Review article

The concept of the quiescent centre and how it found support from work with X-rays. II. The molecular aftermath

Peter W. Barlow

School of Biological Sciences, University of Bristol, Bristol Life Sciences Building, 24 Tyndalls Avenue, Bristol BS8 1TQ, UK
Corresponding author: P.W. Barlow, E-mail: p.w.barlow@bristol.ac.uk, Phone: +44 (0)117 394 1212

Received on June 05, 2015; Accepted on August 18, 2015

Abstract: With the advent of the molecular era of plant biology, the location and activity of the quiescent centre (QC) within the root meristem were reappraised with respect to the transport and distribution of hormones, especially auxin. Later, when methods for probing gene activity became established, the genes and their regulators that were identifiably specific to the QC were also actively studied, at first in relation to the establishment of the root and its QC in the proembryo and later in relation to the interaction of the QC with neighbouring meristem cells. Auxin distribution in and around the QC was found to be associated with co-located oxidative enzymes which established a redox system within the root apex. This system is pivotal in both maintenance of quiescence and the activation of cell proliferation in the QC via the generation of reactive oxygen species (ROS) and their interaction with mitochondria. These and other features of QC biology are summarised.

Keywords: *Arabidopsis thaliana*, determinate root growth, mitochondria, quiescent centre, reactive oxygen species (ROS), stem-cell niche, X-irradiation, *Zea mays*

Introduction

Part I of this paper (Barlow 2015a) outlined the way in which the concept of the QC was developed and consolidated during the most active period of FAL Clowes's research into this zone of the root apical meristem. Accordingly, because of the historical approach taken in that paper, only research publications from the era concerned (mainly the 1960s and 1970s) were cited. Also indicated in Part I was the significance of the QC in relation to both meristem activity and root growth. Moreover, because a QC has been found in roots of almost all those species which have been examined for its presence, it would seem to be a near-universal feature of angiosperm and gymnosperm roots, irrespective of their type of meristem construction (open, closed or intermediate), and is not a novelty discovered by chance in just a few species. However, an exception to this broad generalisation was found in the roots of the cactus *Stenocereus gummosus*, which apparently never possessed a QC at any time during their short growth period (Rodríguez-Rodríguez et al. 2003). Roots of many other species of Cactaceae were later shown to have determinate growth, and these, too, either lacked a QC, or possessed one for only a short time (Shishkova et al. 2007, 2013). Further possible exceptions are the fine roots of trees, which also display determinate growth (Pregitzer 2003), though in these cases, the presence or absence of a QC has not been established one way or the other. Although these last-mentioned features indicate the inherent variability of roots with respect to the behaviour of their apical meristems, it was when the QC came to be thought of as a 'stem cell' compartment from which the entire development of the root issues, from proembryo to adult plant, that its fundamental status was realised. Hence, this stem-cell aspect of the QC, and the special properties which 'stemness' entail (Loeffler and Potten 1997), called for thorough investigation. Furthermore, since QCs are generated *de novo* during the early stages of lateral root development, QCs can also be considered as founders of the entire root system. The present paper, Part II, briefly outlines some of the avenues of research pursued since Clowes's time, especially those from molecular biology, that provide explanatory mechanisms for QC maintenance in the midst of actively dividing cells.

Barlow PW 2015 The concept of the quiescent centre and how it found support from work with X-rays. II. The molecular aftermath. Plant Root 9: 56-67. doi: 10.3117/plantroot.9.56

Novel experimental systems

Experimental and analytical techniques developed considerably in the years following the era of Clowes's research. For example, as a means of detecting DNA synthesis, ^{3}H-thymidine labelling and the ensuing time-consuming autoradiography were replaced by the incorporation into nuclei engaged in DNA synthesis of immunofluorescently marked bromodexoyuridine (BrdU – an analogue of thymidine) and the results visualised almost immediately by fluorescence microscopy (Kerk and Feldman 1995, Jiang et al. 2003). Recently, 5-ethynyl-2'-deoxyuridine (EdU) has been adopted as an alternative to BrdU (Hong et al. 2015, Zhang et al. 2015). Likewise, serial sectioning of fixed and embedded root apices has given way to optical sectioning using confocal microscopy, at least in the case of *Arabidopsis thaliana* (but not for thicker roots like those of *Oryza sativa*, for which semi-thin sections of embedded material roots are still required for analysis), the necessary 'staining' being done by the application of immunofluorescent probes for specific proteins and by fluorescent staining of cell walls and nuclei. Observation of whole-mounted *Arabidopsis* roots has even proceeded to the point where cell divisions within the QC can be revealed in real time, enabling rapid estimation of cell division frequencies (Campilho et al. 2006). Confocal microscopy can also reveal real-time symplasmic movement of assimilates within roots. For example, carboxyfluorescein may be used as a tracer of such movement and, when injected into an *Arabidopsis* cotyledon, can be visualized unloading from the phloem at the tip of the root, moving within the cortex, and accumulating in the proximity of the QC (Oparka et al. 1994).

Model species for studying the QC, meristem, and root cap, now include, besides *Zea mays*, which was favoured by Clowes, *Arabidopsis thaliana* (Scheres and Wolkenfelt 1998) and *Oryza sativa* (Kamiya et al. 2003, Coudert et al. 2010). The last-mentioned species (rice) has the additional feature of possessing diverse types of shoot-borne roots, which are thus available for comparative genetic and developmental studies (Mai et al. 2014, Coudert et al. 2015). All these species have a 'closed' type of root meristem construction. Equally interesting as a model system, therefore, would be roots of 'open' construction, such as *Pisum sativum*, where the location of the QC is less easy to predict on anatomical grounds (except in a rather general way). Also instructive would be not only cases of roots regenerating a new apex together with a new QC following, say, amputation of the original root apex (Sena et al. 2009) but also comparisons between the ontogeny of a regenerating QC within an existing root tip and that of the QC forming during embryogeny (e.g. Clowes 1978a, 1978b).

Defining the QC

The QC was originally defined, and given its most characteristic form, by autoradiography, which revealed a zone of the root tip where ^{3}H-thymidine incorporation into DNA during the S phase of the mitotic cycle occurred at a lower rate than anywhere else in the meristem (see Barlow 2015a). This and other features indicative of low metabolic rates, led the QC to be generally regarded in a negative operational sense (Torrey 1972), even though, apparently, it had the potential for what might be supposed to be the default state of greater activity when the root cap was damaged, for example, (Clowes 1972), or when the meristem was placed under stress or challenged by irradiation with consequent root regeneration (Barlow 2015a). Nevertheless, there is evidently some heterogeneity within the QC for, interestingly, Clowes, in a reply to a question that followed a conference paper delivered by John Torrey, remarked that "there is a gradation across the quiescent centre, with maximum quiescence in the centre, and an increase in the rate of mitosis at the edge" (Torrey 1972, p. 11). The autoradiograph of the QC of *Z. mays* prepared by Barlow (1978, fig. 1b) using ^{14}C-thymidine to mark the nuclei shows this feature quite clearly, and should be compared with the autoradiograph shown in Fig. 2 of the preceding paper (Barlow 2015a): the most quiescent cells reside at the pole of the stelar portion of the QC, whereas an increased proportion of the cortical and epidermal zones within the QC have become labelled. Also, the configuration of nuclear chromatin is different in stelar and cortical/epidermal zones, being more dispersed in the former zone (Barlow 1978, fig 1a, c). Autoradiographs of the QC of cultured *Convolvulus* roots prepared after feeding with ^{3}H-thymidine for increasingly long times have also revealed different degrees of quiescence within the QC zone (Phillips and Torrey 1972), suggesting that a 'deeper' quiescence occurs in a central zone of the QC, at the apex of the stelar complex, as Clowes (in Torrey 1972) had indicated. However, some of the differential responsiveness of the QC of these roots may have arisen from its natural sensitivity to the internal radiation due to the incorporated ^{3}H-thymidine and consequent stimulation of DNA synthesis in the QC zone (see Clowes 1961).

One key observation in line with this research on QC heterogeneity was that treatment of roots with ascorbic acid (AA) also had the property of advancing G1 cells of the QC into S phase and, thus, of diminishing the size of the QC (Liso et al. 1990).

This discovery probably led Kerk and Feldman (1995) to examine the effect more closely and to arrive at the conclusion that the oxidative state of the meristem was important for the regulation of the frequency of mitoses in both root apical meristem (RAM) and QC (see later for discussion of this topic).

A reason for mentioning this operational definition of the QC is that some confusion has arisen over which cells at the root apex actually constitute the QC. We may take the current situation with regard to rice roots as an example. The QC of rice roots, which are similar in structure to those of *Z. mays*, has been carefully described by Rebouillat et al. (2009). These authors draw attention to a group of 'central cells' in the stelar portion of the QC; these cells are part of the QC, as judged from the pattern of labelling using either BrdU or EdU to identify nuclei that have commenced DNA synthesis. However, other descriptions of the rice root QC by Ni et al. (2014) create some confusion. These authors also write about a group of 'central cells' of the QC, but here they are referring to four cells at the pole of the epidermal lineage which abut the cap columella (Ni et al. 2014, their figs 1C and 1E). These cells are said to be the 'quiescent centre', even though it is clear from their illustration of a sectioned root labelled with BrdU that a much larger unlabelled group of cells (which includes the four mentioned epidermal cells) are evidently quiescent also (Ni et al. 2014, their fig. 1A). The BrdU-negative zone in their fig. 1A corresponds to a region expressing high *QUIESCENT-CENTRE SPECIFIC HOMEOBOX* (*QHB*) promoter activity (Ni et al. 2014). The same zone is also marked by *OsSCR1p:OsSCR1:GFP* expression. The signal from each of these probes is weak in cells at the pole of the stele, but is much stronger in a few distal cells of the endodermis, as well as in the mentioned polar epidermal cells (Ni et al. 2014, their fig. 1D), and so these molecular markers do not exactly identify the QC in all respects. *OsSCR* is a rice-plant homologue of the *SCARECROW* gene found in *A. thaliana* (Sabatini et al. 2003).

Similar comments apply to the situation in *A. thaliana*, where the pole-located epidermal cells have been referred to as constituting the 'quiescent centre' (Dolan et al. 1993, Nawy et al. 2005, Lee et al. 2013). It seems that Ni et al. (2014) have taken these observations on *Arabidopsis* by Dolan et al. (1993) as their cue for designating the four mentioned polar epidermal cells in the rice root apex as the QC. It is true that the 3-4 polar epidermal cells of *Arabidopsis* are quiescent; but the QC, operationally speaking, contains more than these four cells and includes a group of central stelar cells which, in *Arabidopsis*, are specified by the gene *WUSCHEL-RELATED HOMEOBOX 5* (*WOX5*) (Sarkar et al. 2007), as well as by the *AGAMOUS-LIKE 24* (*AGL24*) gene (Nawy et al. 2005). The four mentioned *Arabidopsis* epidermal cells are generally referred to as stem cells, and they occupy a 'stem-cell niche'. They are, in fact, a sub-compartment of the QC and do not constitute the QC alone.

It is the use of different methodologies for the definition of the QC which has led to a perceptible fragmentation of the DNA concept. As suggested by Shishkova et al. (2007), the aforementioned four stem cells of *Arabidopsis* roots could be referred to as "QC *sensu* Dolan", or QC^D, for it was Dolan et al (1993) who first drew attention to the presence of these cells of *A. thaliana*, and to a "QC *sensu* Clowes", or QC^C, a larger group of cells where proliferation is slow and which is easily defined according to its low rate of nuclear DNA synthesis. In fact, Shishkova et al. (2007) concluded on theoretical grounds that the *Arabidopsis* QC should be larger than the proposed QC^D, and in this expanded form would more properly define a QC^C.

Further confusion comes with the use of the term 'initial'. The initial cells are often taken to reside outside the QC^D. This is on the grounds of the pattern of the cell lineages which issue from the few cells of the QC^D. However, in QC^C, these so-called initial cells, as defined by the QC^D, would lie within the QC. Initials are defined in operational terms, i.e. they relate to actual cellular proliferation. With respect to the QC^C, the initial cells would lie on, and define, the edge of the proliferatively inactive QC. This contradictory problem of locating the initials has arisen repeatedly, ever since the time when the QC was first proposed; and it has been repeatedly commented upon by Clowes, most recently in Clowes (1976), and earlier in Clowes (1967a). The problem derives from the non-correspondence between the prior development of the cellular patterns and lineages and their contemporary patterns, as judged by molecular markers. It can be solved by returning to Clowes's idea a minimal constructional centre for the root (Clowes 1954), which is defined by the "minimum number of cells required to maintain the [cellular] pattern" Clowes 1954, p. 115) and by redefining the QC in terms of its capacity for cell proliferation.

Insights into the regulation of quiescence

In the time-frame of the research mentioned in Part I of the present paper (Barlow 2015a), only limited insights could have been gained with respect to the controls of cell growth and division operative within the root apical meristem (RAM) and its QC. Naturally, the central topic has concerned the nature of quiescence. One experimental approach adopted by

Clowes (1972) to elucidate the matter was to interfere with the integrity of the root cap and look for effects within the QC. The idea for this approach stemmed from earlier observations that when the root cap meristem was damaged, by radiation, say, cell divisions in the QC were activated (Clowes 1970). Clowes (1972) found that slicing off the distal portion of the cap of a *Z. mays* root tip was sufficient to induce a 5-fold increase in the rate of division in the QC within the space of 5 hours. Many years later, a development of this basic surgical method was to use a focussed laser light beam to ablate selected individual living cap cells of *A. thaliana*. The resulting responses in the QC were observed using confocal microscopy (van den Berg et al. 1995, 1997). By this means it was possible to see directly into the meristem and observe that formerly quiescent cells had been induced to enlarge and divide, and had come to occupy the space left by a laser-ablated columella initial. The experiment also gave insight into the interaction between QC and cap initials, showing that a balance existed in the latter cells between division and differentiation, both of which processes appeared to be regulated by the QC (van den Berg et al. 1997).

Six years before the mentioned surgical work of Clowes (1972), Juniper et al. (1966) had found that it was possible to remove the entire root cap of *Z. mays* from the apex. This operation paved the way for new experiments to investigate the cap's properties and its interaction with the rest of the meristem, in addition to its well known role in gravitropism. For example, the cap could be bisected longitudinally and the two halves separated by the insertion of a mica strip (Shaw and Wilkins 1973) or, alternatively, the cap could be removed and replaced by droplets of buffer solution loaded with growth regulators (Barlow and Pilet 1984). These ectopic applications, at concentrations just effective in maintaining the status quo, were assumed to mimic the chemical properties of the absent cap. Another method was to insert barriers into the RAM, and thereby divert the flow of the presumed growth regulators within the apex (Pilet 1982). All these experiments were attempts to gain insight into the hormonal regulation of QC behaviour (Barlow and Pilet 1984, Műller et al. 1993, 1994). In the end, however, they led to insights of different kinds: into gravitropism and thence to the discovery of PIN proteins (Friml et al. 2002). The latter are now known for their role in defining auxin distribution in and around the apex and setting up conditions for establishing a stem-cell niche of which the QC is a part (Sabatini et al. 1999, Petersson et al. 2009, Ding and Friml 2010).

Then, in the early 1990s, the first attempts were made to characterise gene transcripts within QCs isolated from decapped roots of *Z. mays*, and comparing these with transcripts from the RAM and root cap (Sabelli et al. 1993). More recently, this approach has yielded information about genes whose activity and products are specific to the QC (Nawy et al. 2005, Brady et al. 2007), as well as those which are either up- or down-regulated in the QC relative to their activity in the RAM and root cap (Ponce et al. 2000, Jiang et al. 2006a, 2010).

Genetics and the quiescent centre

In general, the behaviour of any particular cell, or group of cells (such as the QC), is a response to (a) its own inherent and autonomous properties, (b) its interaction with its immediate neighbour cells as well as with the tissues and symplasmic conduits (xylem, phloem) of the organ within which it lies, and (c) its susceptibility to variations in the physical properties of the organism's external environment.

The QC is not known to exist as a single cell but rather as a small group of mitotically inert diploid cells. An exception may be made in the case of roots of certain pteridophytes where the single apical cell takes the place of a QC and where the sequence 'quiescence - DNA synthesis – mitosis - quiescence ...' becomes disturbed and the apical cell becomes endopolyploid. The apical cell may then sometimes divide and produce a sector of polyploid descendents in the RAM (Avanzi and D'Amato 1967). Such a situation gives justification for the idea that, under some circumstances, the QC operates like an 'intermittent centre' rather than a centre that is continuously and completely inert (Clowes 1967b, Barlow 2015b).

The first step towards the development of a QC occurs during embryogeny. In *Arabidopsis thaliana*, this involves the division of a single hypophysis cell to give the precursor cell (or mother cell) from which, after two more divisions, four cells are formed which are precursors of the QC, or which form the QC^{D} (Dolan et al. 1993). Recently, a transcription factor gene, *NO TRANSMITTING TRACT* (*NTT*), and two related genes *WIP DOMAIN PROTEIN 4* (*WIP4*) and *WIP5*, have been reported to be required for the division of the hypophysis cell that leads to QC formation and thence to a root (Crawford et al. 2015). Probably, the QC mother cell produced by this division does at first act autonomously, dividing twice, but thereafter the daughters come under the influence of their location, the 'stem cell niche', a site which is probably prepared concurrently, if not beforehand, by the activities of PIN proteins in the basal portion of the proembryo and products of the *WIP* genes. Nevertheless, the formation of the biochemical and structural milieu of this niche

(Wildwater et al. 2005, Műller and Sheen 2008, Weijers et al. 2006) and the subsequent onset of a behavioural pattern characteristic of these four niche cells are the first steps in establishing the self-perpetuating RAM that emerges at germination, and perhaps also of establishing a true QC, or QC^{C} (see Raghavan 1990). Analysis of the relevant gene networks has predicted that not all the necessary genes and regulators which establish this niche have been discovered (Azpeitia et al. 2010, 2013).

There is, too, the problem of how the RAM achieves the size it does, and how this size relates to the dimensions and proliferative behaviour of the QC. Simplistic schemes in which auxin and cytokinin gradients provide a control of both QC location and meristem size (Torrey 1972, Barlow 1976), were ideas largely derived from the work of Skoog and Miller (1957) and their colleagues on the role of these two hormones in promoting DNA synthesis and cell division. These ideas still have some currency (Grieneisen et al. 2007, Müller and Shen 2008), though once the RAM has been properly established cytokinin may have its main action in (a) limiting the longitudinal extent of the meristem (Takatsuka and Umeda 2014), and (b) in vascular patterning immediately proximal to the QC (Bishopp et al. 2011). Recently, the gene *PHABULOSA* (*PHB*) has been shown to regulate the extent of proliferative activity of the RAM (Sebastian et al., 2015) and thereby set conditions to maintain a steady rate of RAM cell production which is coordinated with the rate of cell production from the initials that lie either in or around the QC. The activity of *PHB* itself is regulated by the QC (Sebastian et al. 2015); and, earlier, the gene *PLETHORA* had been shown to regulate the number of cells in the QC, apparently by monitoring and adjusting the level of auxin in the RAM (Aida et al. 2004).

Meristem function that is coupled to an ability to regulate the plane of cell division is now deemed crucial for cell differentiation (Scheres et al. 1995). The nature of this coupling within the RAM is to be understood in terms of complex pathways of gene regulation and their interaction with natural growth regulators (Sabatini et al. 2003, Sozzani et al. 2010, Lee et al. 2013). These events would occur outside the confines of the QC *sensu* Dolan (QC^{D}), but within the limits of the QC *sensu* Clowes (QC^{C}). Indeed, because the idea of a hormonal control of the RAM originated from conceptually simple beginnings, it is not surprising that the repertoire of hormonal regulators now includes, besides auxin, some which were only suspected in earlier times, such as abscisic acid (Zhang et al. 2010), or were under-appreciated, such as ethylene (Ortega-Martínez et al. 2007), or even unknown at the time of the discovery of the QC, such as jasmonate (Chen et al. 2011) and brassinosteroids (Heyman et al. 2013, 2014, Vilarassa-Blasi et al. 2014, Chaiwanon and Wang 2015).

Steady-state root growth and RAM activity may be only one stage during the life of the root; eventually RAM activity declines and the pool of proliferative cells gradually become exhausted with the passage of time. *Arabidopsis* roots are a case in point. After an early stage of expansion and steady maintenance of size, the RAM ultimately enters a phase of growth which is coupled with an alteration to the structure of the QC, and which indicates a cessation of its former operational state (Baum et al. 2002). This so-called determinate stage of growth is under the control of a new set of genes and regulators (Hernández-Barrera et al. 2011). First attempts have been made (Reyes-Hernández et al. 2014) to uncover the conditions for the switch in RAM morphology and the extent to which the QC is responsible for the change. Earlier, however, Sánchez-Calderon et al. (2005) observed that meristem determinacy was regulated by external phosphate levels, a variable that can (at least in some species) affect the amount of DNA housed within plant cell nuclei (GM Evans 1968). Also of interest is the way in which root apical morphology changes during the life of a root, especially the switch from ‘closed’ to ‘open’ meristem structure (Armstrong and Heimsch 1976, Clowes 1981). Indeed, analogous structural changes have presumably occurred during root phylogeny and account for the various structural types of meristem (Heimsch and Seago 2008). The comparative morphology of root apices revolves around the question of the regulation and orientation of formative divisions (Sozzani et al. 2010). New insights into these problems might come from experimental work, such as exemplified by results of Galhina et al. (2007), where different genes and promoters were able to disturb the pattern of the gene-product gradients within the meristem.

Redox regulation of RAM and QC

Included in the repertoire of root meristem and QC regulators are pathways related to redox states. Crucial to an understanding of the QC and its behaviour is the model due to Kerk and Feldman (1995, see also Jiang et al. 2003). It is hypothesised that there is a redox system within the root apex which regulates auxin levels in both the RAM and the QC, and that disturbance of this balance of oxidants (e.g. auxin) and antioxidants (e.g. ascorbic acid – AA) permits (or not) division of QC cells (de Tullio et al. 2010). Generally, an oxidized state prevails in the QC due to its high auxin content generated by PIN-directed auxin transport (Sabatini et al. 1999).

Auxin has been found to induce reactive oxygen species (ROS) which thereby impose an oxidative stress upon the mitochondria, which exist with a simple structure within the QC cells (Jiang et al. 2006b). The redox state regulates mitochondrial ATP production and is also able to block mitotic cycle transitions and thereby bring about proliferative quiescence (Jiang et al. 2006b). Clowes (1969) had already speculated on the possibility of high auxin content in the QC and had also suggested that oxygen status in the apical zone might account for the quiescence of the QC (see Barlow 2015a). However, he had thought of this in terms of an O_2 deficiency due to an inadequate O_2 diffusion pathway within the root apex, this pathway having originated with O_2 internalised by the shoot. Clowes was therefore only partly correct in thinking that oxygenation may play a role in quiescence and that mitochondria were also involved; his reasoning has now been turned upside-down by the discovery of a redox balance in the QC that is grounded in auxin as oxidant and AA as anti-oxidant. Following the observations of Feldman and his co-workers, other redox regulators such as thioredoxin and glutathione have been described and considered to play additional roles in auxin homeostasis (Bashandy et al. 2010).

There are many situations whereby reactive oxygen species (ROS) and reactive nitrogen species (RNS) – e.g. hydrogen peroxide (H_2O_2), superoxides ($^{\cdot}O_2^-$), NO, etc. – are generated within plant tissues. The biochemical events by which these reactive species arise, and the pathways by which they are neutralised by antioxidants, allow the meristem to achieve a type of homeostasis whereby damage is repaired, growth and proliferation maintained (Riley 1994, de Tullio et al. 2010), and the length of the meristem determined (Tsukagoshi et al. 2010). There are also possibilities, using fluorescence staining methods, of identifying sites of ROS production within cells (Narayanan et al. 1997).

It is probable that ROS and the cellular damage caused thereby (Caputo et al. 2012) are the instigators of the radiation responses of the QC discovered by Clowes, both from the internal beta-radiation imported into the meristem from ^{3}H-thymidine (Clowes 1961) and from radiation received from external X- and gamma-ray sources (Clowes 1965, see also Barlow 2015a). ROS are probably the factors which bear out scepticism about the role of nuclear, as opposed to cytoplasmic, responses to radiation in bringing about the decline of root growth (HJ Evans 1965). However, Evans was thinking of growth impairment in terms of chromosome aberrations and their effect on the mechanics of mitosis and cellular viability whereas, nowadays, the emphasis would be on the radiation induction of biochemical pathways within the cytoplasm that affect redox status due to the presence of single and double DNA-strand breaks (Zhou and Elledge 2000, Chen and Umeda 2015). It is possible that the intermittent nuclear DNA replication in the apical cell of some ferns, which leads to their endopolyploid status, could be a response to chance DNA breaks which, in turn, stimulate redox-like regulation of the nuclear cycle (Adachi et al. 2011).

Some of the potentially lethal radiation and other types of damage received by cells will be mitigated or mediated by properties of the mitochondrial population (Leach et al. 2001, Mikkelson and Wardman 2003), which is known to vary in size and configuration throughout the root apex (Kuroiwa et al. 1992, Fujie et al. 1993, Jiang et al. 2006b) and which, interestingly, shows mtDNA synthesis to proceed rapidly within the very zone of the QC in which nuclear DNA synthesis is most retarded. However, in the QC of *Z. mays* roots the level of mitochondrial transcripts is low (Li et al. 1996). It maybe that, in terms of cellular function, X-ray damage to the DNA of the mitochondrial population within the RAM, and which is mediated by ROS, is of primary importance, and that damage sustained by the nuclear DNA is secondary (Kam and Banati 2013). Noteworthy is that mitochondrial mtDNA, when damaged by ROS, is less easily repaired than similarly damaged nuclear DNA (Yakes and van Houten 1997). Also, radiation-induced changes to mitochondrial membranes and consequent efflux of Ca^+ ions may have rapid effects on intracellular metabolism and induce cascade reactions which effectively amplify the consequences of the damage initially due to ROS (Leach et al. 2001). Even that damage which is due to environmental (Clowes and Stewart 1967) and nutritional factors (Webster and Langenauer 1973), and which may be mediated by ROS, is amplified in a similar way; that is, the ensuing repair processes are activated not only locally but they also reach into the QC as a consequence of this amplification step, possibly using plasmodesmata as a pathway for Ca^+. It may be that here Ca^+ ions are liberated from mitochondria and that these ions are then able to play a role in the immediate onset of prophase in certain cells of the QC (as described in Barlow 2015a). Other related and rapid metabolic changes contribute to the decondensation of the nuclear chromatin (Barlow 1985), especially in the cortical zone of the QC, thus preparing the nuclei here for DNA replication.

By contrast, the redox systems described by Caputo et al. (2012) may confer upon the QC a resistance to radiation damage; and Cruz-Ramírez et al. (2013) express a similar view. The latter authors also make the distinction between the responsiveness of the QC, with its ability to serve as a reservoir of

reparative cells, and the need for the continued maintenance of a stem-cell niche.

Also important is the efficiency with which DNA repair is conducted in the QC and elsewhere (Fulcher and Sablowski 2009). Mainly it is nuclear DNA which has been considered from this point of view, but attention should also be given to mtDNA (see Yakes and van Houten 1997). While repair levels (in either the QC or elsewhere in the RAM) have not been investigated, it seems fairly evident that RAMs in general are subject to considerable stress (accompanied by ROS and RNS production) during their lifetime and rely on genes such as *MAIN* (Wenig et al. 2013) and *MAIN LIKE 1* (Ühlken et al. 2014) to correct any damage to DNA that would ensue from such stress.

Intriguingly, and with the QC specifically in mind, Heyman et al. (2014) raised the possibility that QC stem cells could possess an immortal strand of DNA. Originally proposed by John Cairns (1975), and discussed soon afterwards in relation to the QC of *Z. mays* (Barlow 1978), the 'immortal strand' hypothesis proposes that, in stem cells, there is conservative segregation of DNA strands at mitosis (as opposed to the usual semi-conservative segregation), and that the newly synthesised daughter strand of DNA is directed into the daughter cell by means of a polarisation of mitosis. In the case of the QC, any daughter strand of DNA which has become damaged during the replication process would pass, say, from an initial cell located at the stem cell niche (QC^D) into the daughter of that cell, while the unblemished DNA template, the 'immortal' strand, would be conserved and retained in the other daughter cell, at the stem-cell niche (Cairns 2006, Rando 2007). The presence of genes like *MAIN* and *MERISTEM DISORGANIZATION 1* (Hashimura and Ueguchi 2011) in *Arabidopsis*, and the multitude of DNA repair genes which are general and widespread in organisms, including plants (Britt 1999), suggests that life forms are continually being assailed by potentially lethal conditions which, by their induction of ROS and RSN, threaten the integrity of the nuclear and mitochondrial genomes and the efficiency of cellular metabolic pathways. This damage can be ameliorated only by the appropriate redox systems. Damaging external conditions may even include periodic exposures to radon (^{222}Rn) gas released from the ground, as well as effects due to in-coming cosmic rays from space which create disturbances to the Earth's electromagnetic field (EMF) (reviewed by Barlow et al. 2013). Each of these could induce intracellular free radicals (ROS, RNS), as reviewed by Zakhvataev (2015) and, hence, could impact upon the RAM and the stem cells of the QC. The possibility of EMF effects is evident when weak electric fields are present in the vicinity of the *Zea* root apex (Wawrecki and Zagórska-Marek 2007). Here, descendents of the QC were seen to intrude into the root cap. However, Clowes and Wadekar (1989) and the present author (PW Barlow unpublished) have repeatedly found similar images of intrusive cells arising apparently spontaneously in germinating roots of *Z. mays*.

The quiescent centre in its wider environment

With respect to the totality of QCs present in a multi-branched root system in the context of a whole plant with its corresponding multi-branched shoot system, it can be supposed that the respective stem-cell populations (root and shoot) are in communication, as suggested by Veit (2006), and that, because of this, the architecture of the whole plant could be influenced in accordance with the information exchanged. In other words, there may be a system of general stem-cell homeostasis, whereby a network of hormonal fluxes not only maintains all of the appropriate stem-cell niches of root and shoot, but also initiates new niches where and when appropriate, and even causes some of them to subsequently abort or become suppressed, as in fine root or short root development. This system of homeostasis is rendered feasible because the stem-cell niches of shoots and roots have common maintenance pathways (Tucker and Laux 2007, Sarkar et al. 2007).

Regarding the relationship of QCs and root stem cells with the biosphere, as outlined by de Tullio et al. (2010), the RAM and its QC are both part of this large-scale environment: namely that of the soil, or rhizosphere, to which they contribute mainly through the secretory properties of the root cap (Iijima et al. 2008). The RAM, and perhaps the QC also, are responsive to the soil environment and vice versa. Although a study of the effect of soil-borne *Pseudomonas* bacteria upon roots of *A. thaliana* did not detect any effect upon the QC, the bacteria did alter the form of the root system through manipulation of its auxin levels (Zamioudis et al. 2013). In addition, soil bacteria are sources of indoles, and these compounds have effects on lateral root densities. So, in this sense bacteria within the rhizosphere do contribute to the generation of new stem-cell niches (Bailly et al. 2014) as a condition for subsequent lateral root primordia formation. Similarly, soil structure might influence QC niche formation should impedance of root growth, due to the structure and compaction of the soil, result in contorted roots. Lateral roots would then form preferentially on the convex flank of such roots following a mechanically induced relocation of PIN1 protein and a consequent alteration of auxin flow (Ditengou et al. 2008).

Evolution and the quiescent centre

In the long history of plant life, the root organ displays a distinctive phylogeny (Dolan 2009). Whether this phylogeny has run in parallel with the development of a QC zone within the dividing region of the evolving root organ is not known. The path leading to the evolution of the QC is obscure but might be deduced not only from what can be assumed from the hormonal complement of basal plant forms, but also from the evolutionary history of the genes that underpin root structure supportive of a QC. From an examination of apices of rhizophores and roots of *Selaginella martensii*, a suggestion was put forward that a QC (*sensu* Clowes) could quite easily have arisen at the site of a prospective root bifurcation (Barlow et al. 2004, Barlow 2015b). At this point, however, the question of stem cells – stem-cell niches (QC^D), and proliferatively quiescent zones (QC^C) – arises: i.e. the contrast between QC *sensu* Dolan and QC *sensu* Clowes. Which of these three structures would be a crucial element for Darwinian natural selection? Speculations (or evidence) about QC evolution should take account of the positional and the operational attributes of the QC, and whether, in any of the species studied, the QC might be a continuous or an intermittent QC (Barlow 2015b).

Afterword

That the importance of the QC has been acknowledged in the plant sciences community is evident from the number of citations in the scientific literature: Google Scholar, for example, registered 9,800 'hits' (in May 2015) when key-words "quiescent+centre" and "root+apex" were entered, and 10-times more hits appeared using "quiescent+centre" alone as the key-word. Likewise, Web of Science (Thomson Reuters) revealed >500 records of research papers when "quiescent+centre" was used as the 'Topic' entry. These citations commenced in 1964. It was from about 1995 onwards that mention began of particular genes which define the location of the QC.

Acknowledgements

The author is grateful to Prof JG Dubrovsky for his critical reading of an early version of the present paper and to two anonymous referees for their useful suggestions.

References

Adachi S, Minimisawa K, Okushima Y, Inagaki S, Yoshiyama K, Kondou Y, Kaminuma E, Kawashima M, Toyoda T, Matsui M, Kurihara D, Matsunaga S, Umeda M 2011 Programmed induction of endoreduplication by DNA double-strand breaks in *Arabidopsis*. Proc. Natl. Acad. Sci. USA 108: 10004-10009.

Aida M, Beis D, Heidstra R, Willemsen V, Blilou I, Galinha C, Nussaume L, Noh Y-S, Amasino R, Scheres B 2004 The *PLETHORA* genes mediate patterning of the *Arabidopsis* root stem cell niche. Cell 119: 109-120.

Armstrong JE, Heimsch C 1976 Ontogenetic reorganization of the root meristem in the Compositae. Am. J. Bot. 63: 212-219.

Avanzi S, D'Amato F 1967 New evidence on the organization of the root apex in leptosporangiate ferns. Caryologia 20: 257-264.

Azpeitia E, Benítez M, Vega I, Villareal C, Alvarez-Buylla ER 2010 Single-cell and coupled GRN models of cell patterning in the *Arabidopsis thaliana* root stem cell niche. BMC Systems Biol. 4: article 134.

Azpeitia E, Weinstein N, Benítez M, Mendoza L, Alvarez-Buylla ER 2013 Finding missing interactions of the *Arabidopsis thaliana* root stem cell niche gene regulatory network. Front. Plant Sci. 4: article 110, doi: 103389fpls.2013.00110.

Bailly A, Groenhagen U, Schulz S, Geisler M, Eberl L, Weiskopf L 2014 The inter-kingdom volatile signal indole promotes development by interfering with auxin signalling. Plant J. 80: 758-771.

Barlow PW 1976 Towards an understanding of the behaviour of root meristems. J. Theor. Biol. 57: 433-451.

Barlow PW 1978 The concept of the stem cell in the context of plant growth and development. In: Lord BI, Potten CS, Cole RJ, Eds., Stem cells and tissue homeostasis. Cambridge University Press, Cambridge, pp. 87-113.

Barlow PW 1985 Nuclear chromatin structure in relation to cell differentiation and cell activation in the cap and quiescent centre of *Zea mays* L. J. Exp. Bot. 36: 1492-1503.

Barlow PW 2015a The concept of the quiescent centre and how it found support from work with X-rays. I. Historical perspectives. Plant Root 9: 43-55.

Barlow PW 2015b Origin of the concept of the quiescent centre of plant roots. Protoplasma (submitted).

Barlow PW, Pilet P-E 1984 The effect of abscisic acid on cell growth, cell division and DNA synthesis in the maize root meristem. Physiol. Plant. 62: 125-132.

Barlow PW, Lück HB, Lück J 2004 Pathways towards the evolution of a quiescent centre in roots. Biologia (Bratislava) 59 suppl. 13: 21-32.

Barlow PW, Fisahn J, Yazdanbakhsh N, Moraes TA, Khabarova OV, Gallep CM 2013 *Arabidopsis thaliana* root elongation growth is sensitive to lunisolar tidal acceleration and may also be weakly correlated with geomagnetic variations. Ann. Bot. 111: 859–872.

Bashandy T, Guilleminot J, Vernoux T, Caparros-Ruiz D, Ljung K, Meyer Y, Reichheld J-P 2010 Interplay between the NADP-linked thioredoxin and glutathione systems in *Arabidopsis* auxin signalling. Plant Cell 22: 376-391.

Baum SF, Dubrovsky JG, Rost TL 2002 Apical organization and maturation of the cortex and vascular cylinder in *Arabidopsis thaliana* (Brassicaceae) roots. Am. J. Bot. 89: 908-920.

Bishopp A, Help H, El-Showk S, Weijers D, Scheres B, Friml J, Benková E, Mähönen AP, Helariutta Y 2011 A mutually inhibitory interaction between auxin and cytokinin specifies vascular pattern in roots. Curr. Biol. 21: 917-926.

Brady SM, Orlando DA, Lee J-Y, Wang JY, Koch J, Dinneny JR, Mace D, Ohler U, Benfey PN 2007 A high-resolution root spatiotemporal map reveals dominant expression patterns. Science 318: 801-806.

Britt A 1999 Molecular genetics of DNA repair in higher plants. Trends Plant Sci. 4: 20-25.

Cairns, J 1975 Mutation selection and the natural history of cancer. Nature 255: 197-200.

Cairns J 2006 Cancer and the immortal strand hypothesis. Genetics 174: 1069-1072.

Campilho A, Garcia B, van den Toorn H, van Wijk H, Campilho A, Scheres B 2006 Time-lapse analysis of stem-cell division in the *Arabidopsis thaliana* root meristem. Plant J. 48: 619-627.

Caputo F, Vegliante R, Ghibelli L 2012 Redox modulation of the DNA damage response. Biochem. Pharmacol. 84: 1292-1306.

Chaiwanon J, Wang Z-Y 2015 Spatiotemporal brassinosteroid signalling and antagonism with auxin pattern stem cell dynamics in *Arabidopsis* roots. Curr. Biol. 25: 1031-1042.

Chen P, Umeda M 2015 DNA double-breaks induce the expression of flavin-containing monooxygenase and reduce meristem size in *Arabidopsis thaliana*. Genes Cells 1-11.

Chen Q, Sun J, Zhai Q, Zhou W, Qi L, Xu L, Wang B, Chen R, Jiang H, Qi J, Li X, Palme K, Li C 2011 The basic helix-loop-helix transcription factor MYC2 directly represses *PLETHORA* expression during jasmonate-mediated modulation of the root stem cell niche in *Arabidopsis*. Plant Cell 23: 3335-3352.

Clowes FAL 1954 The promeristem and the minimal construction centre in grass root apices. New Phytol. 53: 108-116.

Clowes FAL 1961 Effects of β-radiation on meristems. Exp. Cell Res. 25: 529-534.

Clowes FAL 1965 Meristems and the effect of radiation on cells. Endeavour 24: 8-12.

Clowes FAL 1967a The functioning of meristems. Sci. Prog. 55: 529-542.

Clowes FAL 1967b The quiescent centre. Phytomorphol. 17: 132-140

Clowes FAL 1969 Anatomical aspects of structure and development. In: Whittington WJ, Ed., Root growth. Butterworths, London, pp. 3-17.

Clowes FAL 1970 The immediate response of the quiescent centre to X-rays. New Phytol. 69: 1-18.

Clowes FAL 1972 Regulation of mitosis in roots by their caps. Nature 235: 143-144.

Clowes FAL 1976 The root apex. In: Yeoman MM, Ed., Cell division in higher plants. Academic Press, London, pp. 253-284.

Clowes FAL 1978a Origin of the quiescent centre in *Zea mays*. New Phytol. 80: 409-419.

Clowes FAL 1978b Origin of quiescence at the root pole of pea embryos. Ann. Bot. 42: 1237-1239.

Clowes FAL 1981 The difference between open and closed meristems. Ann. Bot. 48: 761-767.

Clowes FAL, Stewart HE 1967 Recovery from dormancy in roots. New Phytol 66: 115-123.

Clowes FAL, Wadekar R 1989 Instability in the root meristem of *Zea mays* L. during growth. New Phytol. 111: 19-24.

Coudert Y, Périn C, Courtois B, Khong NG, Gantet P 2010 genetic control of root development in rice, the model cereal. Trends Plant Sci. 15: 219-226.

Coudert Y, Le VAT, Adam H, Bès M, Vignols F, Jouannic S, Guiderdoni E, Gantet P 2015 Identification of CROWN-ROOTLESS 1-regulated genes in rice reveals specific and conserved elements of postembryonic formation. New Phytol. 206: 243-254.

Crawford BCW, Sewell J, Golembeski G, Roshan C, Long JA, Yanofsky MF 2015 Genetic control of distal stem cell fate within root and embryonic meristems. Science 347: 655-659.

Cruz-Ramírez A, Díaz-Triviño S, Wachsman G, Du Y, Arteága-Vázquez M, Zhang H, Benjamins R, Blilou I, Neef AB, Chandler V, Scheres B 2013 A SCARCROW-RETINOBLASTOMA protein network controls protective quiescence in the *Arabidopsis* root stem cell organizer. PLoS Biology 11(11): e1001724.

De Tullio MC, Jiang K, Feldman LJ 2010 Redox regulation of root apical meristem organization: Connecting root development to its environment. Plant Physiol. Biochem. 48: 328-336.

Ding Z, Friml J 2010 Auxin regulates distal stem cell differentiation in *Arabidopsis* roots. Proc. Natl. Acad. Sci. USA 107: 12046-12051.

Ditengou FA, Teale WD, Kochersperger P, Flittner KA, Kneuper I, van der Graaff E, Nziengui H, Pinosa F, Li X, Nitschke R, Laux T, Palme K 2008 Mechanical induction of lateral root initiation in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA 105: 18818-18823.

Dolan L 2009 Body building on land – morphological evolution of land plants. Curr. Opin. Plant Biol. 12: 4-8.

Dolan L, Janmaat K, Willemsen V, Linstead P, Poethig S, Roberts K, Scheres B 1993 Cellular organisation of the *Arabidopsis thaliana* root. Development 119: 71-84.

Evans GM 1968 Nuclear changes in flax. Heredity23: 25-38.

Evans HJ 1965 Effects of radiations on meristematic cells. Rad. Bot. 5: 171-182.

Friml J, Wiśniewska J, Benková E, Mendgen K, Palme K 2002 Lateral relocation of auxin efflux regulator PIN3 mediates tropism in *Arabidopsis*. Nature 415: 806-809.

Fujie M, Kuroiwa H, Kawano S, Kuroiwa T 1993 Studies on the behaviour of organelles and their nucleoids in the root apical meristem of *Arabidopsis thaliana* (L.) Col. Planta 189: 443-452.

Fulcher N, Sablowski R 2009 Hypersensitivity to DNA damage in plant stem cell niches. Proc. Natl. Acad. Sci. USA 106: 20984-20988.

Galinha C, Hofhuis H, Luijten M, Willemsen V, Blilou I, Heidstra R, Scheres B 2007 PLETHORA proteins as dose-dependent master regulators of *Arabidopsis* root development. Nature 449: 1053-1057.

Grieneisen VA, Xu J, Marée AFM, Hogeweg P, Scheres B 2007 Auxin transport is sufficient to generate a maximum and gradient guiding root growth. Nature 449: 1008-1013.

Hashimura Y, Ueguchi C 2011 The *Arabidopsis MERISTEM DISORGANIZATION 1* gene is required for the maintenance of stem cells through the reduction of DNA damage. Plant J. 68: 657-669.

Heimsch C, Seago JL Jr 2008 Organization of the root apical meristem in angiosperms. Am. J. Bot. 95: 1-21.

Hernández-Barrera A, Ugartechea-Chirino Y, Shishkova S, Napsucialy-Mendivil S, Soukup A, Reyes-Hernández V, Dong G, Dubrovsky JG 2011 Apical meristem exhaustion during determinate primary root growth in the *moots koom 1* mutant of *Arabidopsis thaliana*. Planta 234: 1163-1177.

Heyman J, Cools T, Vandenbussche F, Heyndrickx KS, van Leene J, Vercauteren I, Vanderauwera S, Vandepoele K, de Jaeger G, van der Staeten D, de Veylder L 2013 ERF115 controls root quiescent cell division and stem cell replenishment. Science 342: 860-863.

Heyman J, Kumpf RP, de Veylder L 2014 A quiescent path to plant longevity. Trends Cell Biol. 24: 443-448.

Hong JH, Chu H, Ghosh D, Gong X, Xu J 2015 A quantitative analysis of stem cell homeostasis in the *Arabidopsis* columella root cap. Front. Plant Sci. 6: article 206. Doi: 10.3389/fpls.2015.00206.

Iijima M, Morita S, Barlow PW 2008 Structure and function of the root cap. Plant Product. Sci. 11: 17-27.

Jiang K, Meng YL, Feldman LJ 2003 Quiescent center formation in maize roots is associated with an auxin-regulated oxidizing environment. Development 130: 1429-1438.

Jiang K, Zhang S, Lee S, Tsai G, Kim K, Huang H, Chilcott C, Zhu T, Feldman LJ 2006a Transcription profile analyses identify genes and pathways central to root cap functions in maize. Plant Mol. Biol. 60: 343-363.

Jiang K, Ballinger T, Li D, Zhang S, Feldman LJ 2006b A role for mitochondria in the establishment and maintenance of the maize root quiescent center. Plant Physiol. 140: 1118-1125.

Jiang K, Zhu T, Diao Z, Huang H, Feldman LJ 2010 The maize root stem cell niche: a partnership between two sister cell populations. Planta 231: 411-424.

Juniper BE, Groves S, Landau-Schacher B, Audus LJ 1966 Root cap and the perception of gravity. Nature 209: 93-94.

Kam WW-Y, Banati RB 2013 Effects of ionizing radiation on mitochondria. Free Radical Biol. Med. 65: 607-619.

Kamiya N, Nagasaki H, Morikami A, Sato Y, Matsuoka M 2003 Isolation and characterization of a rice *WUSCHEL*-type homeobox gene that is specifically expressed in the central cells of a quiescent centre in the root apical meristem. Plant J. 35: 429-441.

Kerk NM, Feldman LJ 1995 A biochemical model for the initiation and maintenance of the quiescent center: implications for organization of root meristems. Development 121: 2825-2833.

Kuroiwa T, Fujie M, Kuroiwa H 1992 Studies on the behaviour of mitochondrial DNA. Synthesis of mitochondrial DNA in a specific zone just above the quiescent center in the root meristem of *Pelargonium zonale*. J. Cell Sci. 101: 483-493.

Leach JK, Van Tuyle G, Lin P-S, Schmidt-Ullrich R, Mikkelsen RB 2001 Ionizing radiation-induced, mitochondria-dependent generation of reactive oxygen/nitrogen. Cancer Res. 63: 3894-3901.

Lee Y, Lee WS, Kim S-H 2013 Hormonal regulation of stem cell maintenance in roots. J. Exp. Bot. 64: 1153-1165.

Li X-Q, Zhang M, Brown GG 1996 Cell-specific expression of mitochondrial transcripts in maize seedlings. Plant Cell 8: 1961-1975.

Liso R, Innocenti AM, Bitonti MB, Arrigoni O 1988 Ascorbic acid-induced progression of quiescent centre cells from G1 to S phase. New Phytol. 110: 469-471.

Loeffler M, Potten CS 1997 Stem cells and cellular pedigrees – a conceptual introduction. In: Potten CS, Ed., Stem cells. Academic Press, London, pp. 1-27.

Mai CD, Phung NTP, To HTM, Gonin M, Hoang GT, Nguyen KL, Do VN, Courtois B, Gantet P 2014 Genes controlling root development of rice. Rice 7: article 30.

Mikkelsen RB, Wardman P 2003 Biological chemistry of reactive oxygen and nitrogen and radiation-induced signal transduction mechanisms. Oncogene 22: 5734-5754.

Müller B, Sheen J 2008 Cytokinin and auxin interaction in root stem-cell specification during early embryogenesis. Nature 453: 1094-1098.

Müller ML, Pilet P-E, Barlow PW 1993 An excision and squash technique for analysis of the cell cycle in the root quiescent centre of maize. Physiol. Plant. 87: 305-312.

Müller ML, Pilet P-E, Barlow PW 1994 Effect of abscisic acid on the cell cycle in the growing maize root. Planta 195: 10-16.

Narayanan PK, Goodwin EH, Lehnert BE 1997 α particles initiate biological production of superoxide anions and hydrogen peroxide in human cells. Cancer Res. 57: 3963-3971.

Nawy T, Lee J-Y, Colinas J, Wang JY, Thongrod SC, Malamy JE, Birnbaum K, Benfey PN 2005 Transcriptional profile of the *Arabidopsis* root. Plant Cell 17: 1908-1925.

Ni J, Shen Y, Zhang Y, Wu P 2014 Definition and stabilization of the quiescent centre in rice roots. Plant Biol. 16: 1014-1019.

Oparka KJ, Duckett CM, Prior DAM, Fisher DB 1994 Real-time imaging of phloem unloading in the root tip of *Arabidopsis*. Plant J. 6: 759-766.

Ortega-Martínez O, Pernas M, Carol RJ, Dolan L 2007 Ethylene modulates stem cell division in the *Arabidopsis thaliana* root. Science 317: 507-510.

Petersson SV, Johansson AI, Kowalczyk M, Makoveychuk A, Wang JY, Moritz T, Grebe M, Benfey PN, Sandberg G, Ljung K 2009 An auxin gradient and maximum in the *Arabidopsis* root apex shown by high-resolution cell-specific analysis of IAA distribution and synthesis. Plant Cell 21: 1659-1668.

Phillips HL Jr, Torrey JG 1971 The quiescent center in cultured roots of *Convolvulus arvensis* L. Am. J. Bot. 58: 665-671.

Pilet PE 1982 Abscisic acid, one of the endogenous growth inhibitors regulating root gravireaction. In: Wareing PF, Ed., Plant growth substances 1982. Academic Press, London, pp. 529-536.

Ponce G, Luján R, Campos ME, Reyes A, Nieto-Sotelo J, Feldman LJ, Cassab GI 2000 Three maize root-specific genes are not correctly expressed in regenerated caps in the absence of the quiescent centre. Planta 211: 23-33.

Pregitzer KS 2002 Fine roots of trees – a new perspective. New Phytol. 154: 267-273.

Raghavan V 1990 origin of the quiescent center in the root of *Capsella bursa-pastoris* (L.) Medik. Planta 181: 62-70.

Rando TA 2007 The immortal strand hypothesis: segregation and reconstruction. Cell 129: 1239-1243.

Rebouillat J, Dievart A, Verdeil JL, Escoute J, Giese G, Breitler JC, Gantet P, Espeout S, Guiderdoni E, Périn C 2009 Molecular genetics of rice root development. Rice 2: 15-34.

Reyes-Hernández JB, Srivastava AC, Ugartachea-Chirino Y, Shishkova S, Ramos-Parra PA, Lira-Ruan V, Díaz de la Garza RI, Dong G, Moon, J-C, Blancaflor EB, Dubrovsky JG 2014 The root indeterminancy-to-determinancy developmental switch is operated through a folate-dependent pathway in *Arabidopsis thaliana*. New Phytol. 202: 1223-1236.

Riley PA 1994. Free radical in biology: oxidative stress and the effect of ionizing radiation. Int. J. Radiat. Biol. 65: 27-33.

Rodríguez-Rodríguez JF, Shishkova S, Napsucialy-Mendivil, Dubrovsky JG 2003 Apical meristem organization and lack of establishement of the quiescent center in Cactaceae roots with determinate growth. Planta 217: 849-857.

Sabatini S, Heidstra R, Wildwater M, Scheres 2003 SCARECROW is involved in positioning the stem cell niche in the *Arabidopsis* root meristem. Genes Develop.

17: 354-358.

Sabatini S, Beis D, Wolkenfelt H, Murfett J, Guilfoyle T, Malamy J, Benfey P, Leyser O, Bechtold N, Weisbeek P, Scheres B 1999 An auxin-dependent distal organizer of pattern and polarity in the *Arabidopsis* root. Cell 99: 463-472.

Sabelli PA, Burgess SR, Carbajosa JV, Parker JS, Halford NG, Shewry PR, Barlow PW 1993 A molecular characterization of cell populations in the maize root apex. In: Ormrod JC, Francis D, Eds., Molecular and cell biology of the plant cell cycle. Kluwer Academic Publishers, Dordrecht, pp. 97-109.

Sánchez-Calderon L, López-Bucio J, Chacón-López A, Cruz-Ramirez A, Nieto-Jacobo F, Dubrovsky JG, Herrera-Estrella L 2005 Phosphate starvation induces a determinate developmental program in roots of *Arabidopsis thaliana*. Plant Cell Physiol. 46: 174-184.

Sarkar AK, Luijten M, Miyashima S, Lenhard M, Hashimoto T, Nakajima K, Scheres B, Heidstra R, Laux T 2007 Conserved factors regulate signalling in *Arabidopsis thaliana* shoot and root stem cell organizers. Nature 446: 811-814.

Scheres B, Wolkenfelt H 1998 The *Arabidopsis* root as a model to study plant development. Plant Physiol. Biochem. 36: 21-32.

Scheres B, di Laurenzio L, Willemsen V, Hauser M-T, Janmaat K, Weisbeek P, Benfey PN 1995 Mutations affecting the radial organisation of the *Arabidopsis* root display specific defects throughout the embryonic axis. Development 121: 53-62.

Sebastian J, Ryu KH, Zhou J, Tarkowská P, Cho Y-H, Yoo S-D, Kim E-S, Lee J-Y 2015 PHABULOSA controls the quiescent center-independent root meristem activities in *Arabidopsis thaliana*. PLoS Genet. 11: e1004973.

Sena G, Wang X, Liu H-Y, Hofhuis H, Birnbaum KD 2009 Organ regeneration does not require a functional stem cell niche in plants. Nature 457: 1150-1154.

Shaw S, Wilkins MB 1973 Source and lateral transport of growth inhibitors in geotropically stimulated roots of *Zea mays* and *Pisum sativum*. Planta 109: 11-26.

Shishkova S, Rost TL, Dubrovsky JG 2007 Determinate root growth and meristem maintenance in angiosperms. Ann. Bot. 101: 319-340.

Shishkova S, Las Peñas ML, Napsucialy-Mendivil S, Matvienko M, Kozik A, Montiel J, Patiño A, Dubrovsky JG 2013 Determinate primary root growth as an adaptation to aridity in Cactaceae: towards an understanding of the evolution and genetic control of the trait. Ann. Bot. 112: 239-252.

Skoog F, Miller CO 1957 Chemical regulation of growth and organ formation in plant tissue cultured in vitro. Symp. Soc. Exp. Biol. 11: 118-131.

Sozzani R, Cui H, Moreno-Risueño MA, Busch W, van Norman JM, Vernoux T, Brady SM, Dewitte W, Murray JAH, Benfey PN 2010 Spatiotemporal regulation of cell-cycle genes by SHORTROOT links patterning and growth. Nature 466: 128-132.

Takatsuka H, Umeda M 2014 Hormonal control of cell division and elongation along different trajectories in roots. J. Exp. Bot. 65: 2633-2643.

Torrey JG 1972 On the initiation of organization in the root apex. In: Miller MW, Kuehnert CC, Eds., The dynamics of meristem cell populations. Plenum Press, New York–London, pp. 1-10.

Tsukagoshi H, Busch W, Benfey PN 2010 Transcriptional regulation of ROS controls transition from proliferation to differentiation in the root. Cell 143: 606-616.

Tucker MR, Laux T 2007 Connecting the paths in plant stem cell regulation. Trends Cell Biol. 17: 403-410.

Ühlken C, Horvath B, Stadler R, Sauer N, Weingartner M 2014 MAIN-LIKE1 is a crucial factor for correct cell division and differentiation in *Arabidopsis thaliana*. Plant J. 78: 107-120.

Van den Berg C, Willemsen V, Hage W, Weisbeek P, Scheres B 1995 Cell fate in the *Arabidopsis* root meristem determined by directional signalling. Nature 378: 62-65.

Van den berg C, Willemsen W, Hendricks G, Weisbeek P, Scheres B 1997 Short-range control of cell differentiation in the *Arabidopsis* root meristem. Nature 390: 287-289.

Veit B 2006 Stem cell signalling networks in plants Plant Mol. Biol. 60: 793-810.

Vilarrasi-Blasi J, González-García M-P, Frigola D, Fàbregas N, Alexiou KG, López-Bigas N, Rivas S, Jauneau A, Lohmann JU, Benfey PN, Ibañes M, Caño-Delgado AI 2014 Regulation of plant stem cell quiescence by a brassinosteroid signalling module. Development. Cell 30: 36-47.

Wawrecki W, Zagórska-Marek B 2007 Influence of a weak DC field on root meristem architecture. Ann. Bot. 100: 791-796.

Webster PL, Langenauer HD 1973 experimental control of the activity of the quiescent centre in excised root tips of *Zea mays*. Planta 112: 91-100.

Weijers D, Schlereth A, Ehrismann JS, Schwank G, Kientz M, Jürgens G 2006 Auxin triggers transient local signalling for cell specification in *Arabidopsis* embryogenesis. Development. Cell 10: 265-270.

Wenig U, Meyer S, Stadler R, Fischer S, Werner D, Lauter A, Melzer M, Hoth S, Weingartner M, Sauer N 2013 Identification of MAIN, a factor involved in genome stability in the meristems of *Arabidopsis thaliana*. Plant J. 75: 469-483.

Wildwater M, Campilho A, Perez-Perez JM, Heidstra R, Blilou I, Korthout H, Chatterjee J, Mariconti L, Gruissem W, Scheres B 2005 The *RETINOBLASTOMA-RELATED* gene regulates stem cell maintenance in *Arabidopsis* roots. Cell 123: 1337-1349.

Yakes FM, van Houten B 1997 Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. Proc. Natl. Acad. Sci. 94: 514-519.

Zakhvataev VE 2015 Tidal variations of radon activity as a possible factor synchronizing biological processes. Biophysics 60: 140-156.

Zamioudis C, Mastranesti P, Dhonukshe P, Blilou I, Pieterse CMJ 2013 Unravelling root developmental programs initiated by beneficial *Pseudomonas* spp. bacteria. Plant Physiol. 162: 304-318.

Zhang H, Han W, de Smet I, Talboys P, Loya R, Hassan A, Rong H, Jürgens G, Knox JP, Wang M-H 2010 ABA promotes quiescence of the quiescent centre and suppresses stem cell differentiation in the *Arabidopsis* primary root meristem. Plant J. 64: 764-774.

Zhang Y, Jiao Y, Liu Z, Zhu Y-X 2015 ROW1 maintains quiescent centre identity by confining *WOX5* expression to specific cells. Nat. Commun. 6: 6003, doi: 10.1038/ncomms7003

Zhou B-B S, Elledge SJ 2000 The DNA damage response: putting checkpoints in perspective. Nature 408: 433-439.

Peter William Barlow is presently Honorary Research Fellow at the School of Biological Sciences, Bristol University.