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Recent progress in the study of primary root development and physiology: Emphasis on the stem cell biology

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ABSTRACT

Stem cells are clonogenic precursors whose daughter cells can either remain stem cells or undergo differentiation. These cells are influenced by positional information from the stem cell niche. Auxin has a determining role on the establishment and maintenance of the stem cell. Auxin might maintain the stem cell status by auxin-induced oxidative stress in the quiescent center (QC). Several transcription factors are found to specify the QC, and one of them, RB-like protein, shares the common controlling mechanism with the RB protein. Recent techniques open the possibility to understand the root development more specifically and globally. Recent trend to use the RNAi technology and to understand the mechanism of miRNA (and siRNA) in plants helps to find other controlling mechanisms of root development.

Key words : root apical meristem, initials, stem cell, stem cell niche, quiescent center (QC), auxin, redox, oxidative stress

Introduction

Both in plant and animal systems, stem cell research has been one of hot areas for the last few years. Pluripotency (or totipotency) has been studied for a long time to answer the question how the cell's fate is determined, in the animal system, especially in the medical field, this study contributes to fulfill the human desire to cure many diseases. Plant stem cells and animal stem cells have common characteristics of being influenced by the microenvironment, the stem cell niche. In this review, the recent findings in plant stem cell research in the root system will be discussed.

Apical anatomy of the plant root

A huge progress in the study of development of root comes from mutant characterizations and analyses of cell- and tissue-specific gene expression in Arabidopsis (12 and references therein). Also, findings from peas and corn also contributed to the understanding of root development (17, 39).

Fig 1 shows the apical region of Arabidopsis root. The Arabidospis root has a simple, concentric structure. From

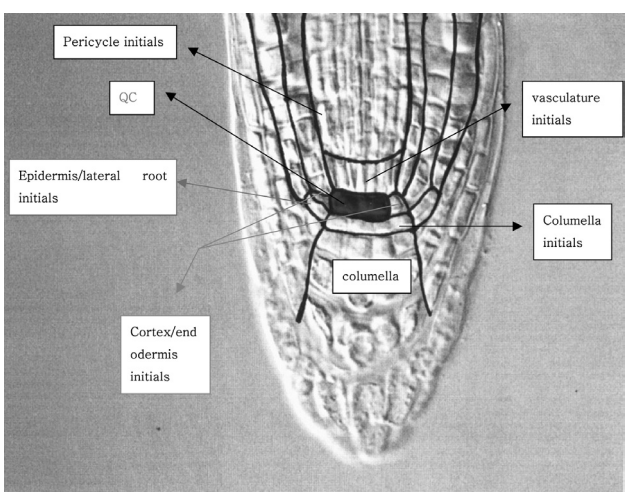

Fig 1. Organization of the root meristem

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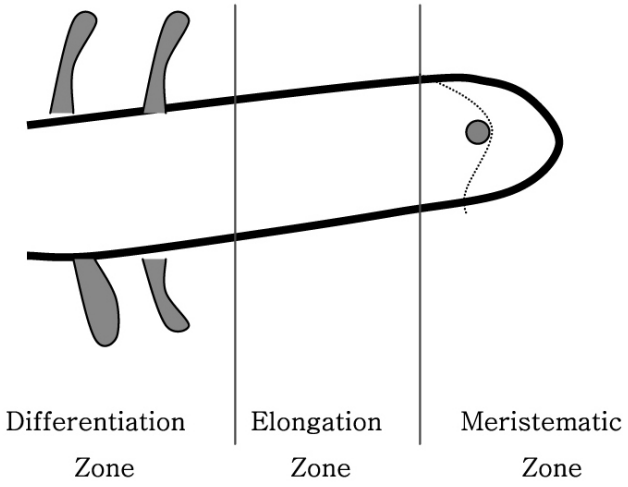

Fig 2. Linear development from the tip to the base (i.e. right to left) Circle: QC; Extrusions: root hairs

the outside to the inside, epidermis, cortex, endodermis, pericycle, vasculature are sequentially layered. There are three distinct zones representing the two developmental stages (22), meristematic zone at the root tip followed by the elongation zone, finally, the differentiation zone (Fig 2).

Stem cell and stem cell niche concept in plants

Stem cells are clonogenic precursors whose daughter cells can either remain stem cells or undergo differentiation (49). They show ability for unlimited proliferation, self-maintenance and self renewal so that they have the potential to differentiate descendants. Their stem cell fates are transient because their fate can be altered by cues from the environment (10). Local regulators from the surrounding of the stem cell control the stem cell's fate. This microenvironment is called 'stem cell niche'. Plant cell's niches are the meristems. Stem cells divide infrequently, and their descendants do not directly differentiate, but constitute an intermediate cell population of more rapidly dividing progenitors. This population has cells whose differential potential is somewhat restricted. Stem cell division is basically asymmetric because two different types of progeny are made. There are two different types of strategy to achieve this asymmetric division (79). One is divisional (invariant) asymmetry where, after division, one cell remains as stem cell another cell differentiates or become a transit amplifying cell. Another strategy is populational (or environmental) asymmetry where daughter cells have to be either stem cells or differentiating cells. The later strategy is under more extrinsic control than the former one.

The stem cells in the root meristem is also called "root initials" and they give rise to all the cell types in each layer by divisions to produce clonally related files of cells. The initial cells, as in divisional asymmetry, divide and make one initial cell and one transit amplifying cell. The later cell divides further and differentiates.

Quiescence center (QC)

The initial cells surround mitotically inactive cells positioned at the point of lineage convergence, and this is demonstrated by using radiolabeled DNA precursors and autoradiography. Clowes termed these cells as quiescent center, QC (13, 14, 16). He also showed that there were mitotically active cells around the QC and that the location of these cells could shift as the size of the QC changed (Fig 3). This means that an initial cell is defined by the location within the root apical meristem (RAM), but not by any inherent properties or lineage. A daughter cell from an initial

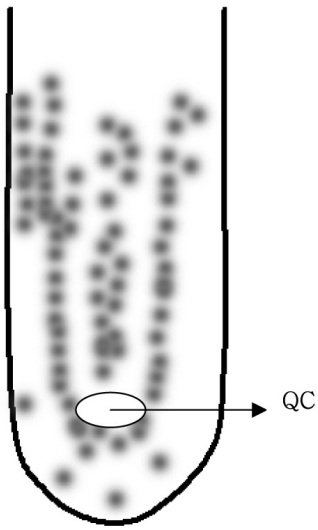

Fig 3. Schematic diagram of an autoradiograph of the ^{3}H-labeled maize root tip. The gray dots show cells performing active cell division (DNA replication.). Notice that there is no dot in the QC region. [redrawn from (39)].

cell, which has contact with the QC remains as an initial cell and another daughter cell which is separated from the QC differentiates. In this case, positional information determines the stem cell state. QC cells send an unknown signal which helps the initials to maintain the stem cell status. This idea was elegantly tested by using laser ablation experiment in Arabidopsis (83, 84). The ablated cells can be replaced by their surrounding cells, which then acquire the identity.

This brings the concept of stem cell niche by Schofield who mentioned that stem cells were located in the microenvironments that provide signals to maintain their undifferentiated cell state (74). Recently, this concept was applied to animals (78) and plants (1, 49). QC and initial cells in the root form the stem cell niche. There are different views whether the QC cells should be regarded as stem cells or not. It has been thought that QC cells are stem cell organizers (49, 1, 79). In a recent review, QC cells were considered as stem cells by dividing the initials into functional initials and structural initials (40, 5). Anyway, QC cells are one of the most important cells in root development and can act as integrators for many processes and events requisite for root meristem establishment and maintenance (40).

The function of root cap

There is a seemingly insignificant and small organ which is called a root cap at the very end of the root. This small organ has many biochemical and biophysical processes that are very important for the survival of the whole plant (77). Root cap perceives gravity and transduces the signal to the growth zone of root proper in the form of second signal. This second signal has been thought to involve a mobile plant hormone, auxin. This hormone redistributes in the root after gravistimulation, which causes differential growth in the elongation zone just behind the root meristem. Another role of the root cap might be the regulation of mitosis in the root, because after removal of the root cap caused cell division in the QC (15).

Derivatives from the asymmetric division of hypophysis are both QC (upper) and root cap (lower). QC is established in the octant stage of embryogenesis (73). Therefore, it is reasonable to ask whether root cap is required for root meristem establishment and maintenance. The answer is yes. There is no cap-less primary root in nature (86) and there is no root cap defected mutant whose mutation affected the hypophysis in Arabidopsis (91). The importance of root cap in the meristem function was shown by a genetic method, instead of using laser ablation method (82, Fig 4). They used a cholera toxin gene attached to the root cap specific promoter (promoter of Arabidopsis *RCP1*) to kill the root cap. Also, when the root cap is removed, the QC activates and the meristem is altered (25). Root meristem function is regulated by the root cap border cells in pea (92). The regulation of cap size and pattern of cap cell differentiation are related the distribution of auxin and pattern of sugar metabolism (6 and references therein). Existence of communication between the root cap and QC was suggested from the decapping experiments (25, 50) and from laser ablation (83, 84). Auxin might be one of responsible molecules for this communication (40, 6). One of the recent findings in this field is the involvement of micro- RNA targeted auxin response factors (87). Overexpression of micro-RNA, miR160, repressed the expression of ARF10 and ARF16. ARF10 and ARF16 restrict the stem cell niche, promote columella cell differentiation, and play an important role in root cap development.

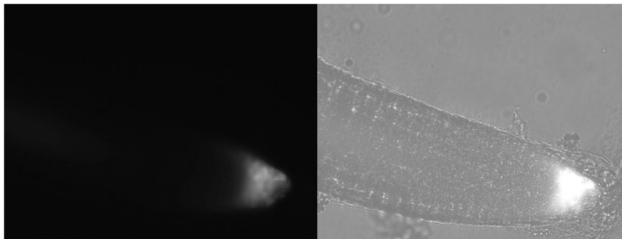

Fig 4. Expression of the RCP1 promoter:: GFP at the root cap (left) and the same photograph when the UV filter is removed [Unpublished personal datum]

The role of auxin and transcription factors involved

One of the most important factors that pattern the early embryo is auxin. Auxin satisfies the criteria of a hormone, because it can transport long-distance and triggers the developmental events (26, 81). Auxin is a morphogen which di-

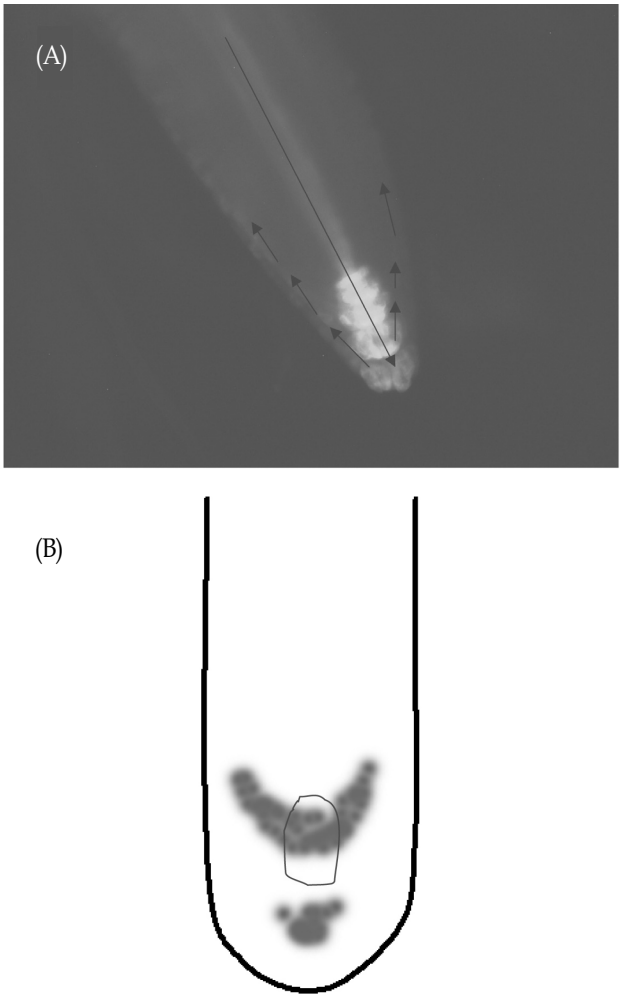

Fig 5. DR5::GFP response (A) and diagram of the root tip showing the direction of polar auxin transport (red arrows). The GFP signal shows the 'auxin maximum'. Bottom photograph (B) shows the change of auxin distribution (brushed dots) when an auxin transport inhibitor, NPA, is treated. The red circle indicates the auxin maximum when NPA is absent.

rects the differentiation and determination of plant form (88), and a neurotransmitter-like substance which can move intercellularly via vesicular trafficking (7, 3, 4). For the last criterion, F-actin is involved in the process (7 and references therein).

Auxin is polarly transported and its carrier or response mutants show defects in the root. This feature is due to the spatially distinct acropetal and basipetal auxin transport system in the root tip (Fig 5). Although it is not known of its mechanism, auxin maximum forms near to the root tip showing strong accumulation of auxin within the QC (67). This maximum can be visualized by using a synthetic auxin-responsive promoter (DR5) driving the GUS or GFP reporter gene. Application of high doses of auxin or treatment of auxin transport blockers cause dramatic morphogenic alterations, such as ectopic QC and stem cell formation. Auxin might position the stem cell niche in the developing root, because the auxin maximum matches the stem cell niche in the root. When the auxin maximum artificially moved upward in the root, the QC followed the auxin maximum, reforming the endodermal cell file (67). Recently, two AP2 type transcription factors, *PLT1* and *PLT2* are cloned and analyzed for their function (1). These genes are required for the QC specification and stem cell activity and they are all induced by auxin. Auxin accumulation in the embryo activates ARF genes, *MONOPTEROS* (*ARF5*) (34), *BODENLOS* (*BDL, IAA12*) (32) and then activates the transcription of *PLT* genes. These transcription factors will upregulate the QC specifying genes such as *SHORTROOT* (*SHR*) (8) and *SCARECROW* (*SCR*) (71, 68, 21) that are members of GRAS family of transcription factors. Therefore, auxin could act as a signal for all SCR-expressing cells to acquire QC identity (68). Besides *PLT* genes, other auxin-related genes such as *AUX1* (80), *AXR1* (67), *AXR3* (67), *AXR6* (36), *PIN1* (30), *PIN3* (29), *PIN4* (28), *HOBBIT* (*HBT*) (91, 11) play important roles in root development.

The role of other transcription factors

WOX5 and QHB

WUSCHEL (WUS) is an atypical homeodomain protein that specifies the stem cells in the shoot apical meristem. The WUS-like homeodomain proteins, WOX5 and QHB, are found in Arabidopsis and rice, respectively (31, 44). Both specifically express in the QC cells and are thought to be important in QC specification.

RBR

QC cells are arrested at the G1 phase of the cell cycle, and this G1 restriction point is regulated by retinoblastoma protein (89). Root meristem-specific knock-out of the RB pro-

tein by RNAi led to supernumerary stem cells, and its overexpression dissipated stem cells prior to arresting other mitotic cells (90). Their results suggested that RBR regulated the size of the stem cell population.

Possible role of redox on root development and other physiological responses

Many cellular processes and their direction depend on redox state or redox homeostasis (70, 27). Reactive oxygen species play an important role as messengers in plants and vertebrates (33, 56). Also antioxidants such as ascorbate, glutathione, and tocopherol are redox buffers that influence the redox state in the cell and tissue (27). The levels and ratios of the reduced and oxidized forms of redox couples (i.e. GSH/GSSG, AA/DHA) determine the tissue's overall redox status. Redox (or ROS) influences many physiological and developmental processes in plants (Table 1).

Regarding the root apical meristem development, redox status of the QC has an important role for its maintenance and activation. The QC is more oxidized than the neighboring cells which divide more rapidly than the QC cells (41, 46, 51, 69). It was proposed that auxin changed the redox state of the QC and then affected the cell cycle (36). Genetic studies on an Arabidopsis mutant, *rml*1, showed that the root meristem formed normally, but its derivatives disorganized after germination (85). There was a mutation in the gamma-glutamylcysteine synthetase gene which is the first enzyme in glutathione synthesis. This mutant had low glutathione level compared to that of wild type. It provided genetic evidence that redox state affects the cell division and organization. AA treatment increases the rate of cell division in plant cells (46, 51, 52), and DHA treatment showed the opposite effect to the AA treatment (62, 63). Cells in G1 state of the cell cycle are sensitive to oxidative stress so that they are arrested at that stage (64, 37, 43). This has to do with the QC cells because they are arrested at the G1 stage and they have low level of reduced glutathione (GSH) and AA (41). Facts that ascorbate oxidase (AAO) can degrade IAA and can be transcriptionally activated by auxin led to the proposal that natural rhythmic relationship among the level of auxin, AAO level and the activity of cell division at the QC could be established (40, 41, 45). In this case, the root apex and the QC should catabolize IAA to create an auxin sink for more auxin (39, 45, 61).

The correlation between high level of auxin and oxidative stress came from several findings that auxin can generate H_2O_2, O^-, and other ROS (42, 60, 75, 76) and it induces NADH oxidase (53, 55).

Table 1. Physiological or developmental processes affected by oxidative stress

Physiological or developmental process involved	References
Cell cycle progression and cell division	(35), (48), (59), (64), (20)
Apoptosis	(19)
Leaf elongation	(65), (76)
Somatic embryogenesis	(23), (57), (18), (54)
Gravitropism	(42)
2,3,5-tribenzoic acid (TIBA)-like disturbance of auxin distribution	(58)
Maintenance of QC	(39)

Recent approaches using root genomics

Root genomics become one of powerful tools to understand the developmental network in root. A number of genes have been identified, which are important for pattern formation, cell cycle, hormone signaling etc. to understand the developmental processes in the root. However, to understand the regulatory networks that determine the cell identity, direction of cell division etc, much more information is needed using techniques for separating tissues in the root and also performing the global expression analysis (72). Because root has a mixture of tissues with different developmental stages from the tip to the basal part of the plant, it is essential to obtain RNA from specific stages and/or cell types. Fluorescence-activated cell sorting (FACS) is used to purify the stage-specific or tissue-specific cells after their promoter is fused to GFP (33). This method was adopted by Birnbaum *et al*. (9) using protoplasting the root tissues. For enriching the tissue-specific RNA, cell-type specific epitope-tagged RNA binding proteins (66), laser-assisted microdissection of specific cells or tissues (24, 2) could be used. After enriching RNAs, microarray technology or serial analy-

sis of gene expression (SAGE) can be used to analyze the gene expression pattern.

Conclusion

QC and root initials are in the stem cell niche that influences each other in both directions. The responsible signal or molecule that inhibits differentiation of the initials is not known, but plant hormone, auxin, could specify the QC status. Downstream of auxin, there are several transcription factors which play critical role on QC specification and cell cycle arrest at the G1 stage. One of the factors that maintain the QC status is auxin-induced oxidative stress and auxin catabolism. Improving technologies will help us to understand the root development globally.

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