Searching for Nitrogen Sensing Systems in Higher Plants

Man-Wah Li¹ • Hon-Ming Lam¹,²*

¹ Molecular Biotechnology Program, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China
² Department of Biology, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China

Corresponding author: * honming@cuhk.edu.hk

ABSTRACT

Nitrogen is an essential element of all life forms. With limited mobility, most plants cannot actively hunt for additional nitrogen resources apart from the substratum they rooted. To efficiently utilize the finite nitrogen resource, plants need to adopt a set of nitrogen sensing systems to manage the nitrogen status from time to time. To cope with diverse situations, different systems were evolved to perceive different forms of signals (from inorganic nitrogen to organic nitrogen) at different levels (from intracellular to the whole plant). The integrated effects of these signals ensure efficient uptake, transportation, assimilation, utilization, and storage of important nitrogen resources. Several nitrogen sensing systems were documented in microorganisms and animals. In this review, we summarized the recent progress of similar systems found in plants, including: (i) PII-mediated nitrogen regulatory system; (ii) general amino acid control; (iii) ionotropic glutamate receptors; (iv) cytokinin His-Asp phosphorelay system; and (v) NIT2 system.

Keywords: cytokinins His-Asp phosphorelay, general amino acid control, glutamate receptors, nitrogen signaling, PII, signal transduction

INTRODUCTION

Plants are sessile living organisms which depend on the limited nutrients in the confined substratum where they rooted. Changes of nutrient availability may happen either as a long-term subtle change or an unpredictable rapid event. Coordinated adjustment to nutrient status is the key of success in the community.

Nitrogen (N) is one of the major constituents of life. It is essential for the biosynthesis of important biomolecules such as amino acids, nucleic acids, chlorophyll, phytohormones as well as other N-containing metabolites. Though nitrogen gas (N₂) makes up 78% of the Earth’s atmosphere, it cannot be directly used by plants. Plants mainly acquire N in the form of nitrate from the soil and assimilate the acquired N resources into amino acids via reduction processes. Some plants can undergo symbiotic N fixation to “fix” N₂ into amino acids. Both processes are extremely energy demanding. The ultimate energy source is coming from respiratory oxidation of photosynthetic products while, on the other hand, N is an essential constituent of the photosynthetic machinery. An efficient coordination between N (acquisition, assimilation, and catabolism) and carbon (C) (photosynthesis and respiration) pathways is essential for the survival of plants.

Monitoring systems for N availability is therefore strategically important. N sensing systems were first reported in microorganisms and animals, including: (i) PII-mediated nitrogen regulatory system in Escherichia coli and cyanobacteria sensing the balance between C and N metabolites; (ii) general amino acid control (GAAC) in yeast sensing amino acid starvation conditions; (iii) ionotropic glutamate receptors (iGLRs) in animal nervous system responsive to organic N in the form of amino acids; (iv) cytokinin His-Asp phosphorelay system in bacteria mediates signal initiated by inorganic nitrate; and (v) NIT2 system responsive to both organic and inorganic forms of N signals.

Although the plants have evolved a unique mode of living, increasing evidences suggest that plants have adopted N sensing systems homologous to the systems in bacteria, yeast and animals described above. In this review, we summarized some current developments in this aspect.

PII-mediated nitrogen regulatory system

PII-mediated nitrogen sensing has been thoroughly studied in E. coli and cyanobacteria and identified as a system to regulate cellular C:N balance. In the glutamine synthetase/glutamate oxoglutamate aminotransferase (GS/GOGAT) cycle, ammonium (NH₄⁺) generated via nitrate/nitrite reduction is first incorporated into glutamate (Glu) to form glutamine (Gln). The Gln will then react with 2-oxoglutarate (2KG) to form two Glu molecules. Therefore, NH₄⁺ deficiency will lead to an accumulation of 2KG (N-limiting) while high level of Gln will be built up with surplus of NH₄⁺ (N-rich). The PII system regu-
lates the C:N balance largely base on this equilibrium.

In _E. coli_, N-limiting conditions (high 2KG, low Gln) will stimulate binding of 2KG and ATP to PII and stimulate PII uridylylation by the uridylyltransferase/uridylyl-removing enzyme (UTase/UR) at Tyr-51. PII-UMP will attempt to (i) deadylylate the inactive adenyllylated glutamine synthetase (GS) by stimulating adenyltransferase (ATase); (ii) stimulate NRI phosphorylation by removing PII from NRI (NRI kinase) to activate the transcription of the _gnlA_ gene (encoding GS) (Ninfa and Atkinson 2000).

Distinct from PII of _E. coli_, PII in the photosynthetic cyanobacteria (Fig. 1) is post-translationally regulated by phosphorylation on serine-49 (Ser-49) instead of uridylylation. When Ser-49 is substituted with alanine (Ala), PII-mediated regulation of nitrate and nitrite uptake will be lost (de Marsac et al. 2001).

When organic N becomes limiting (high 2KG, absence of NH$_4^+$, presence of NO$_3^-$ or NO$_2^-$), cyanobacterial PII will be phosphorylated upon synergistic binding of ATP and 2KG (Forchhammer and Demarsac 1994; Forchhammer and Hedler 1997; Kobayashi et al. 2005). Under such conditions, dephosphorylation of PII is prevented (Ruppert et al. 2002) to keep the switch in the “ON” state. Dephosphorylation of PII-P from _Synchococcus_ sp. strain PCC7972 is catalyzed by a type-2C protein phosphatase (phosphor-PII (PII-P) phosphatase: PphA) under low ATP concentration independent of 2KG (Ruppert et al. 2002). PII and PphA are also important for the regulation of NO$_3^-$ uptake in _Synechocystis_ sp. strain PCC6803 when the reducing power is low, under low light conditions (Klof and Forchhammer 2005).

Under normal growth conditions, the PII interaction protein X (PipX) binds to the unphosphorylated PII. However, N deprivation triggers phosphorylation of PII to release the PipX protein from the protein complex. Favored by the presence of 2KG, the free PipX will then bind to the transcriptional factors NtcA (Espinosa et al. 2006) and up-regulate the expression of _gnlB_ (encoding PII: Lee et al. 1999), _gnlA_ (encoding GS: Vazquez-Bermudez et al. 2002), and _nirA-nrtABCD-narB_ (encoding nitrite reductase, high affinity-transporter permease, nitrate reductase) in the _nir_ operon (Luque et al. 1992) as well as _ntcA_ itself.

N-acetyl-L-glutamate kinase (NAGK) is an enzyme that involved in arginine (Arg) biosynthetic pathway using Glu as a precursor. NAGK is the key target enzyme for feedback inhibition by excessive Arg. In _Synechococcus elongates_ strain PCC7972, when N is not limiting, unphosphorylated PII binds NAGK and greatly reduces the feedback inhibition of Arg biosynthesis. Conversely, when N is insufficient, NAGK will be released from phosphorylated PII and the feedback inhibition was restored (Maheswaran et al. 2004). Such control is useful in manipulating C:N balance since Arg is the amino acid having the lowest C:N ratio.

Plant PII homologues were first identified in _Arabidopsis thaliana_ (encoded by _AtGLB1_) and _Ricinus communis_ (castor bean) and exhibit more than 50% homology to PII proteins from _E. coli_ and cyanobacteria (Hsieh et al. 1998). Subsequent EST search revealed the presence of PII-like proteins in over 20 plant species including _Oryza sativa_ (rice), _Lycopersicon esculentum_ (tomato), _Glycine max_ (soybean), _Medicago sativa_ (alfalfa) and so on (Moorehead and Smith 2003). The plant PII proteins are nuclear-encoded and targeted to chloroplast (Hsieh et al. 1998). Resemblance of the PII proteins in _E. coli_ and cyanobacteria, the plant PII-like proteins are homotrimers with subunit of around 16.8kDa (Smith et al. 2003).

In _A. thaliana_ (Burillo et al. 2004) and rice (Sugiyama et al. 2004), NAGK is a potential interacting partner of plant PII. Recent studies have further proved that the biosynthesis of Arg in _A. thaliana_ is also regulated by the interaction between NAGK and PII, similar to that in cyanobacteria (Ferrario-Mery et al. 2006) while PII-NAGK crystal structure revealed structurally how NAGK is regulated by PII and Arg (Mizuno et al. 2007). In addition, plant PII proteins can bind to 2KG or ATP at their chloroplastic physiological concentrations (Smith et al. 2003). Overexpression of _AtGLB1_ impairs plant ability in sensing and metabolizing Gln, leading to an unusual accumulation of anthocyanin in seedlings grown on medium containing high sucrose and using Gln as the sole N source (Hsieh et al. 1998). Moreover, _A. thaliana_ PII mutants show increased sensitivity to

- 2-oxoglutarate
- ATP

![Fig. 1 Model of PII-mediated nitrogen regulatory system in cyanobacteria. When N is not limiting (signified as low ATP and 2-oxoglutarate (2KG) levels), PII interacts with NtcA and activates transcription of target genes involved in N uptake, metabolism, and regulation while free NAGK is the target for feedback inhibition in the Arg biosynthetic pathway. Plant homologues of PII and NAGK have been identified and proven to be involved in the regulation of Arg biosynthesis.](image)
nitrogen sensing. Li and Lam

Nitrogen sensing. Li and Lam

nitrite toxicity and become defective in monitoring C:N balance under N-limiting or excessive NH$_4^+$ conditions (Ferrario-Mery et al. 2005). All these findings support the involvement of PII in the N sensing in plants.

Despite these advances, there are still many black boxes that have not been unveiled in the plant PII-mediated N regulatory system. First, the NRI/NRII two-component system of _E. coli_ and the NtcA system of cyanobacteria have not been discovered in plants. The mechanism of how plant PII proteins convert the N depletion signal into transcription control remained unknown. Moreover, phosphorylation/de-phosphorylation has yet to be demonstrated in plant PII proteins. Although the Ser residue needed for phosphorylation is conserved in the T-loop of _A. thaliana_ PII protein, crystal structure reveals that the Ser residue is not exposed to the surface (Smith et al. 2003). Such exposure is important for protein-protein interactions to take place.

**General amino acid control**

General amino acid control (GAAC) refers to the phenomenon of cross-pathway transcription regulation of amino acid biosynthesis upon amino acid starvation in _Saccharomyces cerevisiae_ (yeast) (Fig. 2). Details of the yeast GAAC can be found in a comprehensive review by Hinnebusch (2005).

Uncharged tRNAs, which accumulated as a result of amino acid starvation, will bind to the histidyl-tRNA synthetase (HisRS) related domain of the GCN2 (general control non-repressible 2) protein to activate the juxtaposed kinase domain. The binding of uncharged tRNA to GCN2 is facilitated by the interaction of GCN2 with the GCN1/GCN20 (two elongation factor 3 (EF3)-like proteins) complex. The activated GCN2 phosphorylates the α subunit of eukaryotic translation initiation factor 2 (eIF2α), leading to a subsequent global inhibition of protein translation and reprogramming of transcriptome.

The eIF2 consists of α, β, and γ subunits. GTP-bound eIF2 together with methionyl initiator tRNA (Met-tRNA$_{Met}^{Met}$) to form a ternary complex (TC: eIF2/GTP/Met-tRNA$_{Met}^{Met}$). The TC assembles 40S ribosomal protein to form the 43S pre-initiation complex which scans along the mRNA from the 5’ cap for the AUG start codon. At which, GTP will be hydrolysed by eIF5 to GDP during the formation of the 48S complex. Translation will begin after re-recruitment of the 60S ribosomal subunit by the 48S complex. GDP-bound eIF2 will be converted back to the GTP-bound form by the guanine-nucleotide exchange factor (eIF2B) to initiate the next round of translation.

After phosphorylation by GCN2 during amino acid starvation, eIF2α will inhibit the exchange of GDP with GTP and reduce the availability of eIF2-GTP for TC formation, resulting in a halt of the global translation. In contrast, such condition will activate the translation of the transcriptional factor GCN4 (Hinnebusch 2005). Four upstream open reading frames (uORFs) are found located in the 5’ untranslated region (5’UTR) of _GCN4_ mRNA. Translation of these four genes with uORFs have been found in plants.

---

**Fig. 2 Model of general amino acid control in yeast.** The kinase activity of GCN2 is activated by binding to uncharged tRNAs accumulated upon amino acid starvation. The binding of uncharged tRNAs is facilitated by the binding of GCN1/GCN20 complex to GCN2. The activated GCN2 phosphorylates the α subunit of eukaryotic translation initiation factor 2 (eIF2α) and inhibits the exchange of GDP on the eIF2γ with GTP. This will reduce the availability of eIF2-GTP for the formation of ternary complex (TC) involved in translation initiation. When there is no starvation, TC formation is not limited by the phosphorylated eIF2α, abundant TC allows translation of the upstream open reading frame (uORFs) of GCN4 which leads to pre-mature disintegration of the ribosome complex before translating the _GCN4 ORF_. Reduction of TC during amino acid starvation allows the ribosome to bypass the uORFs of GCN4 and allows the translation of the _GCN4 ORF_. GCN4 protein is a transcription factor acting on GCRE motif in the promoter of target genes. GCN4 regulates the expression of several hundreds of genes, including a number of amino acid biosynthetic genes. When N supply is scarce, GCN2 is dephosphorylated by SIT4 which is a component of the TOR pathway. Homologues of GCN1, GCN2, GCN20, eIF2α, GCRE-like motif and genes with uORFs have been found in plants.
uORFs will lead to the pre-mature release of ribosomes and make the translation of GCN4 ORF impossible. Shortage of TC during starvation allows ribosomes to bypass uORF4 before re-binding to TC, enabling the translation of the ORF of GCN4.

GCN4 is a transcription factor that regulates the expression of a large number of genes to deal with adverse conditions. Microarray analysis revealed at least 539 GCN4 target genes that are involved in 12 different amino acid biosynthetic pathways, amino acid precursor biosynthesis, vitamin/cofactor biosynthesis, peroxisome biogenesis, glycolen metabolism, purine biosynthesis and so on (Natarajan et al. 2001). GCN4 regulates the expression of these genes by acting on the GCN4-responsive element (GCRE: 5’TGAATC3’) in their promoters (Arndt and Fink 1991). The expression of GCN2 itself is also upregulated by overexpressing GCN4 (Rousseau et al. 1988). Other proteins including GCN5 (a histone acetyltransferase), ADA2 (a transcriptional adaptor protein), MBF1 (multiprotein bridging factor 1), and TBP (TATA-box binding protein) also participate in gene expression regulation mediated by GCN4 (Barlev et al. 1995; Takemaru et al. 1998; Kuo et al. 2000; Swanson et al. 2003). On the other hand, GCN2 is also under the regulation of the TOR (target of rapamycin) pathway. Under non-starvation conditions, GCN2 is phosphorylated at Ser-577 to reduce its affinity towards uncharged tRNAs. At the same time, the downstream effector TAP42 of the TOR pathway binds to SIT4 (a type-2A protein phosphatase), preventing it from dephosphorylating GCN2. In contrast, during starvation conditions, SIT4 will be released from TAP42 and from dephosphorylating GCN2. In doing so, GCN2 will be phosphorylated at Ser-51 by the yeast bridging factor 1, and TBP (T A TA-box binding protein) (GCRE: 5’/397TGACTC3’/397) in their promoters (Arndt and Fink 1991). This result suggests the existence of a nervous system in plant, a gene family encoding 20 putative iGLRs is present in the genome of A. thaliana (Lam et al. 1998; Lacombe et al. 2001; Chiu et al. 2002), supporting the notion that iGLRs appeared before the divergence of plant and animal (Chiu et al. 1999). By the percentage identity of amino acid, the 20 iGLRs from A. thaliana can be clustered into 3 clades similar to those in animals (Chiu et al. 2002). Subsequent studies reveal the possible roles of these in plants (Fig. 3).

Several amino acids (alanine, asparagine, cysteine, glutamate, glycine and serine) were shown to trigger rapid and drastic changes in cytosolic Ca2+ concentration, using the A. thaliana aequorin reporter lines (Demnison and Spalding 2000; Dubos et al. 2003; Qi et al. 2006). In addition, impaired Ca2+ utilization and increased sensitivity to ionomic stress by the constitutive expression of AtGLR1.2.3.2 in A. thaliana (Lacombe et al. 2001) suggests the existence of amino acid-gated Ca2+ channels. Further studies revealed the absence of amino acids induced membrane depolarization in glr3.3 knockout lines, supporting the relationship between plant iGLRs and the Ca2+ influx (Qi et al. 2006). Consecutive Glu treatment of A. thaliana at pH 7.7 leads to desensitization (Qi et al. 2006), similar to the regulation of synaptic Ca2+ signaling in the animal central nervous system (Jones and Westbrook 1996). This further supports the ligand-related receptor relationship. Alternatively, AtGLR1.1 was found to be expressed in the Arabidopsis root (Fig. 3). To test if plant iGLRs participate in signal transduction as their counterparts in animals, A. thaliana was subjected to the animal iGLR antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX: Lam et al. 1998) and agonist S(+)-β-D-aminoisopropionic acid (BMAA: Brenner et al. 2000) treatments. DNQX treatment blocked the light induction of chlorophyll synthesis and light inhibition of hypocotyl elongation, while BMAA treatment caused hypocotyl elongation and inhibited cotyledon opening.

Using amino acids as the signaling molecules, it is likely that plant iGLRs can take part in regulation C and N metabolisms. Underexpression of AtGLR1.1 alters the C/N sensitivity of the plant during seed germination (Kang and Turano 2003). Reduced transcript levels of genes encoding C and N metabolic enzymes including cytosolic glutamine synthetase (GS), aspartate transaminase (AAT2), nitrate reductase (NRI), nitrite reductase (NIR), nitrate transporter (CHL1), NADP-dependent isocitrate dehydrogenase (ICDH), and hexose kinase 1 (HXX1) were observed in the antiAtGLR1.1 line (Kang and Turano 2003), suggesting a regulatory role of AtGLR1.1 in C and N metabolisms.

Abscisic acid (ABA) is a plant hormone that regulates germination and stomatal closure and can mediate the regulatory effects of nitrate on root branching in A. thaliana. Underexpression of AtGLR1.1 results in the upregulation of
ABA biosynthetic gene (zeaxanthin epoxidase: ABA1) while DNQX treatment leads to an accumulation of ABA and a reduction in germination rate (Kang and Turano 2003). Kang and Turano (2003) proposed a working model in which N signals (NO₃⁻ or amino acids) act as the positive signals stimulating AtGLR1.1 to inhibit the ABA biosynthesis and ease the ABA induced physiological changes, while C signals (sucrose) act negatively toward AtGLR1.1 to reduce the expressions of C and N metabolic genes and elevate ABA biosynthesis which in turn inhibits seed germination.

Decomposition of organic matter in the soil, plant root exudates and microbial actions on roots release amino acids into the rhizosphere. The growth of primary roots but not lateral roots is strongly inhibited by Glu in the medium (Filleur et al. 2005). All 20 iGLRs from A. thaliana are strongly expressed in roots while 5 of them are root specific (Chiu et al. 2002). iGLRs in roots may take part in sensing the change of amino acid composition in soil (Filleur 2005; Qi 2006), leading to the adjustment of root architecture for colonizing the nutrient-rich patch of soil (Filleur et al. 2005).

γ-aminobutyric acid (GABA) is recently found to be a long-distance signal in up-regulation of nitrate uptake in Brassica napus L. (Beuve et al. 2004). However, the present of plant GABA receptors is yet to be confirmed. Sequence analysis showed that the N-terminal region of plant iGLRs are related to the GABA₉ receptors, suggesting that plant iGLRs may also participate in shoot-to-root signaling mediated by GABA (Turano et al. 2001).

Other than N sensing, plant iGLRs may also play roles in environmental stress responses. For examples, AtGLR3.4 can be induced through touch, cold, and the applications of GABA, aspartate, malate as well as membrane-permeable weak acids (Meyerhoff et al. 2005). Treatment of either the Ca²⁺ channel blocker gadolinium or the iGLR antagonist 2-amino-5-phosphonopentanoate can eliminate the effects of Glu, resembling the toxicity of Al³⁺ on cortical microtubules depolymerization and root growth inhibition (Sivaguru et al. 2003).

**Cytokinins His-Asp phosphorelay**

Cytokinins are a type of important plant hormones that involved in the regulation of diverse biological processes such as cell division and differentiation, chloroplast biosynthesis, senescence and signal transduction. Naturally occurred cytokinins are adenine-type cytokinins derived from adenine and can be further classified into isopentenoid cytokinins and aromatic cytokinins.

Cytokinin may also mediate N signal between root and leaf (Fig. 4). Replenishment of nitrate to maize trigger accumulation of certain cytokinins species in roots by recycling of the stored form and de novo synthesis (Takei et al. 2001). This is supported by the observation that a cytokinin biosynthetic gene, isopentenyl transferase 3 gene (ATIPT3), was induced by nitrate replenishment (Miyawaki et al. 2004). Mass flow in xylem induced by absorption of nitrate brings along the accumulated cytokinins to the leaves (Takei et al. 2001) at which the signal will be perceived by the His-Asp phosphorelay.

The His-Asp phosphorelay was first identified in bacteria which comprises of two components, the histidine protein kinase (HPK) and the response regulator (RR) (Stock et al. 1989). Upon stimulation, HPK will autophosphorylate a highly conserved His residue within the transmitter domain using an ATP, and then pass the phosphoryl group to a conserved Asp residue on the receiver domain of RR, which will in turn trigger the downstream responses (Stock et al. 2000). Multi-step His-Asp phosphorelay was later found to have an extra component, the histidine-phosphotransfer protein (HTP), which helps relaying the phosphoryl group between the HPKs and RRs (Appleby et al. 1996). Hybrid HPKs having extra (one or more) receiver domains fused to the C-terminus of the transmitter domain were also found such that the phosphoryl group in the multi-step His-Asp phosphorelay must pass through several rounds of phosphorylation to reach the RRs (Appleby et al. 1996).

In plants, multi-step His-Asp phosphorelay is adopted to perceive the cytokinins signals. The N-terminal of the plant HPKs consists of multiple transmembrane regions forming a cyclases/histidine kinase-associated sensory extracellular (CHASE) domain as the docking site of cytokinins (Anantharaman and Aravind 2001).

Cytokinin-responsive HPKs have been identified in A. thaliana (AHK2, AHK3, AHK4/CRE1/WOL: Inoue et al. 2001; Ueguchi et al. 2001), maize (ZmHK1, ZmHK2, ZmHK3a/b: Yonekura-Sakakibara et al. 2004), and rice (OHK2-5: Ito and Kurata 2006). AHK4 is responsive to only trans-zeatin (Inoue et al. 2001) while ZmHK1 is responsive to zeatin in both of its cis and trans forms (Yonekura-Sakakibara et al. 2004). Furthermore, AHK3 and AHK5 are also responsive to cytokinins.
AHK4 exhibit differential binding affinity toward different cytokinins and such preference is pH-, salt-, and temperature dependent (Romanov et al. 2006). Together, these data implicate that different plant HPKs may have diverse specificities towards different cytokinins and may function in response to different kinds of environment stimulations.

Multiple HPTs are also present in different plant species including A. thaliana (AHPI-5; Imamura et al. 1999), rice (OHP1-2; Ito and Kurata 2006), and maize (ZmHP1-3; Sakakibara et al. 1998; Sakakibara et al. 1999; Asakura et al. 2003). AHPS relocalize into nucleus upon cytokinins treatment (Hwang and Sheen 2001) without an upregulation in transcription (Tanaka et al. 2004), suggesting that plant HPTs act as the shuttles to pass the cytokinins signals from HPKs on the membrane to the RRs and cytokinins response factors (CRFs) (see below) in the nucleus. High-order but not single ahp mutations reduce the plant sensitivity toward cytokinins (Hutchison et al. 2006). AHPI-3,5 are proposed to be the positive regulators of the cytokinins response. On the other hand, AHPI4 is a negative regulator since cytokinins sensitivity reduction in high-order ahp mutants can be slightly reverted with an additional ahp4 mutation (Hutchison et al. 2006). Pseudo-HPTs lacking the conserved His residue for phosphorylation (AHPI6; Mahonen et al. 2006) and alternatively spliced AHPI5 (AHPSL1; Hadilova and Brzobohaty 2007) are also putative negative regulators in the cytokinins responses.

Downstream of HPTs are the RRs and CRFs. Multiple RRs are present in A. thaliana (ARRs: Hwang et al. 2002), rice (OsRRs: Ito and Kurata 2006), and maize (ZmRRs: Sakakibara et al. 1998; Sakakibara et al. 1999), CRFs are recently reported to be components of the cytokinins His-Asp phosphorelay in A. thaliana (Rashotte et al. 2006).

Based on sequence homology, physiological functions and modes of induction, RRs are further divided into type-A RRs and type-B RRs. Type-B RRs are transcriptional factors containing the GARP DNA-binding domain which activate genes with upstream GARP motif (Riechmann et al. 2000). Targets of type-B RRs are involved in diverse regulatory functions. For example, the targets of ARR1 (a type-B RR) include genes encoding type-A RRs, CRFs, cytokinins metabolic enzymes, putative disease resistance response proteins, and structural proteins (Taniguchi et al. 2007).

Functions of type-A RRs may be quite varied. For example, ARR4 from A. thaliana can bind to seemingly unrelated targets such as DNA-binding proteins (Yamada et al. 2004) and phytochrome B (Sweere et al. 2001). GARP motif is commonly found on the promoter of type-A RRs (Sakai et al. 2001). In A. thaliana, type-A RRs are transcriptionally induced by Type-B RRs in response to cytokinins and nitrate (Taniguchi et al. 1998). Despite that the expression of type-A RRs can be induced by nitrate in maize plants, expression of type-A RRs in detached leaves can only be induced by cytokinins but not nitrate (Sakakibara et al. 1998; Sakakibara et al. 1999). This supports the notion that nitrate signal is transferred to effectors through the His-Asp phosphorelay mediated by cytokinins.
CRFs belong to the family of AP2 transcription factors and are transcriptionally induced by cytokinins in a type-B RRs dependent manner. Like the HPTs, CRFs also relocate to the nucleus upon cytokinins treatment (Rashotte et al. 2006). Loss of function analysis showed that CRFs control mainly the development of cotyledons, leaves, and embryos while microarray data revealed that a large portion of genes regulated by CRFs are overlapping with genes induced during early cytokinins transcriptional response and a portion of genes that are regulated by type-B RRs. Redundancy and overlapping functions have been shown in every steps of the His-Asp phosphorelay in A. thaliana by knockout assays, including AHKs (Higuchi et al. 2004; Nishimura et al. 2004; Riefler et al. 2006), AHPs (Hutchison et al. 2006), type-A RRs (To et al. 2004), type-B RRs (Mason et al. 2005); and CRFs (Rashotte et al. 2006). Such high redundancy may help to fine-tune the cytokinins His-Asp phosphorelay signaling pathway toward different situations (Hutchison et al. 2006).

Existence of HPTs and hybrid HPKs containing additional receiver domains may help in the signal transduction by: (i) providing multiple points of regulation such as transcription control of each unlinked components or cross-talk with other signaling pathways (Appleby et al. 1996); and (ii) increasing tolerance to noise ratio without affecting the rapid response toward the actual stimulation (Kim and Cho 2006).

Correlation between N signals and responses of cytokinins His-Asp phosphorelay has not yet been concretely proven. Meanwhile, gene induction overlapped in nitrate response, cytokinins response, RRs induced response, and CRFs induced response does not seem to relate directly to primary N metabolism. However, cytokinins His-Asp phosphorelay signaling pathway is induced upon sufficient supply of N that signifies a proper time for growth and development. Induction of a whole array of genes could therefore be a preparation for growth under rich nutrient supply.

**Nit2 System**

The major nitrogen regulatory protein NIT2 is a GATA transcription factor in *Neurospora crassa* (Chiang and Marzluf 1994). NIT2 contains a single zinc finger DNA-binding domain (Fu and Marzluf 1990) that regulates the expression of many structural genes as shown in Fig. 5, including nit-3 (nitrate reductase; NR) and nit-6 (nitrite reductase; NiR) which are important for N metabolism (Marzluf 1997).

When primary N sources (NH4+, Gln and Glu) are available, the negative regulator NMR (nitrogen metabolite repression) will interact with NIT2 to neutralize its DNA-binding activity, leading to the loss of transcription activation (Xiao et al. 1995; Pan et al. 1997). Either deletion or mutation of the NMR binding regions on NIT2 will result in the loss of N repression (Pan et al. 1997). In contrast, when primary N sources are limiting, NIT2 will bind to the NIT2 sites (GATA elements) upstream of the target genes (Marzluf 1997). At the same time, the pathway-specific regulatory factor NIT4 will act on the NIT4 sites located adjacent to the NIT2 sites and interact with NIT2 to derepress the gene expression (Feng and Marzluf 1998; Mo and Marzluf 2003), resulting in a shift to utilize secondary N resources (nitrate and nitrite). However, the exact N sensing mechanism of NIT2 is still unclear.

Most plants obtain N in the form of nitrate from soil which is than converted into NH4+ by the sequential reduction reactions by NR and NiR before assimilated into amino acids. The NIT2-like regulatory system may be involved in the regulation of NR and NiR genes. *In vivo* DMS footprinting and electrophoretic mobility shift assays using the *Neurospora* NIT2 revealed the existence of putative nitrate-responsive NIT2 binding site on the promoter of the spinach NR gene (Rastogi et al. 1997). The plant homologue of NIT2 (NITL1; NIT2-Like protein), exhibiting a 60% homology to the zinc finger DNA-binding domain of NIT2, was identified in *Nicotiana plumbaginifolia* by cross-hybridization (Danielvedele and Caboche 1993). However, the function of NITL1 has not been fully elucidated.

**Conclusion**

In this review, we have discussed five putative plant N sensing systems, responding to different signals. IGLRs are receptors using amino acids as ligands while GAAC is activated during amino acid depletion. Cytokinin His-Asp phosphorelay mediates signals upon replenishment of N (N sufficient) while PII responses to low C:N ratio (N deficient). Despite that the function of the plant NIT2 system is still largely unknown, the mode of its activation seems to be quite distinct from the other four systems. Previously, the search of plant N sensing systems based heavily on the identification of components homologous to that found in bacteria, fungi, and animals. Individual N sensing system was often investigated independently. With the rise of system biology and the gradual revealing of missing links of each system, the future focus of researches will switch to the interactions between systems, giving rise to a detailed and macroscopic view of the N sensing mechanisms in plants. The VirtualPlants (http://virtualplant-prod.bio.nyu.edu/cgi-bin/virtualplant.cgi) system recently developed pro-

---

Fig. 5 Model of NIT2 system in *Neurospora crassa*. In the presence of primary N sources (ammonium, glutamine, and glutamate), NMR (nitrogen metabolite repression) binds to NIT2. This prevents NIT2 from binding to the GATA4 element. On the other hand, when secondary N sources (nitrate and nitrite) instead of the primary N sources are available, NIT2 will be released from NMR and bind to the GATA4 element in the promoter of NIT2 target genes. Concurrently, NIT4 will bind to NIT4 binding site and interacting with NIT2 to enhance the production of enzymes such as nitrate reductase (NR) and nitrite reductase (NiR) for efficient utilization of the available N sources. Plant NIT2-like protein (NLT1) and NIT2 bind site (GATA element) on the promoter of plant genes have been identified.
vides a sophisticated tool and platform to integrate every single system in silico to reveal the interactions and cross-talks therein.

ACKNOWLEDGEMENTS

This work was supported by the Hong Kong UGC AoE Plant & Agricultural Biotechnology Project AoE-B/07/09.

REFERENCES

Anantharaman V, Aravind L (2001) The CHASE domain: a predicted ligand-binding module in plant cytokinin receptors and other eukaryotic and bacte-

Arndt K, Fink GR (1986) G protein, a positive transcription factor in yeast, binds general control promoters at all 5'TGACTC3' sequences. Proceedings of the National Academy of Sciences USA 83, 8516-8520


20

Dynamic Soil, Dynamic Plant 2 (1), 13-22 © 2008 Global Science Books
regulator NtcA regulates transcription of the signal transducer PII (GlnB) and influences its phosphorylation level in response to nitrogen and carbon supplies in the Cyanobacterium *Synechococcus* sp strain PCC 7942. Journal of Bacteriology 181, 2697-2702.


Turano FJ, Panta GR, Allard MW, van Berkum P (2001) The putative glutamate receptors from plants are related to two superfamilies of animal neurotransmitter receptors via distinct evolutionary mechanisms. Molecular Biology and Evolution 18, 1417-1420


