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ROLE OF PHYTOHORMONES IN THE CONTROL OF SYMBIOTIC NODULE DEVELOPMENT IN LEGUME PLANTS. II. AUXINS

(review)

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Abstract

This review refers to analysis of the modern data about the role of auxins in the regulation of nitrogen-fixing nodule development in the legume plants. The interaction of these hormones with cytokinins and components of signaling cascade activated by Nod factors during nodule organogenesis in legume plants with different types of nodules are discussed. Emphasis is being given to the participation of auxins in the control of initiation and early stages of nodule development. In early works the analysis of transgenic plants containing the fusions between promoters regulated by auxins and reporter genes, showed the accumulation of auxins in the places of nodule primordia development. It indicates a direct effect of these plant hormones on the process (U. Mathesius et al., 1998; C. Pacios-Bras et al., 2003). These studies became the basis for the suggestion that the auxin maximum in the cells giving later nodule primordia precedes these organs appearance. The creation of such peaks is dependent on Nod factors and cytokine response activation in plants, but it is differently regulated in legumes with determined (regulation of auxin biosynthesis) and non-determined (control of auxin transport with flavonoids) type of nodules (A.P. Wasson et al., 2006; J. Plet et al., 2011; T. Suzaki et al., 2012). The effect of cytokinins on the auxin transport (auxin transporters PINs) during the formation of nodules has much in common with the participation of cytokinins in the control of lateral root development program, which may indicate an evolutionary relationship of two morphogenetic programs. In legume nodules and lateral roots development programs the initial stages have much in common, since both structures are formed as a result of the reactivation of differentiated root cells and have the features of structural similarity. However further divergence leads to the formation of structurally and functionally distinct organs that can be associated with unequal role that cytokinins and auxins play in controlling these processes. The review also examined the role of the balance of auxin and cytokinin hormones in determining the program of development of root cells. We discuss the experimental data using exogenous hormones, which showed that the founder-cells, giving rise to lateral roots, characterized by high plasticity of development processes, determined by the balance of auxins and cytokinins (L. Laplaze et al., 2007; Chatfield et al., 2013). However, an analysis of the data leads to the conclusion that the role of auxin in the control later stages nodulation in legumes is little investigated.

Keywords: legume-rhizobial symbiosis, auxins, phytohormonal balance, nodule organogenesis

Symbiotic interaction between the legumes and the *Rhizobiales* bacteria leads to appearance of nitrogen-fixing root nodules due to de novo organogenesis. This is initiated by lipo-chitooligosaccharides (Nod factors), the signals excreted by rhizobia [1]. Nodule development results from root cell reactivation, differentiation and transition to a division to produce a cluster of dividing cells which forms a symbiotic root nodule primordium. Nodulation in legumes is a unique model to study de novo body formation as influenced by an external regulation (in this particular case, by the signals produced by rhizobia).

There are numerous physiological and genetic evidences that the changes in phytohormone balance induced by Nod factors are necessary to successfully develop the root nodules [2-7]. These changes are mediated by regulation of the

genes encoding plant phytohormones' biosynthesis, activation and transport [5, 8]. Hormones produced by rhizobia are able to impact on the efficacy of symbiosis but not nodule formation [9].

This review is focused on the role of auxins in nodulation and also on the auxin-to-cytokinin balance as an essential component of root cell development program in legumes.

Auxins are involved in control of nodule initiation and initial stage of development. According to recent studies, the Nod factor-activated signaling cascade stimulates a cytokinin response in the cells of root cortex [10, 11]. In alfalfa *Medicago truncatula*, pea *Pisum sativum*, white clover *Trifolium repens* which produce an indeterminate type of nodules the cells of root pericycle, endodermis and inner cortical layer are cytokinin-responding and involved in the appearance of nodular primordia [5]. In contrast, in *Lotus japonicas* and soybean *Glycine max*, the legumes which have determinate nodules, a cytokinin response occurs in the cells of outer cortical layer [6].

However, experimental data indicate an important role of auxins in the regulation of initiation and development of nodules on the roots of legumes [4, 5, 7, 12, 13]. It has been known long enough that the nodule-like structures appeared in the legumes treated with inhibitors of polar transport of auxins, indicating these plant hormones to participate in root nodulation [4, 14]. Using genetic constructs containing auxin-regulated promoters *GH3:GUS*, *DR5:GUS* fused to the reporters, a local auxin accumulation was detected at the sites of primordia generation as an evidence for the direct effect of these plant hormones [17-19].

Rhizobial inoculation and treatment with Nod-factors led to disruption of local auxin accumulation and polar auxin transport (PAT) in the legumes producing indeterminate nodules [17, 18]. Two possible causes for PAT inhibition have been experimentally revealed. Firstly, the flavonoids which synthesis is activated by Nod-factors affect the PAT at the site of inoculation [17, 18, 20-22]. On the other hand, the PAT inhibition is possibly due to the effect of cytokinin on the distribution of PIN (PIN-FORMED) proteins, the transporters of auxins, in the root conducting system. Unlike wild type plants, the mutant *cre1* deficient in cytokinin receptor exhibits no change in PIN protein localization in response to inoculation [5, 23]. No changes in the PIN distribution would result in no PAT inhibition.

An in-depth analysis of the mutant *cre1* allowed a molecular level understanding of Nod-factors, auxin and cytokinin interaction in the plants with indeterminate nodules. Mutant *cre1* does not respond to rhizobial inoculation by stimulated synthesis of flavonoids, and, as a result, no changes in PIN localization occur and the polar auxin transport is not disrupted [24]. However, the treatment of *cre1* mutant by exogenous flavonoids and PAT inhibitors restores nodulation [24]. Therefore, signaling pathway activation by Nod-factors stimulates the cytokinin receptor leading to expression of the genes encoding flavonoids. In turn, the flavonoids affect PIN proteins resulting in the inhibition of auxin transport. As a result, locally accumulated auxin triggers the initiation of the root nodule primordia formation and stimulates proliferation of pericycle, endodermis and inner cortical layer cells giving rise the nodules.

Ability of flavonoids to act as auxin transport regulators, in particular to affect PIN synthesis [25] and localization [26], has been shown for other plant species. Thus, in plants with indeterminate nodules the activity of Nod factors and cytokinins precedes the changes in auxin level and localization during root nodule development. Cytokinins influence the flavonoid synthesis and distribution of PIN proteins that is essential to form a local auxin gradient and to develop root nodule, a lateral organ generated de novo.

In the legumes with determinate nodules (*L. japonicus*) an auxin transport inhibition response to rhizobial inoculation was not observed, however, an increased auxin level at the site of nodule generation was shown experimentally with the use of auxin-regulated constructs [27, 28]. Moreover, the increased auxin correlated with the induced expression of gene *TRYPTOPHAN AMINOTRANSFERASE RELATED 1 (TAR1)* involved in auxin biosynthesis control [29, 30]. Additionally, at initiation of organogenesis in *L. japonicus* the cytokinin action preceded auxin accumulation which, according to the authors' view, stimulated root cell proliferation affecting cell cycle regulators [29, 30].

Indeed, the role of cytokinins and auxins in the control of organogenesis may be due to their mutual effects on one another. Numerous publications show that cytokinins can regulate morphogenesis influencing the transport and metabolism of auxins. Hormone transport is crucial in the formation of auxin gradient that causes cell specialization and regulation of their proliferation and differentiation [31]. A case study of auxin transport in plants has been conducted for indole-3-acetic acid (IAA). In plant cells the IAA influx is mediated by proteins AUX1/LAX (AUXIN RESISTANT 1/LIKE AUX 1) and PGP (P-glycoprotein of the family MDR — multidrug resistance) [32]. An efflux of auxins from cell is regulated by PIN and PGP proteins embedded in the cell membrane diametrically to transporters AUX1/LAX [32]. In legumes the changes in auxin transport and biosynthesis occur after cytokinin involvement in the control of root nodulation. These data suggest that cytokinins can affect auxins in the early development of root nodules [4].

The mutual effect of cytokinin and auxin during organogenesis is quite well studied [33–36]. For example, cytokinins similarly regulate synthesis, localization and selective degradation of PIN proteins at formation of lateral roots in *Arabidopsis*, a model plant [33–36]. It is these mechanisms are found for the cytokinins to probably regulate auxin gradient formation during the development of lateral roots [36, 37]. Cytokinins and auxins are known to differently affect shoot and root development: shoot formation is cytokinin-stimulated while root formation is auxins-stimulated. In shoot apical meristem of *Arabidopsis* cytokinins stimulate cell proliferation and auxins, in contrast, affect cell differentiation. The root meristem auxins are necessary to maintain its activity, while cytokinins inhibit the development of root meristem [38, 39]. In this, the cytokinins affect root cell differentiation by inhibition of the stimulatory effect of auxins on cell division, i.e. the hormones act in close concert with each other. The balance between cell division and differentiation and, as a result, the control of meristem size are due to cytokinin-dependent stimulation of the auxin response repressors Aux/IAA. That suppresses expression of PINs (the auxin transporters) and leads to a change in the cytokinin-to-auxin balance in the root zone where the transition from cell division to differentiation occurs [38, 39]. These findings are in line with auxin-stimulated proliferation of the root cells producing nodule primordia in legumes.

Role of the auxin-to-cytokinin balance in the program of root cell development. In recent years, a lot of works report on a mutual influence of cytokinin and auxin, indicative of the role of their balance in determining cell development program. Exactly the balance may determine the choice of a development program. For example, it is known that in *Arabidopsis* lateral roots are derived from specific founder cells of pericycle as a result of high auxin level [40]. The exogenous auxin IAA led to an increase in the number of lateral roots [41]. On the contrary, the exogenous cytokinin 6-benzylaminopurine (6-BAP) blocks development of lateral root primordia (the regularity of cell division in primordium is impaired, leading to its flattening)

[37]. Moreover, under certain conditions the exogenous cytokinin, when used at the early lateral root primordia stage, is capable of causing a complete change of the development program, resulting in lateral root primordia transformation into active shoot meristem [42]. Thus, the experiments with exogenous hormones suggest that founder cells, giving rise to lateral roots, are characterized by high plasticity, and the vector of their development is determined by the balance of auxins and cytokinins.

In legume the development programs for nodules and lateral roots have much in common in the initial stages, since both organs derive from differentiated root cells after their reactivation and have the features of structural similarity [43-47]. However further divergence results in structurally and functionally distinct bodies that can be associated with different role the cytokinins and auxins, as regulators, play to control these processes. This has led some authors to the hypothesis that the nodules program has evolved on the basis of the program of lateral root formation [48]. This assumption is evidenced by the data that the Nod factors can stimulate not only the nodule, but the lateral root [49, 50]. Additionally, the lateral root growth stimulation by Nod factors depends on the major genes (*NFP*, *DMI1*, *DMI2*, *DMI3* and *NSP2*) which control Nod factor-signaling cascade after the reception of Nod factors. The same genes regulate the activation of early nodulins and initial root cortex cell divisions at nodulation.

A comparative analysis of the root transcriptome in *M. truncatula* wild type plants and mutant *sickle* treated with Nod factors confirm this assumption. The mutant *sickle* is characterized by an increased susceptibility to inoculation and more intense response to Nod factors due to ethylene resistance [51, 52]. In wild type plants, and especially in mutant the exogenous Nod factors greatly increases the expression of genes that control root development (mostly these are the homologs of the *Arabidopsis* model plant genes) [53]. In *Arabidopsis* many of these genes are involved in control of cell division and formation of lateral root primordia, as well as the maintenance of stem cell pool and meristem structure of formed root center. The authors provide evidence that the increase in the expression of these genes is associated with the root nodule development. A significant increase in the number of root nodule primordia, but not lateral roots in mutant *sickle* indicate that induction of expression of the gene set is associated mainly with nodulation [53]. This supports the hypothesis that in legumes the same genes may control the initial stages of development in lateral roots and root nodules.

Thus, the appearance of nodules is preceded by maximum auxin accumulation in the cells which will produce root nodule primordia. Reaching auxin peaks depends on Nod factors and activation of cytokinin response, but differs in legumes with determinant nodules (auxin level control due to regulation of its biosynthesis) and indeterminant nodules (auxin transport regulation by flavonoids). The effect of cytokinins on PINs (the auxin transporters) and auxin transport at root nodule formation has much in common with the role of cytokinins in programming lateral root development that may indicate an evolutionary relationship of two these morphogenetic programs.

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