Multiple routes communicating nitrogen availability from roots to shoots: a signal transduction pathway mediated by cytokinin

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Abstract

In higher plants, inorganic nitrogen has crucial effects on growth and development, providing cellular components and modulating gene expression. To date, not only nitrogen assimilatory genes but also a substantial number of genes with other functions have been shown to be selectively regulated by the availability of nitrogen. In terms of the communicating substance(s) between root and shoot, accumulating evidence suggests that nitrate itself is the primary signal molecule triggering the activation of transcription of nitrate assimilation and related genes. On the other hand, some of the genes involved in photosynthesis, cell cycling and translation machinery are also regulated, at least in part, by nitrate and other nitrogen sources and, in some cases, the effect can be mimicked by cytokinin treatment. Spatial and temporal studies on the accumulation levels and the translocation of cytokinin in response to nitrate replenishment in maize showed subsequent accumulation of various cytokinin species in the roots, xylem sap and leaves. In Arabidopsis thaliana, trans-zeatin riboside-5'-monophosphate and/or trans-zeatin riboside also accumulated in the roots in response to nitrate resupply. These studies suggest that cytokinin metabolism and translocation could be commonly modulated by nitrogen availability in higher plants. Thus, in addition to nitrate, cytokinin could be another root-to-shoot signal communicating nitrogen availability.

Key words: Arabidopsis thaliana, communication, cytokinin, His–Asp phosphorelay, nitrate assimilation, nitrogen availability, Zea mays.

Introduction

The availability of inorganic nitrogen in the rhizosphere is a crucial chemical factor governing the growth rate and developmental pattern of higher plants. Plants constantly sense nutrient availability and modulate their metabolic activities and development to adapt efficiently to the nutritional status. This adaptation is conducted by modulating not only nitrogen metabolism but also other metabolic pathways and morphogenic responses. The variety of responses implies that expression of a number of genes could be regulated by nitrogen availability and, in fact, many examples have been reported (Stitt, 1999; Wang et al., 2000). An integrated network with intracellular, intercellular and interorgan signalling must be required to orchestrate such a series of changes in gene expression in a whole plant body.

For most higher plants, nitrate is the major source of inorganic nitrogen. Although it depends on the plant species and their growth conditions, a large proportion

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of the nitrate ions taken up from the soil are translocated to the leaf, and assimilated and metabolized into various organic compounds utilizing reductant provided by photosynthesis. The reduced nitrogen compounds are incorporated into various biomacromolecules, such as proteins and nucleic acids. The photosynthetic apparatus and translation machinery are the major allocation sites of nitrogen in the leaf cell and thus it can be assumed that distribution to these components is affected by the availability of nutrients in the soil.

The expression of a series of genes involved in utilization of nitrate is triggered by the sensing of the nitrate ion itself. That is to say, nitrate can function as both an assimilation substrate and a signalling molecule. Recent studies have revealed some pieces of the network of expression of nitrate-regulated genes in plant cells (Crawford, 1995; Stitt, 1999; Wang et al., 2000).

In addition to nitrate-specific regulation, recent studies have indicated the existence of another route communicating the availability of nitrogen from roots to the leaves mediated by cytokinin, a plant hormone (Simpson et al., 1982; Samuelson and Larsson, 1993; Sakakibara et al., 1998; Takei et al., 2001b). The compounds, whose content is correlated with nitrogen availability, are translocated from root-to-shoot concomitantly with nitrate ions and regulate the pattern of expression of various genes. Furthermore, in terms of the signal transduction to the target genes, the involvement of a His–Asp phosphotransfer system has been implicated (Sakakibara et al., 1998; Taniguchi et al., 1998; Imamura et al., 1999; Sakakibara et al., 2000). Multiple signalling pathways communicated by nitrate and by cytokinin seem to be temporally and spatially co-ordinated with nitrogen metabolism and play a pivotal role in the integrated response to the nutritional environment at the whole plant level.

As the recent findings on the function of nitrate ion as a gene regulatory signal have been adequately summarized by Stitt (Stitt, 1999), this article mainly focuses on another pathway of nitrogen signal transduction from roots to shoots mediated by the plant hormone, cytokinin.

**Nitrate is the primary signal for nitrate assimilatory and related genes**

As mentioned above, a representative signal molecule of the inorganic nitrogen source is the nitrate ion itself. It functions not only as a metabolic substrate for assimilation, but also as a signal for modulating the pattern of growth and development by means of regulating the expression of various genes. Genes whose expression is specifically responsive to nitrate can be referred to as ‘nitrate-specific responsive genes’. A profile of these genes is summarized in Fig. 1. To date, it has been revealed that they include not only genes involved in nitrate uptake (Tsay et al., 1993) and reduction (Vincentz et al., 1993), but also genes involved in ammonia assimilation (Redinbaugh and Campbell, 1993; Sakakibara et al., 1997), the supplying of reductant (Ritchie et al., 1994; Matsumura et al., 1997), biosynthesis of cofactor(s) (Sakakibara et al., 1996), provision of carbon-skeleton for nitrogen assimilation (Scheible et al., 1997), and root architecture (Zhang and Forde, 1998). Previous studies have provided some common properties of the regulatory processes: (1) the response occurred rapidly, requiring only μM concentrations of nitrate, without new synthesis of protein (CHX-insensitive) (Gowri et al., 1992; Redinbaugh and Campbell, 1993; Sakakibara et al., 1996, 1997), (2) treatment with some drugs such as okadaic acid, EGTA or La³⁺ inhibited the nitrate-dependent expression (Sakakibara et al., 1997; Sueyoshi et al., 1999), implying that influx of extracellular calcium ions and protein phosphorylation is involved in the signalling pathway. However, the biochemical nature of the signal perception and transduction pathway of the nitrate-specific response from the cell surface to the nuclei, has been poorly characterized. Detailed analysis is clearly required on the specificity of the nitrogen compounds triggering the expressions of the genes.

**The effect of nitrogen can be replaced by other compounds**

On the other hand, genes involved in amino acid metabolism (Zhao et al., 1998; Guyer et al., 1995), storage
proteins (Staswick et al., 1991; Müller et al., 1997), photosynthesis (Sugiharto and Sugiyama, 1992), cell cycling (Soni et al., 1995), and transcription/translation machinery (Suzuki et al., 1994) are regulated by multiple nitrogen sources, nitrate, ammonium ions and/or amino acids. Namely, they do not have specificity for the responsive nitrogen molecule. Such genes could be referred to as ‘genes broadly responsive to nitrogen’ (Fig. 1). The regulation pattern of these genes is varied, and various control systems could be involved in the response. The response of these genes occurs against broad changes of nitrogen availability, and emerge relatively slowly: several hours through day(s) after change of the status. The C4 photosynthesis gene for PEP carboxylase requires cytosolic protein synthesis (CHX-sensitive) for up-regulation by replenishment of the nitrogen source (Suzuki et al., 1994).

In amino acid metabolism, cross-pathway regulation that is well-characterized in yeast (Deflorge et al., 1975) seems to be conserved in higher plants (Zhao et al., 1998; Guyer et al., 1995). Such regulation may be a prototype for the systematic response of multicellular eukaryotes to nutritional stress.

For broadly responsive genes, the nitrogen effect can be replaced by other compound(s). For instance, nitrogen-dependent expression of genes for vegetative storage protein (Vspx, β) of soybean can be mimicked by the administration of methyljasmonate to the petiole of a detached leaf (Staswick et al., 1991). Application of cytokinin can mimic the nitrogen-dependent regulation of gene expression in photosynthesis (Sugiharto et al., 1992), cell cycling (Soni et al., 1995; Riou-Khamlichi et al., 1999) and translation machinery (Suzuki et al., 1994). The major site of the biosynthesis of cytokinin is believed to be non-photosynthetic tissues, such as root tips (Feldman, 1975), apical meristems (Koda and Okazawa, 1980) and immature seeds (Blackwell and Horgan, 1994). The translocation of the hormone from non-photosynthetic tissues to photosynthetic tissues might be an important factor regulating the expression of specific genes in the living cell.

Cytokinin accumulation and transport are positively regulated by nitrogen replenishment

There are several reports suggesting that the accumulation of cytokinin is closely correlated with the nitrogen status of the plants, such as Urtica dioica (Wagner and Beck, 1993), barley (Samuelson and Larsson, 1993) and maize (Takei et al., 2001b). These studies suggest that cytokinin metabolism and translocation could be modulated by the nitrogen nutritional status. A remarkable finding from these studies is that the increase in cytokinin concentration occurred following the change of status from deficient to sufficient (Samuelson and Larsson, 1993; Takei et al., 2001b). Namely, cytokinin accumulation and translocation occurred after sensing a change in nitrogen availability.

In maize roots, following the addition of nitrate to nitrogen-depleted maize plants, isopentenyladenosine-5'-monophosphate (iPMP) started to accumulate in roots within 1 h, preceding accumulation of trans-zeatin riboside-5'-monophosphate (ZMP), trans-zeatin riboside (ZR) and trans-zeatin (Z) (Takei et al., 2001b). iPMP is the first molecule to be synthesized in cytokinin metabolism, suggesting that cytokinin was synthesized de novo in response to nitrate resupply.

When changes in Z-O-glucoside (ZOG), an inactive and storage form of cytokinin, were determined, the concentration gradually decreased after replenishment of nitrate in the maize roots (Fig. 2). This reciprocal pattern against the accumulation of an active species implies that, in addition to biosynthesis, conversion from a storage form to an active form, also contributes to the accumulation of cytokinins in roots.

In the xylem, following the application of nitrate, both the exudation rate and the concentration of the cytokinins increased, with ZR being the dominant species in the sap (Takei et al., 2001b). In leaf tissue, the accumulation of Z, which was the dominant form, started to increase 4 h after nitrate resupply to plants and the level was maintained for at least 24 h (Takei et al., 2001b). The spatial and temporal changes in the molecular species and accumulation levels strongly suggest nitrogen-dependent translocation of cytokinins from roots to shoots. However, the

![Fig. 2. Changes in the accumulation level of t-zeatin-O-glucoside (ZOG) in maize root during recovery from nitrogen starvation. 16 mM KNO₃ (closed circles) or 0.08 mM KNO₃ and 16 mM KCl (open circles) was resupplied to nitrogen-limited maize, and the roots were harvested at the indicated times (for details of methodology for hydroponic cultivation, see Takei et al., 2001b). Cytokinin-O-glucoside fraction was prepared as described previously (Faiss et al., 1997). ZOG species were identified and measurement by liquid chromatography/mass spectrometry analysis (Takei et al., 2001b). g FW, g fresh weight.](image-url)
biochemical mechanism catalysing the conversion of the cytokinin species has not been identified at present.

To confirm the root-to-shoot signal translocation, the xylem sap was collected after nitrate resupply and then applied to detached maize leaves (Fig. 3). Treatment with the xylem sap from nitrogen-resupplied maize plants resulted in the accumulation of the ZmRR1 transcript (lanes 4–6), whereas that from nitrogen-starved plants did not (lanes 1–3). It should be noted that ZmRR1 is a cytokinin-responsive gene that functions as a response regulator involving nitrogen signalling in the maize leaf (Sakakibara et al., 1998, 1999). This result suggests that the root-to-shoot xylem flow contains a signalling substance(s) to induce the cytokinin-responsive gene. Furthermore, in preliminary results, when cytokinin species were removed from the xylem sap by the treatment with a polyclonal antibody against cytokinins, the inducible effect was greatly diminished (data not shown). This is consistent with the hypothesis that cytokinin synthesized in the roots is the signalling substance(s) communicating nitrogen availability to the leaves.

The effect of nitrogen replenishment was also examined in Arabidopsis thaliana (Fig. 4). The accumulation level of ZMP and/or ZR in the roots was also significantly increased 3 h after the administration of nitrate. These results suggest that the hormone response to nutritional availability may be a common phenomenon in higher plants.

In barley roots, nitrogen-dependent accumulation of cytokinin was inhibited by treatment with methionine sulfoximine, a potent inhibitor of glutamine synthetase (Samuelson and Larsson, 1993), suggesting that the metabolic flow of nitrogen via glutamine is involved in the early step of the response.

Very recently, genes for adenylate isopentenyltransferase (IPT), a cytokinin biosynthesis enzyme, have been identified in A. thaliana (Takei et al., 2001a). The enzyme is encoded by a small multigene family that includes both IPT and tRNA isopentenyltransferase (tRNA-IPT). It is assumed that the expression of each gene is differentially regulated in terms of the expression site and the induction stimulus. Expression of some of the genes might be regulated by the nitrogen availability.

**His–Asp phosphorelay is involved in the signal transduction in the leaves**

The signal transduction system referred to as the ‘two-component system’ or ‘His–Asp phosphorelay
system’ has recently been uncovered in the plant kingdom (Chang and Stewart, 1998; Kakimoto, 1998; Sakakibara et al., 2000). This system is widely used as a microbial signalling pathway (Parkinson and Kofoid, 1992; Mizuno, 1998), and recently, it has been revealed that some eukaryotes such as yeast (Posas et al., 1996), filamentous fungi (Alex et al., 1996) and plants are equipped with this communication system. Emerging characters of the phosphorelay system in plants include sensor (His–protein kinase) domains, His-containing phosphotransfer (HPt) domains, and receiver (response regulator) domains (Mizuno, 1998). Recent studies implicate that the phosphorelay may be widely used for sensing the external and/or internal environment involving ethylene (Bleecker, 1999), cytokinin (Inoue et al., 2001; Suzuki et al., 2001), and osmolarity (Urao et al., 1999). In maize and A. thaliana, some response regulators have been found to be up-regulated by both cytokinins and nitrate (Sakakibara et al., 1998, 1999; Taniguchi et al., 1998; Imamura et al., 1999). These findings strongly suggest that the His–Asp phosphorelay operates in an inorganic nitrogen-signalling pathway mediated by cytokinin in plants.

In maize, expression of some nitrogen-responsive genes, such as ZmRR1 and ZmRR2 encoding maize response regulators, induced in leaves by the resupply of nitrogen to nitrogen-depleted plants (Sakakibara et al., 1998, 1999). In detached leaves, the effect can be replaced by treatment with cytokinin, but not by inorganic nitrogen sources (Sakakibara et al., 1998), suggesting that the actual signal of the nitrogen availability is cytokinin(s). In the whole plant, supplement of ammonium ions to the nitrogen-depleted maize also induced the ZmRR1 transcript, as is the case with nitrate ions (Sakakibara et al., 1998). Although the changes in the amounts of cytokinin species during ammonium administration have not been determined, this result suggests that supplement of ammonium ions also increased the translocation of cytokinins to the leaf.

At near physiological concentrations, Z, ZR and ZMP could induce ZmRR1 expression in the detached leaf system, suggesting that such species function as the inducer(s); iP also had a slight effect on the accumulation of the transcript (Takei et al., 2001b). This induction was transcriptionally regulated (D’Agostino et al., 2000; Deji et al., 2000).

The current scheme of the multiple pathways communicating nitrogen availability in the whole plant is illustrated in Fig. 5. Active cytokinin species, that are newly synthesized or converted from a storage form, are translocated from the root to the shoot with further conversion to various active species, and Z is a possible signal substance communicating nitrogen availability from the roots to the shoots. In the leaf cell, the His–Asp phosphorelay system transduces the signal to the target gene(s) or protein(s). Although some of the key factor(s) involving the signal transduction process have emerged, the whole body of the communicating system is still unclear at present. It will be necessary to reveal the downstream target genes (or proteins) of the His–Asp phosphorelay system in order to understand the physiological function in the nitrogen signalling pathway mediated by cytokinin.

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