greater oxygen requirement of mitotic spindle assembly and function.

Water potential also influences the nuclear cycle. Exposing roots to different water potentials (e.g. by placing them in varying concentrations of polyethylene glycol (PEG) or mannitol) prolongs the nuclear cycle (González-Bernáldez, López-Sáez & García-Ferrero, 1968; Murín, 1979). This may be the result of a decrease in the rate of turgor-

driven growth which, in turn, may directly influence the rate of nuclear progression through the cycle; there could also be direct effects on processes such as DNA synthesis. Yee & Rost (1982) found that mitosis and DNA synthesis in roots of intact bean (*Vicia faba*) plants could recover from a continuous treatment with 20% PEG 6000 (osmotic potential -0.9MPa), but only when the cotyledons were present.

Nucleolar structure

A nucleolar cycle runs in parallel with the chromosome replication cycle (De la Torre & Giménez-Martín, 1982). Early in mitosis the nucleolus disintegrates and its complement of ribonucleoprotein (RNP) is liberated into the cytoplasm, though some remains on the surface of the mitotic chromosomes. When the chromosomes reassemble within a new nuclear membrane at the end of telophase, the nucleolus begins to reform by the coalescence of the RNP material on the chromosomes. The genes for ribosomal RNA (rRNA), which are essential for meristematic cell growth, then become active; the new nucleolus forms at their loci on the chromosomes and continue to grow throughout interphase.

Departures from the cyclical disintegration and reorganization of the nucleolus can be induced by low temperature. In roots of *Allium cepa* (Giménez-Martín *et al.*, 1977), *Brodiaea uniflora* (Sato & Sato, 1984) and *Salix* hybrids (Ehrenberg, 1946) grown at 4–11°C the nucleolus does not disintegrate at prophase but persists into metaphase. A consequence of its persistence is that the nucleolus is freed into the cytoplasm at anaphase. How long it can remain in the cytoplasm, or whether it has any function there, is not known. Nucleolar persistence is also induced by drugs that inhibit, or cause aberrant, RNA (but not protein) synthesis (Moreno Díaz de la Espina, Fernández-Gómez & Risueño, 1979), suggesting that the normal course of nucleolar disintegration at prophase depends on RNA made during the G_2 phase (Fernández-Gómez, Moreno Díaz de la Espina & Risueño, 1978).

Temperature also influences nucleolar size and structure. The volume of nucleoli in root cells of onion grown at 10°C is about twice that of nucleoli at 25°C (Morcillo, Krimer & De la Torre, 1978). Most of this increase results from enlargement of the granular component (Fig. 9) and is accompanied by an increased amount of nucleolar RNA polymerase and a greater abundance of ribosomes in the cytoplasm. However, the over-production of rRNA at the lower temperature is somehow compensated for at a post-transcriptional stage since the amount of protein synthesised per cell

division cycle is similar to that at 25°C (De la Torre, Morcillo & Krimer, 1981).

Heat and cold shocks affect nucleolar structure. Six days after transferring young rootlets of maize from 16° to 4°C there is a loss of the granular component, condensation of nucleolus-associated chromatin and the appearance of unusually large (55 nm diameter) RNP particles (Crèvecouer, Deltour & Bronchart, 1983). Transfer of rootlets to 46°C for 3-5 h also disperses the granular component and transforms it into particles 80-140 nm in diameter (Fransolet *et al.*, 1979). Similar results have been obtained for nucleoli of onion transferred from 15°C to 44°C for 3 h (Risueño *et al.*, 1973). A transfer to 35°C is less disruptive, but does cause the appearance of nucleolus-like bodies in the cytoplasm, probably as the result of an upset in the usual nucleolar disintegration/reaggregation events at mitosis (Díez *et al.*, 1971.)

Transfer of onion roots to a near-anoxic environment causes the

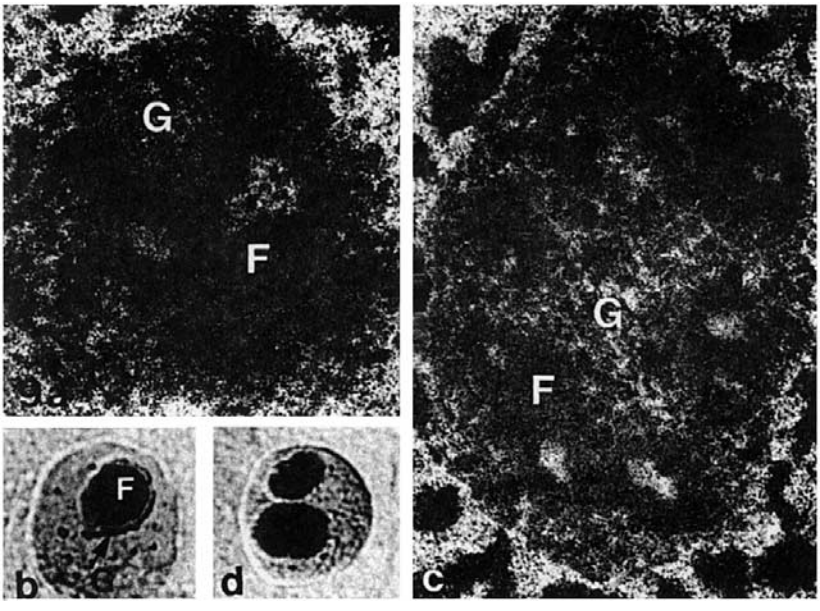


Fig. 9(a,b). Electron and light micrographs showing the segregation of the fibrillar (F) and granular (G) components of a nucleolus in a root meristem cell of *Allium cepa* induced by 3 h of anoxia. (c,d) Electron and light micrographs of a nucleolus in an adequately oxygenated control root showing the typical intermingling of the fibrillar and granular components. Photographs kindly provided by Dr. S. Moreno Díaz de la Espina.

fibrillar component of the nucleolus to segregate from the granular component (Fernández-Gómez, Moreno Díaz de la Espina & Risueño, 1984) (Fig. 9). The effect is complete by 11 h after transfer, but then begins an almost equally complete reversal of segregation. From 18 h a second phase of segregation occurs and is maintained until the roots die at 36 h. The second phase of segregation is completely and rapidly reversible if roots are returned to oxygen-saturated water. Nucleolar segregation is characteristic of treatments that affect rRNA synthesis. The apparent adaptation of the nucleoli to anoxic conditions between the 11th and 18th h is of particular interest since a comparable, though more rapid, adaptation of RNA metabolism has been found in germinating rice embryos transferred from ambient to anoxic conditions (Aspart *et al.*, 1983). Here, after an initial slowing of RNA synthesis, new messenger- and r-RNA molecules are made; they have, however, a different pattern of post-transcriptional processing.

CELL DIVISION AND CELL PATTERNS

The activities of the division cycle described in the preceding section occur in any cell in a root meristem. But meristematic cells do not function in isolation; they are members of a cellular ensemble with a precise organisation and I shall now discuss aspects of division and differentiation as they apply to the generation of the 3-dimensional structure of the root.

Patterns of cell division

It is useful to consider first the relatively simple root of the water-fern, *Azolla pinnata* (Fig. 10) so that the apparent complexity of roots in higher plants can be put in perspective. All cells of the *Azolla* root derive from a single tetrahedral apical cell; the root cap is descended from its acroscopic face, and the three basiscopic descendents of the apical cell undergo a sequence of longitudinal divisions in which each new wall partitions a mother cell at a precise position. These divisions lead to the establishment of every longitudinal file of cells found in the mature root. Only when these files are established do the cells divide transversely. The number of transverse divisions in any file depends on the position of that file within the root. The two types of division, the longitudinal followed by the transverse, are called respectively 'formative' and 'proliferative' (Gunning, Hughes & Hardham, 1978).

The precise positioning of the new cell walls in both formative and proliferative divisions is preceded by the correspondingly precise