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Research article

Redox regulation of root apical meristem organization: Connecting root development to its environment

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ABSTRACT

Post-embryonic root growth relies on the proliferative activity of the root apical meristem (RAM), consisting, in part, of cells with juvenile characteristics (stem cells). It is generally, but erroneously held that the RAM indefinitely produces new cells throughout the lifespan of a plant, resulting in indeterminate root growth. On the contrary, convincing data, mainly from the lab of Thomas L. Rost, show in all species analyzed so far, including *Arabidopsis*, that RAM organization changes over time in parallel with both a cessation of the production of new cells, and a consequent reduction in root growth, even under optimal conditions. In addition, RAM organization evolved to become highly plastic and dynamic in response to environmental triggers (e.g. water and nutrient availability, pollutants). Under unfavourable conditions, the RAM is rapidly reorganized, and, as a result of the cessation of new cell production at the root tip, root growth is altered, and lateral root production is enhanced, thus providing the plant additional strategies to overcome the stress. It is now becoming increasingly clear that this environment-responsive developmental plasticity is linked to reactive oxygen/nitrogen species, antioxidants, and related enzymes, which form part of a complex signalling module specifically operating in the regulation of RAM functioning, in strict relationship with hormonal control of root development exerted by auxin, gibberellins and cytokinins. In turn, such redox/hormone crosstalk regulates gene expression.

1. Introduction

Plant primary (axial) growth is dependent on the presence of actively proliferating cells (meristems) at the shoot and root apex, respectively. In recent years root apical meristem (RAM) organization has been investigated as a "simple" model system to study stem cell biology [58]. However, the apparent simplicity of RAM organization is the result of complex interactions between genetic, hormonal and biochemical factors strictly connected and intermingled. Indeed, RAM establishment and maintenance during plant life is definitely more dynamic and plastic than one could conclude from observing the stereotypical production of new cells in young roots of the model plant *Arabidopsis* under experimental conditions. In addition, it is increasingly clear that RAM organization is very sensitive to environmental cues and that it is able to change accordingly, thus altering the basic "simple" developmental program in order to ensure plant survival under unfavourable conditions.

Such dynamic behaviour of the RAM can be explained most insightfully in terms of the efficient integration of an array of signals in the whole plant during development. In this framework there is mounting evidence of a pivotal role of redox-mediated signalling in RAM organization and dynamics. This article is aimed at summarizing our present knowledge of this interesting, emerging field of research, in parallel with a general discussion on RAM evolution, structure, and function.

1.1. Origin and structure of the root apical meristem

Root appearance and development were critical steps in plant adaptation to terrestrial environments. No roots occurred in early, small-sized land plants, like *Rhynia* and *Cooksonia* [59], but increase in plant size and wider land colonization could only occur in parallel with proper development of a root system, ensuring, among other functions, anchorage to the soil and nutrient uptake. Cells produced in the RAM undergo differentiation and eventually

Abbreviations: ACO, 1-Aminocyclopropane 1-carboxylate oxidase; ASC, Ascorbic acid; DHA, Dehydroascorbic acid; GalL, L-galactono-γ-lactone; GSH, Glutathione (γ-glutamyl-cysteinyl-glycine); GSSG, Glutathione disulfide; IAA, Indole Acetic Acid (auxin); QC, Quiescent center; RAM, Root apical meristem; RNS, Reactive nitrogen species; ROS, Reactive oxygen species.

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form the specialized tissues of the primary root. Whether the RAM was derived from a dichotomy of the shoot apical meristem (SAM) remains a matter of debate. Interestingly, two related *Arabidopsis* transcription factors expressed in the RAM, (*WOX*5) and the SAM (*WUSCHEL*), respectively, seem to have the same function in maintaining meristem organization [77]. The work of Ueda et al. [92] reporting mutations affecting both RAMs and SAMs supports the idea that the RAM originated as a result of SAM transformation. Nevertheless, the obvious differences observed between the RAM and SAM suggest early divergence and distinct evolution of the two organs.

Based on paleobotanical evidence, root structures evolved independently at least twice, in Lycophytina and Euphyllophytina [50]. In lycopods and ferns the production of new cells is accomplished by a single, three-sided apical cell [22], whose division yields initials undergoing differentiation. From time to time division of an apical cell produces a second apical cell which keeps dividing, thus yielding a bifurcating structure. In Euphyllophytina, RAM organization underwent a complex evolution. In Gymnosperms and some basal Angiosperms (including Magnoliids), a group of apparently unspecialized common initials produces all cells, whereas different patterns of RAM organization are observed in more recently evolved plants, with discrete groups of initials operating in a coordinated fashion [37,39]. Notably, in their outstanding work, Heimsch and Seago [39] describe no less than 15 different RAM types in Angiosperms, suggesting that RAM evolution was not a straightforward process, but rather a fascinating series of attempts, some of which resulted in successful root development.

While *Arabidopsis* has undoubted advantages for molecular studies of root development, limiting our views on RAM organization to this plant deprives us of the richness and diversity of developmental pathways and processes that lead to RAM patterning, maintenance and plasticity, and might even lead to the misconception that the typical *Arabidopsis* RAM structure is ubiquitous in plants. Among the different RAM types a major distinction is made between “open” and “closed” meristems [97]. In open meristems initials are not separated by distinct boundaries, whereas groups of initials are clearly distinguishable in closed meristems. Interestingly, in some species changes from a closed to an open RAM have been observed at different developmental stages [39]. The functional differences between the two types of RAM definitely deserve more investigation. Roots with closed apical organization do not release border cells, thereby suggesting that roots with different apical organization types have different relationships to soil-borne micro-organisms [38]. The *Arabidopsis* RAM, as for other *Brassicaceae,* [39] is a three-tiered, closed RAM, with separate tiers for the stele, cortex and columella (Fig. 1). The tiny roots of *Arabidopsis* have an exceptionally small RAM, with a low number of initials. All three groups of actively proliferating initials are in close contact with the cells characterized by relatively slower mitotic activity, forming the quiescent center (QC), although the work of Baum and others [9,10] shows that cell division activity can be observed in the central initial cells in *Arabidopsis* and that there rarely, if ever is a period when they are inactive. Four to seven cells in the *Arabidopsis* RAM form the core of the QC, but also cells in the immediate vicinity of the core have a relatively extended cell cycle [15,33], and should be considered as QC cells *sensu* Clowes [19], in contrast to the tendency of considering just the core cells as proper QC cells [26,80]. In species with larger primary roots, having a much higher number of both QC and initial cells [45], the QC is made up of a heterogeneous population of relatively fast- and slow-cycling cells. Barlow [7] suggested a distinction between “structural initials” for QC slow-cycling cells, and “functional initials” for relatively faster-cycling, surrounding cells. The QC surely has a pivotal role in RAM organization and maintenance [8,45], although its regulation is only partially understood. As it will be discussed subsequently, the QC likely serves as the focus of a complex and dynamic redox regulation of RAM organization. It is worth mentioning that, according to some researchers, initial cells and the QC are equivalent to animal stem cells and the stem cell niche, respectively (see e.g. [58,80]). Although caution is advisable in drawing oversimplified parallels, especially in the light of recent findings obtained in animal studies [3], clearly both plants and animals share the necessity of balancing the production of new cells and their differentiation. As well, the existence of similar or related molecular mechanisms involved in maintaining meristem/stem cell identity cannot be excluded.

1.2. Changes in RAM organization and cessation of growth

The notion that the RAM continuously produces new cells, resulting in indeterminate root growth throughout the plant's lifespan is another widespread misconception. Primary roots of some plants (e.g. some *Cactaceae*) show constitutive, determinate growth, i.e. they are genetically programmed to stop producing new cells a few days after germination, opening the way to an increased production of lateral roots [28]. Haustorial roots of parasitic plants also have constitutive, determinate growth, and cessation of growth occurs when RAM organization changes, giving rise to a specialized structure (a haustorium) which, penetrating the host tissues and connecting to its vascular system, captures water and nutrients [98]. Early studies on the genus *Striga* showed that determinate growth and changes in RAM structure are induced by a “haustoria inducing factor”, but the RAM can reorganize and resume growth upon its removal [84]. This data is of particular interest, since the “haustoria inducing factor” might be related to strigolactones, a recently identified class of phytohormones whose biological role is still under investigation [93].

The above-mentioned cases of determinate root growth might seem particular to very specialized plants, but a closer look at available (although often neglected) data shows that beyond any doubt the plasticity of RAM organization and determinate growth are a general rule among plants. As an example, indeterminate growth of the primary root of *Arabidopsis* grown under optimal conditions ceases after 4 or 5 weeks, in parallel with changes in RAM organization [10,24], as has also been reported for several other species [16]. Interestingly, growth cessation can occur much earlier in response to specific environmental conditions. Phosphate deficiency induces early growth arrest by causing progressive differentiation of all RAM cells [75]. However, if the root is returned to P-sufficient medium (1 mM) when some meristematic cells are still present, RAM organization and growth can be re-established. Pea root tips are also known to reorganize in different patterns, depending on how much of the tip is excised [70], providing yet another excellent example of root tip plasticity. Altered RAM organization and irreversible determinate growth are also induced in roots infected with pathogenic or mycorrhizal fungi, even though the fungal hyphae are not in contact with the root tip [34], suggesting the existence of a long-distance signalling mechanism inducing growth arrest and early differentiation of RAM cells.

Several lines of evidence suggest that RAM maintenance and indeterminate growth are strictly dependent on the establishment and functionality of the QC. This was clearly observed in the above-mentioned *Cactaceae* showing constitutively determinate growth, in which the QC is present for a very short time, if any [71]. Also, in conditions of P deficiency, which induce cessation of growth within 14 d after germination, QC identity (as evaluated by the expression of the marker gene *QC46::GUS*) was lost by day 8, and cell proliferation in the RAM (expression of *CycB1-1::GUS*) ceased shortly

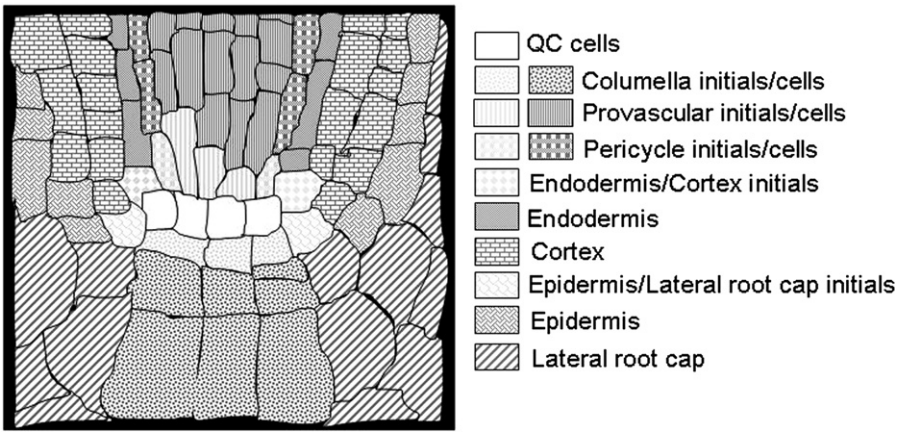

Fig. 1. Schematic representation of the *Arabidopsis* root apical meristem. The four quiescent center (QC) cells (structural initials *sensu* Barlow) are in physical contact with all functional initials (light gray). The QC *sensu* Clowes does not include columella initials [83].

thereafter [75]. In the next section, the origin and maintenance of the QC will be discussed.

1.3. Auxin and PLETHORA genes as main players in RAM organization

More than a century after its discovery the mechanisms of QC formation and maintenance, let alone its function, are still far from understood [8]. First, it should be borne in mind that the "quiescence" of QC cells is defined in relation to the surrounding fast-cycling cells, but the QC is far from inactive. Almost all maize QC cells divide within 120 h from incubation, as shown by the use of labelled thymidine [83], and it has been demonstrated that QC cells derivatives can serve as a source of new initials [10,53]. This feature of the QC is a remarkable difference in comparison to animal "stem cell niches", which, as far as we know, cannot replace stem cells proper [78]. In parallel with its function as a strategic reservoir of initials, elegant experiments of laser ablation of targeted QC cells in *Arabidopsis* showed that initials adjacent to the ablated cell underwent rapid differentiation, demonstrating that QC cells produce a short-range signal (still unknown, but apparently in part related to *WOX*5 expression, [77]) inhibiting differentiation, i.e. maintaining meristematic activity ("stemness") of adjacent cells [94]. These data strongly support the notion that indeterminate growth can only occur if the QC is present and active, since cell differentiation leads to rapid RAM exhaustion. Several attempts have been made to identify the genes responsible for the identity of QC cells. Forward genetic approaches were unsuccessful, so other strategies were devised.

In an ambitious attempt to unravel the complete transcriptome of QC cells, Nawy et al. [58] analyzed mRNAs from protoplasts expressing the *AGL*42 gene, coding for a MADS-box transcription factor which had been found to be expressed preferentially (but not exclusively) in QC cells, leading to the identification of 290 putative QC-enriched transcripts (62 of unknown function). The transcription factor *PLETHORA*1 (*PLT*1, At3g20840), which had been previously identified for its expression in the QC [2] (see below), was included in the 290, but, surprisingly, *PLT*2 (At1g51190) and *PLT*3 (At5g10510), also highly expressed in the QC [35], were not reported by Nawy et al. [58]. Although protoplasting appeared to be the only methodology for working with the tiny *Arabidopsis* QC, this approach could have been a source of error, since the procedure used [13], employing a highly complex fungal cell walls digesting protein mixture, is very likely to also induce changes in gene expression. Otherwise it would be very difficult to explain why small-sized, thin-walled, slowly-dividing QC cells should express at high rates cell wall related genes (such as those encoding extensin, expansin, and cellulose synthase) or disease-resistance genes (perhaps induced by fungal enzymes used to degrade the cell wall?) [58]. Interestingly, in spite of such inconsistencies, this putative QC transcriptome has been recently used as a blueprint of QC formation in regenerating *Arabidopsis* roots [80].

Recent transcriptome data of the maize QC [48], obtained without protoplasting, in part parallels the *Arabidopsis* expression profile, and includes transcripts associated with transcriptional regulators, DNA replication/repair and auxin. Two groupings not reported for the *Arabidopsis* QC, but detected in the maize QC, include a relatively large number of upregulated transcripts for mitochondrial/plastid genes, and a relatively large group of transcripts associated with regulating redox status, whereas only a thioredoxin was reported by Nawy et al. [58]. Additionally, for maize, and not reported for *Arabidopsis*, were a large number of down-regulated transcripts, encoding enzymes that function in various metabolic pathways, including proteins involved with sugar metabolism/glycolysis (e.g. glyceraldhyde-3-phosphate dehydrogenase, vacuolar ATPase), and a range of transcription factors, including six which encode GATA types of zinc finger proteins [48].

Additionally, for *Arabidopsis,* other smaller-scale analyses of genes preferentially expressed in the QC provided information not confounded by the protoplasting approach. A promoter trap analysis, with random insertion of T-DNA harbouring a promoter-less *β-glucuronidase* (*GUS*) reporter gene, identified in the QC two *PLETHORA* (*PLT*) genes coding for AP-2 type transcription factors [2]. Compared to WT plants, double *plt* mutants show more tiers of columella cells with abundant starch granules, a sign of early differentiation, and reduced or absent expression of three QC specific markers (*QC25*, *QC46* and *QC184*), suggesting loss of cell identity [2]. Further studies identified two more *PLT*-related genes, *PLT*3 and *BABYBOOM* (*BBM*), whose expression partly overlaps the *PLT*1 and *PLT*2 expression domain [35]. The combined expression of the four *PLT* genes defines a gradient apparently instructive for the different cell functions within the RAM: high expression of all 4 genes defines QC cells, whereas lower dosage occurs in relatively faster-cycling cells, and lowest *PLT* expression within the RAM occurs in cells starting the differentiation program [35]. Therefore, "structural initials" *sensu* Barlow would be those cells with higher total *PLT* expression, whereas lower expression would occur in neighbouring "functional initials". Of course, the existence of a gradient excludes the possibility of tracing a rigid barrier between the two kinds of cells. On the contrary, this possibility of gradually

changing identity (and behaviour) according to *PLT* expression dynamics could be another relevant factor for RAM plasticity, ultimately traceable to an underlying auxin gradient.

Having demonstrated that graded expression of *PLT* genes acts as the master regulator of RAM organization, it remains to be established which factor(s) operate upstream and downstream of *PLT* expression. There is no doubt that auxin transport has a key role in guiding both embryonic and post-embryonic development [73]. Auxin, synthesized in the aerial part of the plant, is transported to the root tip *via* AUX (influx) and PIN (efflux) facilitators [32]. A relevant auxin amount is also synthesized in the root apex [63], and has been shown by Feldman [31] to influence the movement of acropetally (polarly) transported auxin in the root. When the auxin-sensitive synthetic promoter DR5 is used to drive the expression of Green Fluorescence Protein (GFP), maximum auxin accumulation is observed in QC and columella cells (Fig. 2A). Interestingly, according to recent data [63], using an innovative approach for IAA measurement in different cells of the *Arabidopsis* root, columella cells would have much lower IAA than could be inferred from DR5::GFP signal, whereas QC cells apparently accumulate the highest auxin amount. This is probably due to the fact that the GFP turnover in columella cells is slower than IAA degradation itself, yielding a persistent signal. However, auxin levels reported [63] give a picture taken at one point in time, and surely auxin levels are dynamic in the root. In addition, the auxin measurements are from large populations (150 000+) of cells [63]. So these are averages and again do not allow us to relate auxin concentrations/changes to specific events.

Auxin triggers the expression of *PLT* genes [2,35]. Consistently, homozygous triple *plt*1*plt*2*plt*3 mutants show little or no expression of *PIN*1, *PIN*2 and *PIN*3 genes [35], indicating that PLT proteins and auxin fluxes are interdependent, so that graded auxin distribution in the RAM induces graded *PLT* expression and different behaviour of RAM cells [36].

This apparently simple model is complicated by the identification of many other genes coding for transcription factors, whose expression or repression affect RAM size and organization, apparently in an auxin-independent manner. *SHORTROOT* (*SHR*) and *SCARECROW* (*SCR*), encoding two transcription factors expressed in procambial and endodermal/QC cells, respectively, and initially identified as regulators of radial patterning [11], are also involved in QC specification [74], in connection with *JACKDAW* [100]. Also, the *RETINOBLASTOMA-RELATED* (*RBR*) gene regulates the size of the stem cell pool, possibly in cooperation with *SCR* [103]. Other genes involved in RAM organization have been identified, but their actual position in the overall mechanism is still to be assessed. As an example, the *OBERON* genes encode auxin-regulated transcription factors [87]. Additionally, *HOBBIT* (homologous to *CDC27* [81]), the putative helicase *TEBICHI* [41], *TONSOKU* (a regulator of cell cycle progression, [85], and the cell cycle switch gene *CCS52A* [95], all participating to the regulation of the RAM size and organization, are likely to act downstream of transcription factors. Conceivably,

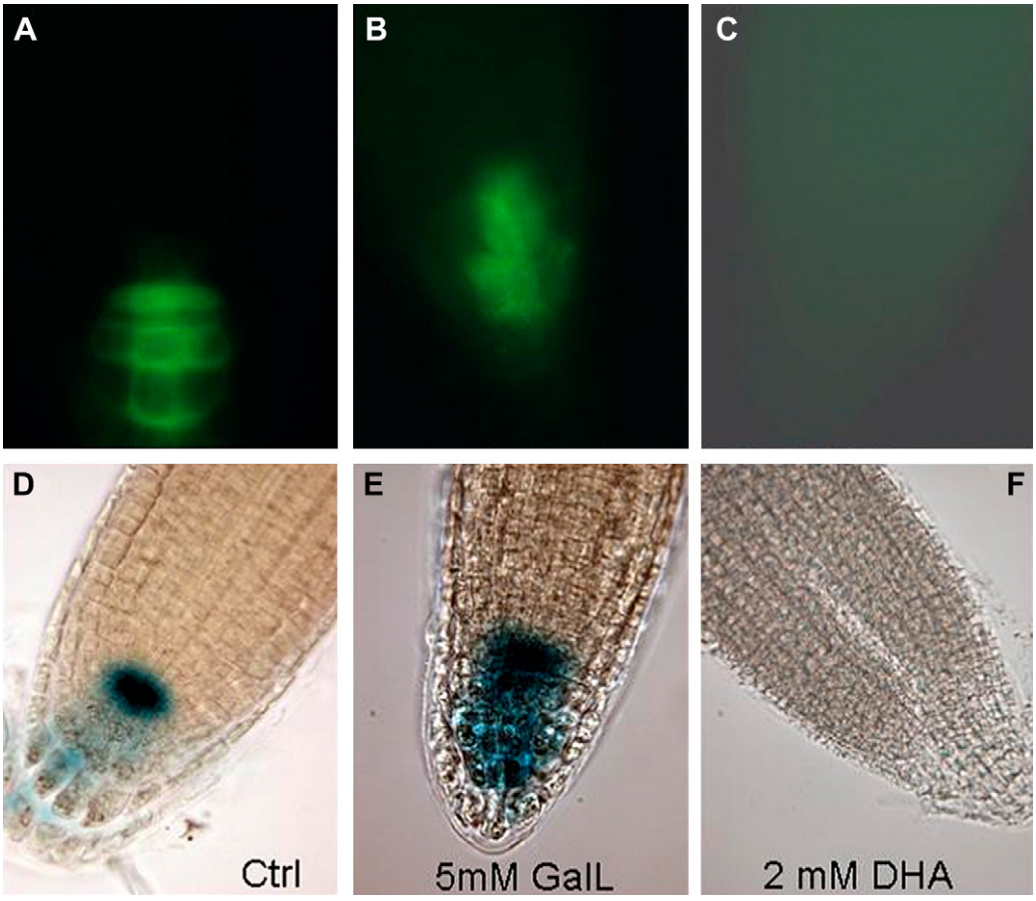

Fig. 2. Effects of ascorbate (ASC) redox changes on auxin distribution in the root apical meristem, and quiescent center identity. *Arabidopsis* seedlings (1 week-old) expressing the gene coding for the Green Fluorescent Protein (GFP) under the auxin-inducible DR5 promoter (panels A,B,C), and seedlings expressing the β-glucuronidase (GUS) gene under the QC184 promoter (D,E,F) were incubated for 16 h in distilled water (Ctrl) (A,D), in a solution of the ASC precursor L-galactono-γ-lactone (GalL) (B,E), or in a solution of dehydroascorbate (DHA) (C,F). Additional controls in D-galactono-γ-lactone, the GalL isomer which is not used for ASC biosynthesis, were not significantly different from controls in water.

many more players are still to be discovered. Another complicating factor is the relationship between auxin and other hormones in regulating different aspects of root organization. The influence of cytokinins [23], gibberellin [91], and ethylene [64] on RAM organization also has been documented.

Coexistence and interaction of different regulative pathways is definitely no surprise, given the importance of RAM organization in plant life. Disentangling the relationship between all these players will be a challenging task for the future. At the present, the two best-characterized pieces of the jigsaw puzzle remain auxin distribution and *PLT* genes, but the identity of factors upstream and downstream auxin transport and the expression of PLT transcription factors is still largely unknown.

1.4. Presence and distribution of redox components in the RAM

Several lines of evidence point to a redox-dependent regulation of RAM development. The concepts of redox status and redox regulation are discussed in detail elsewhere in this special issue. It is now clear that reactive oxygen and nitrogen species (ROS and RNS, respectively) are not just harmful molecules; on the contrary, they act effectively as molecular signals in biological systems, in cooperation with antioxidants. The water-soluble, low-molecular weight sugar ascorbate (ASC), and the cysteine-containing tripeptide glutathione (GSH) are by far the two most studied antioxidants in both plants and animals. ASC and GSH, together with their oxidized forms dehydroascorbate (DHA) and glutathione disulfide (GSSG), respectively, form two redox couples strictly connected within a network of both enzymic and non-enzymic reactions (Fig. 3).

Initial evidence for ASC functioning in root growth and development came from early studies by Mary Elizabeth Reid, who first suggested a possible correlation between ASC content and cell size in the cowpea root [68,69]. Chinoy [17] further explored the connection between ASC localization and plant development. Later, Arrigoni and co-workers [55] observed that lowering ASC content

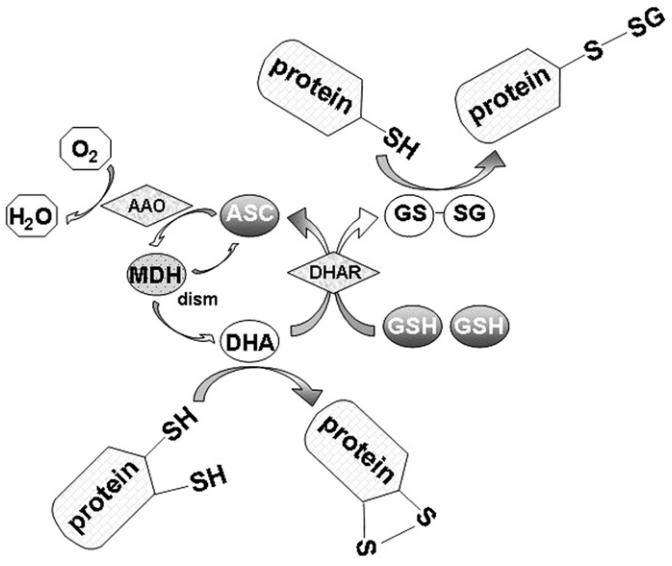

Fig. 3. The ascorbate (ASC) and glutathione (GSH) redox couples. ASC oxidized to monodehydroascorbate (MDH) by ASC oxidase (AAO) or by direct reaction with different oxidants, undergoes non-catalyzed dismutation (dism), yielding ASC and its oxidized form dehydroascorbate (DHA). DHA can be reduced back to ASC either by reacting with protein thiols (forming intramolecular disulfide bridges), or by reacting with glutathione (GSH) in a reaction catalyzed by DHA reductase (DHAR). Protein S-glutathionylation (formation of a mixed disulfide) occurs when glutathione disulfide (GSSG), the oxidized form of GSH, reacts with protein thiols [23].

in onion root meristematic cells blocked cell cycle progression in G_1. Conversely, cell division is stimulated by the ASC precursor L-galactono-γ-lactone (GalL) [5,18]. Notably, ASC administration not only induced cell proliferation in the meristem proper, but also caused new DNA synthesis in 80% of QC cells [42,56]. Kerk and Feldman [51] showed that maize QC cells have low ASC content, in parallel with high expression and activity of the enzyme ASC oxidase (EC 1.10.3.3), and proposed a mechanism of QC maintenance based on targeted ASC oxidase-mediated ASC depletion in QC cells.

In parallel with studies on ASC, also GSH involvement with the mechanism controlling root development and RAM organization was demonstrated. Mutations in the *ROOT MERISTEMLESS*1 (*RML*1) gene of *Arabidopsis*, coding for an enzyme of the GSH biosynthetic pathway, cause a dramatic phenotype characterized by a loss of primary root meristem organization and the ability to form post-embryonic roots [96]. GSH administration rescues the mutant [96]. Both ASC and GSH affect root length and mitotic activity in *Arabidopsis* roots, although to a different extent, with GSH having a clearer effect [76]. However it should be noted that in the condition used by Sanchez Fernandez et al. [76], ASC could have been partly oxidized to dehydroascorbate (DHA), impairing its effects on growth. Both root hair length and density were significantly affected by GSH (and the thiol-reducing agent dithiothreitol), but not by ASC, suggesting distinct roles for these two redox components.

Detailed studies on ASC localization in root cells of *Cucurbita maxima* using the silver nitrate method [57] demonstrated an ordered, intracellular distribution mainly at the plasma membrane-cell wall interface and, most interestingly, around the nuclear membrane and in nucleoli. ASC was found in almost all root cells, excepting in a limited area corresponding to QC cells, thus supporting previous studies on maize roots [51].

Localization of GSH has been studied in *Arabidopsis* roots using derivatization with the fluorescent dye monobromobimane. GSH is mainly associated with the apical meristem and, in the epidermis, with the trichoblast cell files. It is especially worth noting that GSH is clearly observed in endodermal cells, but definitely not detectable in QC cells [76]. Taken together, data on ASC and GSH distribution in root meristems suggest that redox components might be part of the overall mechanism of RAM organization, in parallel and/or in connection with the two other levels (hormonal and transcriptional control) previously discussed.

The identification of a connection between auxin distribution and redox status in the maize RAM [44] was an important step forward in our understanding of the general mechanism of RAM organization and maintenance. Under normal growth conditions, maize QC cells showed usual auxin maxima in QC and columella cells, in parallel with higher oxidant (superoxide ion and hydrogen peroxide) and lower antioxidant (ASC, GSH) content as compared to the proximal meristem. Perturbing auxin distribution either by removing the root cap, or by using the auxin transport inhibitor naptylphtalamic acid (NPA) caused marked changes in mitotic activity in the RAM, with loss of the QC. NPA treatment induced a dramatic drop in the ASC/DHA ratio in the proximal meristem (from 300:1 to 2.3:1!) and concomitant increase of the ratio in QC cells (from 1:18 to 2:1). Interestingly, less marked changes occurred in GSH content and GSH/GSSG ratio in NPA-treated QC cells. NPA also decreased overall ROS content in the QC, an effect reversed after removal of the inhibitor, i.e. with re-establishment of normal auxin flow [44]. This data suggests that auxin distribution, by affecting both ROS and antioxidant content, imposes a graded oxidized environment, apparently in parallel with the above-discussed *PLT* expression gradient [35], although a causal relationship between these two levels is not demonstrated. Exogenous auxin

(1 μM) affects ASC redox status in tomato roots, shifting the ratio towards the oxidized form DHA [89]. Administration of either auxin or DHA induces root growth arrest and production of lateral roots, i.e. changes the developmental program towards determinate growth [89].

We have previously discussed that *PLT* expression is auxin regulated, and can, in turn, regulate auxin transport [35]. To evaluate whether a similar self-reinforcing mechanism also occurs with auxin-dependent alteration of redox state, we investigated the effects of redox components (namely, DHA and the ASC precursor GalL) on auxin distribution in roots of *Arabidopsis* plants expressing the *DR*5::*GFP* construct. Our data (Fig. 2B,C) show that GalL caused a wider distribution of the auxin-responsive signal in the RAM. Moreover, DHA treatment resulted in auxin misplacement, since its usual accumulation in QC and columella cells is no longer evident. Additionally, the expression pattern of the QC identity marker gene *QC*184 in the *QC184::GUS Arabidopsis* line was significantly expanded by treatment with GalL, whereas no expression was observed after treatment with DHA (Fig. 2D–F). This data further suggests a regulative loop between redox and auxin, and a possible significant role of redox in QC specification/maintenance.

1.5. Potential targets of redox regulation

There is convincing evidence that auxin acts upstream of the redox balance in regulating RAM organization and activity. However, what now remains to be assessed is: (i) how does auxin induce ROS and antioxidant production; (ii) which components are actually under redox control?

The mechanism of auxin perception and functioning has been dissected in its basic components [86]. By binding to the TIR1 receptor auxin removes transcriptional repression of a large array of selected genes, possessing the Auxin Response Factor (ARF) signature. In parallel with such mechanism of action, auxin is known to induce the generation of two main ROS, namely hydrogen peroxide [14,49] and superoxide ion [79]. There is also increasing evidence that the ROS/antioxidant balance is the basis for the activation of several enzymes and transcription factors in both animals [60,88] and plants [25], due to the presence of redox-sensitive cysteine residues, whose oxidation/reduction is responsible for changes in protein conformation and activity. This is the case for many regulative proteins, such as thioredoxins, peroxiredoxins, and glutaredoxins [25]. It should be considered that both DHA and GSSG may directly react with thiol-containing proteins (Fig. 3). Changes in redox conditions in cellular microenvironments have been measured *in vivo* using a ROS-sensitive GFP [46]. Such changes can shift the activity of specific proteins, such as glutathione reductase, glucose-6-phosphate dehydrogenase, adenylylsulfate reductase, superoxide dismutase, and glutamate–cysteine ligase [40].

Auxin likely affects gene expression at two different levels; by removing repression, and by acting *via* redox regulation. The two mechanisms would reinforce each other.

Another interesting aspect of auxin-related redox regulation is the activity of the above-mentioned enzyme, ASC oxidase. In tomato roots activity of the enzyme is enhanced by exogenous auxin only in the root tip, but not in the elongation zone [89]. Abundant ASC oxidase protein has been found in QC cells of both maize [51] and pumpkin [57] roots, suggesting a specific role in redox regulation. Notably, the reaction catalyzed by ASC oxidase might have a double function in redox regulation, on one side by oxidizing ASC to DHA, and on the other hand by reducing molecular oxygen to water, thus removing the main cause of ROS generation (O_2). Maintaining low oxygen levels within the QC could be especially important to preserve totipotency, as has been demonstrated for animal embryonic stem cells [29,101]. Probably in connection with potential low oxygen content, mitochondria functionality in maize QC cells is apparently impaired [47]. Mitochondria also show a peculiar distribution within RAM tissues, being much less abundant in central QC cells (the structural initials) [47]. Interestingly, a role for mitochondrial function in animal stem cell maintenance has been hypothesized [62]. In parallel with decreasing oxygen content, ASC oxidase activity also shifts the ASC redox balance towards an oxidized state, which, as mentioned above, is typical of QC cells. According to several reports, DHA, which is formed as a consequence of an ASC oxidase-catalyzed reaction, is able to slow cell cycle progression [65]. This mechanism could contribute to the exceptionally long cell cycle of QC cells. It should be borne in mind that DHA is a very special molecule, able to directly interact with GSH and thiol-containing proteins, and therefore potentially very important in regulating the activity of redox-sensitive proteins (Fig. 3). A third possible function of ASC oxidase could be its ability to catalyze auxin oxidative decarboxylation [52]. Such activity is apparently pH dependent, and could be part of a feedback mechanism controlling auxin levels.

Although no ASC oxidase or related transcript was found in QC cells by Nawy et al. [58], a significant expression of the maize orthologue has been detected [48], and, according to a recent report, at least one of the four Arabidopsis ASC-oxidase genes is significantly upregulated in the organizing zone of the shoot apical meristem [104].

Ethylene production has also been identified as a key regulator of cell division in the RAM [61]. This is another interesting issue, since the last enzyme in ethylene biosynthesis, aminocyclopropane carboxylate oxidase (ACO), uses ASC as a co-substrate [6]. ASC oxidase, by consuming ASC, could also have a role in regulating ACO activity, and therefore RAM structure.

A final consideration on redox regulation is the hypothesis that redox can even affect cell-to-cell communication in the RAM [12]. This view is based on the fact that ROS accumulation occurring in *Arabidopsis* plants mutated in a gene coding for thioredoxin-m3, affects plasmodesmal trafficking. The implications of such findings in the transfer of factors involved in positional signalling inducing RAM organization are self-evident.

1.6. Redox dynamics in the RAM during development and in response to stress

The regulatory mechanism underlying the apparently simple organization of the RAM is indeed very complicated. The evolutionary significance of such complexity probably resides in the fact that different pathways evolved side by side and interdependently, suggesting that organization of development, rather than being "intelligently designed", results from the recruitment of established regulative pathways to a different context.

The contribution of redox regulation to RAM organization is to be found in its intrinsic dynamic nature, and its ability to connect plants with their surrounding environment. Changes in redox are very fast and reliable signals indicating that "something is happening", and can induce a proper adaptive response.

Changes in environmental conditions have been long known to affect RAM organization. Clowes and Stewart [20] showed that exposure of *Zea* roots to 5 °C temperature drastically reduced the rate of root elongation (from 11 mm/day to zero) and mitotic rates (from 11.6% down to 0.5% by the 4th day of cold). Upon return of the roots to favourable temperatures, meristem reorganization was observed, including the activation of the cells of the QC. In nature, low temperature-induced dormancy in which organization of an active meristem disappears is reported for roots of *Libocedrus* [102]. As in maize, this situation in the long-lived incense cedar roots

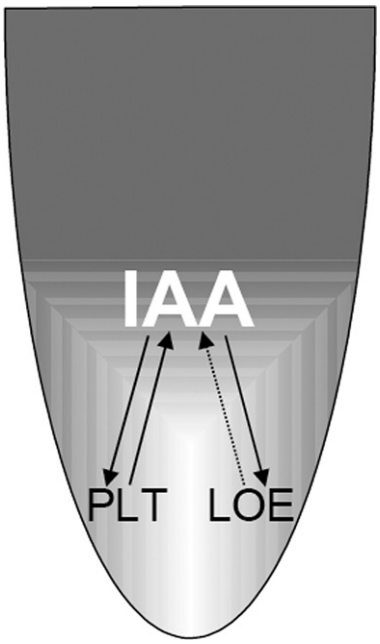

Fig. 4. Hypothetical model of the multiple control of RAM organization. Auxin (IAA) targeted accumulation in the QC induces both the expression of *PLETHORA* (*PLT*) genes and a localized oxidizing environment (LOE), driving RAM organization and functioning. Available evidence suggests that PLT, and possibly also oxidizing conditions (arrow with dashed line), in turn can regulate IAA transport and accumulation. The possibility that PLT expression is affected by redox has not been investigated so far.

presumably involves a comparable return to normal development by activation of the QC cells, which then restore the proximal and distal meristems. Notably, low temperature stress induces a massive hydrogen peroxide production in cucumber [54] and maize [4] roots, which likely affects RAM organization.

Changes in nutritional conditions also result in altered QC dimensions. This is the case when roots of maize are cultured in media supplemented with various amounts of sucrose [30]. Starvation of roots in culture led to QC activation, marked mitotic activity, and change in QC size [99]. Also in this case, a close relationship between sugar sensing and ROS production has been identified [21].

The case of phosphate deficiency is very clear and paradigmatic. As already discussed, phosphate deficiency induces loss of QC identity and RAM organization, leading to determinate root growth [75]. Consistently, hydrogen peroxide and superoxide ion have a maximum in QC cells in P-sufficient (1 mM) *Arabidopsis* roots, but show complete relocation to cortical and epidermal tissues in P-starved plants [90]. Morphogenetic alterations seen in P-starved plants are also connected to gibberellin perception/action *via* DELLA proteins [43], which in turn also play a role in ROS regulation [1].

The effect of metal stress on root organization is another intriguing issue. The pattern of cell division in the maize RAM was completely altered by short-term (up to 3 h) aluminium stress [27]. After only 5 min exposure to 50 μM Al, cell division was inhibited in the proximal meristem, and proliferative activity was relocated to the distal elongation zone. In rice, (but also in many other species) Al treatment result in increased generation of the superoxide anion and hydrogen peroxide, elevated the amount of malondialdehyde, soluble protein, oxidized GSH and resulted in a decline in the concentrations of thiols and ASC [82].

In summary, there is accumulating evidence for crosstalk, modulation and integration between signalling pathways responding to phytohormones, phosphate, light, sugars, biotic and abiotic stress-related stimuli [72]. Low temperatures, metals, pathogens, nutrient deficiency, all induce targeted production of hydrogen peroxide and/or superoxide. Interestingly, all these diverse forms of stress converge, and affect plant morphology in a similar way, reducing (or blocking) primary root growth and relocating meristematic activity to laterals or to other parts of the root. Potters et al. [66] suggested the name Stress Induced Morphogenic Response (SIMR) to indicate such reorganization of plant shape, and proposed an interesting, although speculative, thermodynamic model, in which not the specific pathway, but the achieved metabolic state, is biologically conserved [67]. Needless to say, ROS and redox equilibria play a pivotal role in SIMR establishment.

2. Conclusions

The introduction and accumulation of oxygen on Earth between 3.2 and 2.4 billion years ago provided organisms with both challenges and new tools for sensing and responding to their environment. Without doubt, the appearance of oxygen underlies the development of an increased range of biochemical reactions, leading to remarkable increases in metabolic complexity in plants. Throughout evolution redox reactions have likely exerted significant selective pressure for the development of novel and more efficient biochemical pathways. The increasing evidence that oxidative stress mediates cellular responses, including signalling, points to the involvement of ROS in a still expanding array of developmental processes.

Here we argue that the complexity of plants, and specifically the establishment and maintenance of root meristems, depends on ROS, and on mechanisms for maintaining ROS homeostasis. In terms of the QC, a more oxidizing environment correlates with reduced cell proliferation, and with RAM establishment and maintenance, whereas a mildly oxidizing or reducing environment correlates with an increase in mitoses and differentiation in the QC. Indeed, redox gradients may underlie gradients in various activities along the root axis, including auxin regulation of root development (Fig. 4). Although we ascribe a special role to auxin, ROS balance in root meristems surely involves interactions with other plant growth regulators. Identification of transcription factors and enzymes specifically targeted by redox regulation is presently underway in many laboratories, and will hopefully soon yield exciting results for our understanding of root growth and development.

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