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Review

Redox regulation of differentiation in symbiotic nitrogen fixation ☆

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ABSTRACT

Background: Nitrogen-fixing symbiosis between *Rhizobium* bacteria and legumes leads to the formation of a new organ, the root nodule. The development of the nodule requires the differentiation of plant root cells to welcome the endosymbiotic bacterial partner. This development includes the formation of an efficient vascular tissue which allows metabolic exchanges between the root and the nodule, the formation of a barrier to oxygen diffusion necessary for the bacterial nitrogenase activity and the enlargement of cells in the infection zone to support the large bacterial population. Inside the plant cell, the bacteria differentiate into bacteroids which are able to reduce atmospheric nitrogen to ammonia needed for plant growth in exchange for carbon sources. Nodule functioning requires a tight regulation of the development of plant cells and bacteria.
Scope of the review: Nodule functioning requires a tight regulation of the development of plant cells and bacteria. The importance of redox control in nodule development and N-fixation is discussed in this review. The involvement of reactive oxygen and nitrogen species and the importance of the antioxidant defense are analyzed.
Major conclusions: Plant differentiation and bacterial differentiation are controlled by reactive oxygen and nitrogen species, enzymes involved in the antioxidant defense and antioxidant compounds.
General significance: The establishment and functioning of nitrogen-fixing symbiosis involve a redox control important for both the plant–bacteria crosstalk and the consideration of environmental parameters. This article is part of a Special Issue entitled Redox regulation of differentiation and de-differentiation.

1. Introduction

Plants have particular growth characteristics, developmental patterns and structural architecture. Plant development takes place largely after embryogenesis occurring during seed formation and maturation. Indeed, plant growth is correlated to the postembryogenic formation of new organs such as roots, leaves, stems and flowers. It is first linked to cell division which is restricted to meristems and to cellular differentiation which occurs in a second step [1,2]. Differentiation changes the meristematic cells into non-dividing cells with specific functions such as vascular tissues [3]. Nevertheless, most of the cells keep their potentiality to dedifferentiate into dividing cells depending on the biotic and abiotic environment [4]. Finally, the plant architecture is partially governed by the search for essential elements involved in plant growth such as light, water or minerals. This has lead to specific development of leaves necessary for light energy reception and gas exchange, or roots that draw nutrients required for plant development [5,6].

Redox regulation plays a major role in plant development and adaptation to biotic and abiotic environment [7–10]. In this context, plants have developed a large number of redox systems essential for the sessile life style. Reactive oxygen and nitrogen species (ROS and RNS) such as superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) or nitric oxide (NO) are redox signaling molecules actively produced by plants in response to their environment. On the other hand, plant antioxidant defense is very efficient with numerous enzyme families such as ascorbate peroxidases, glutathione peroxidases, peroxiredoxins, catalases or superoxide dismutases. Moreover, thioredoxin and glutaredoxin families, which are involved in the redox control of protein activity, are also large multigenic families with more than twenty members in each of them [11,12]. Non-enzymatic antioxidant compounds like NAD(P)H, glutathione and ascorbate are present in the millimolar range in plant cells and allow a reducing environment as well as the efficient functioning of the antioxidant defense [13–15].

Plants often use symbiotic interactions with fungi or bacteria to allow a more efficient nutrition process. Interaction with symbiotic fungi improves plant nutrition in water, phosphate and other nutrients [16]. In exchange, the plants provide carbohydrates to the fungi through photosynthate supply. Similarly, plants from the legume family, including alfalfa, soybean and pea, perform a symbiotic interaction with soil bacteria of the *Rhizobium* family to increase their nitrogen

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amount needed for their growth when mineral nitrogen source is scarce.

Nitrogen fixing symbiosis (NFS) involves multiple processes. The molecular cross-talk between the plant and the bacteria allows the entry of the bacteria into the plant [17]. Plant exudates, such as flavonoids, attract rhizobia and trigger the production of nodulation (Nod) factors by the bacteria. Nod factors (NF) are essential for the recognition of the bacteria by the plants. They enable the entry of the bacteria in the plant as well as the formation of nodule meristem *via* cortical cell dedifferentiation [18,19]. Rhizobia bound to curled root hairs of the infection zone induce cell wall degradation and enter into the root through the infection thread (IT), a channel shaped structure which defines the intracellular path of the bacteria through the epidermis and the cortex (Fig. 1B). The plant infection may also occur *via* an intercellular infection process called crack entry which occurs at lateral root bases [20]. Thereafter, the bacteria are internalized in the cells and form a new organelle-like structure called symbiosome. In the symbiosomes, the bacteria divide and differentiate into the nitrogen-fixing bacteroids. The bacterial nitrogenase reduces atmospheric nitrogen (N_2) into ammonia which is exported to the plant cell cytoplasm and integrated into ureides or asparagine. In return, the plant supplies the energy needed for this reduction (16 ATP for one N_2). The nitrogen/carbon exchange between the nodules and the roots requires the presence of a vascular tissue called vascular bundle for the metabolite transport. Moreover, as the nitrogenase is strongly sensitive to oxygen, a specific oxygen barrier is formed by a cell layer around the infected cells which reduces the level of oxygen (O_2) in the nodule cortex.

Nodules are classified as indeterminate and determinate according to their mode of development [21]. In indeterminate nodules, such as those formed by pea, alfalfa or *Medicago truncatula*, the nodule meristem derives from the inner-cortex dedifferentiation and is persistent during the whole lifetime of the nodule, giving elongated nodules (Fig. 1C). Consequently, the meristematic, infection and nitrogen-fixing zones are present at the same time in the nodule. In determinate nodules, such as the ones from soybean or *Lotus japonicum*, the nodule meristem dedifferentiates from outer cortex cells and is only transiently active. This results in spherical nodules, containing cells in a similar developmental state.

Nodule meristematic cells differentiate into multiple cellular types. The peripheral cell layers contain the epidermis, the cortex, the endodermis and the parenchyma. These cell layers participate in the protection of the internal N_2 fixation zone which contains the infected cells and contribute to the root–nodule exchange with the presence of the vascular bundles. The central zone of the nodule contains the meristematic, infection and nitrogen-fixing zones (Fig. 1C). As already mentioned, these different zones are present, respectively, at the same time in indeterminate nodules and successively in determinate ones. The infection of plant cells by the bacteria requires differentiation of the nodule meristematic cells with DNA endoreduplication cycles. The infected nodule cells enlarge and reach ploidy levels of 32C and 64C, and are 80-fold larger than diploid meristematic cells [22,23]. The endoreduplication and the expansion of the infected cells go together with changes in cellular metabolism which allows the reception of the bacteria and nitrogen assimilation by the plant.

During NFS, differentiation also occurs for the bacterial partner which is converted into nitrogen-fixing bacteroids. As for nodule development, bacteroid differentiation is divergent depending on the host plant [24]. In some legumes, bacteroid morphology is unaffected compared to the free living bacteria with a small rod-like shape whereas in other legumes, bacteroids present an extreme morphological change with an elongated phenotype (5 to 10-fold longer) and sometime a Y-shaped form. The bacteroid enlargement is coupled to the endoreduplication of the bacterial genome and a terminal differentiation which is irreversible and prevents further reproduction. In contrast, the bacteroids which do not show these morphological changes have a similar DNA content to free-living bacteria and are able to divide when extracted from nodules. The different bacteroid phenotype is linked to the plant host as a *Rhizobium* strain 32H1 has a terminal differentiation when colonizing peanut and a reversible differentiation with cowpea.

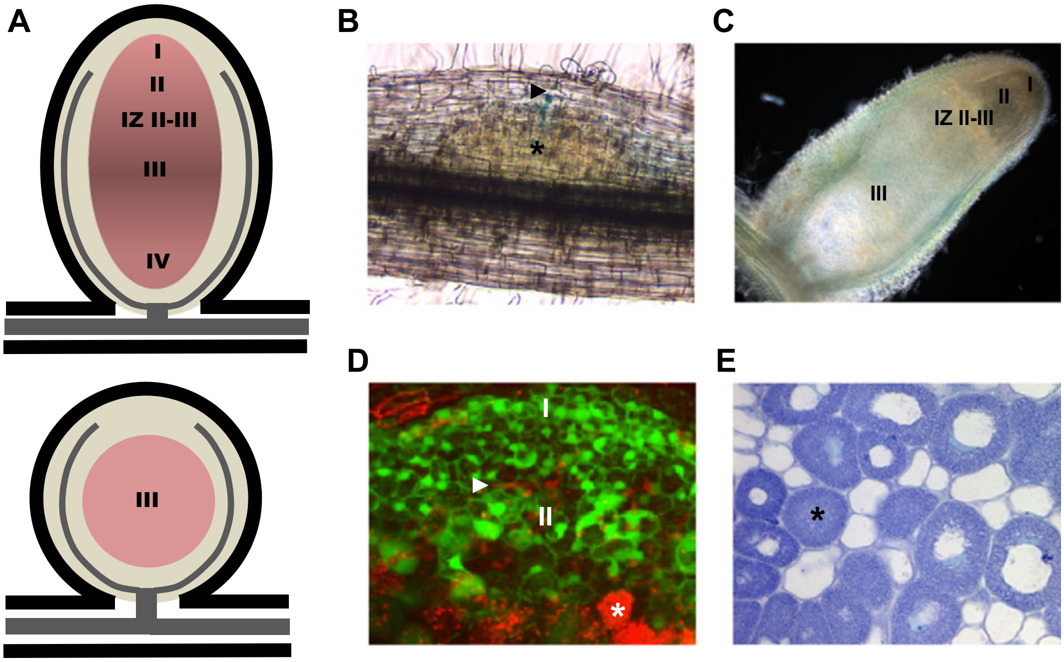

Fig. 1. The different steps of the root nodule formation and root nodule structure. (A) Structure of indeterminate with the apical meristem (I), the infection zone (II), the interzone II–III (IZ II–III), the nitrogen-fixing zone (III) and the senescent zone (IV) and determinate nodules with the nitrogen-fixing zone (III). (B) Development of root nodule with the root nodule meristem (*) and infection thread (►) in blue. (C) Picture of an indeterminate root nodule. (D) Picture of the nodule meristematic zone (I) and the infection zone (II); the plant cell cytosol which increases during cellular differentiation is labeled in green, the infection threads (►) and the infected cells (*) are labeled in red. (E) The nitrogen-cell is fully packed with numerous endosymbiotic bacteria called symbiosomes (*).