Nitrogen and Carbon Nutrient and Metabolite Signaling in Plants

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The effect of nutrients on plant growth and development has been studied for over 350 years since the experiments of van Helmont in 1648 (6). Recent studies on nutrient effects in plants have involved separating their role as building blocks of organic matter or cofactors from their potential role as signaling molecules. The first notion that soil nutrients—or the lack thereof—could be sensed by plants was published in 1906 by Brezeale who documented increased transport of nutrients in response to starvation (9). Fast forward nearly 100 years and transporters for most macronutrients and micronutrients have been cloned using a variety of techniques including microbial complementation, expressed sequence tags, and Arabidopsis mutant isolation. Studies of these cloned transporters have begun to define the molecular basis for the regulation of uptake for many of the macronutrients (NH₄⁺, NO₃⁻, K⁺, Ca²⁺, Mg²⁺, H₂PO₄⁻, SO₄²⁻, and Mn²⁺) and micronutrients (Cl⁻, Zn, Mn, Fe, and Cu). In a number of cases, nutrient availability has been shown to affect the transcription of the transporter gene. A current challenge is to determine if the nutrient (or lack thereof) is sensed or if the signal is a derived metabolite. If one considers how long it has taken researchers to unravel signal transduction pathways for which the signal is known (e.g. hormones, light), uncovering the workings of nutrient/metabolic signaling systems is likely to provide challenges to new researchers well into the new millennium. Even in cases where a signaling metabolite has been defined, such as for Glc in yeast and in plants (10), the signaling cascades are proving to be remarkably complex. For example, Glc signaling in plants has been shown to involve complex cross-talk with hormone signaling pathways (27). Although there is ample evidence for nutrient signaling in relation to a number of macronutrients and micronutrients, we have focused this historical note on N and C nutrient and metabolite sensing in plants. We have attempted to cite early studies that suggested that N or C nutrients, or their derived metabolites, are sensed and to highlight examples where recent molecular-genetic studies have begun to identify components of these sensing/signaling pathways.

N NUTRIENTS AND METABOLITES AS SIGNALS

The beneficial effects of saltpeter (KNO₃) on plant growth has been known since the mid-sixteenth century (9). Since that time, evidence has mounted to support a model in which nitrate acts as a positive signal necessary for the induction of nitrate uptake and its reduction (7). In contrast, the metabolized product(s) of nitrate, ammonium, and its assimilation products Glu and Gln are believed to exert negative effects on nitrate uptake and reduction. Evidence to support this model was first discerned using genetic and molecular approaches in the fungi Neurospora and Aspergillus (3). Molecular-genetic studies in plants have also lent support for nitrate and its downstream metabolites to act as signaling molecules in higher plants (21). However, the role of nitrate as an inducer and ammonium or Gln/Glu as repressor signals is too simplistic a model. For example, nitrate serves to repress the expression of a key enzyme in the starch biosynthesis pathway, presumably to divert C skeletons toward the N-assimilatory pathway (18). Below is a brief review of studies that have provided evidence that nitrate, ammonium, or amino acids may serve as signaling molecules in plants.

NITRATE AS A SIGNAL

In the 1950s, physiological studies showed that nitrate treatment could induce nitrate reductase (NR) activity and nitrate transport, suggesting a role for nitrate (or nitrite) as a signaling molecule. In the 1970s, antibodies were used to show that NR was induced by nitrate. Finally in the 1980s, the gene cloning decade, a molecular basis for this nitrate regulation began to emerge. Nitrate applications were shown to induce the accumulation of mRNAs for NR and for a host of other C- and N-metabolic genes (e.g. SPS, GS, etc.; 3,4). The 1990s saw the cloning of NRT1 (CHL1) and NRT2 nitrate transporter genes, and it was shown that nitrate could induce the expression of both (7, 17, 22). Despite all
these studies, it was still not clear how this signaling pathway worked or whether nitrate or a downstream metabolite was sensed. In 1997, NR mutants of tobacco that contain very low levels of NR activity were used to demonstrate that nitrate was capable of altering gene expression even though it could not be reduced or further metabolized (18). These studies also suggested that nitrate signaling can interact with signals generated further downstream in N metabolism. This was deduced because nitrate treatment of wild-type plants led to transient induction of genes involved in nitrate uptake and metabolism, whereas nitrate induction in the NR mutants was sustained. These findings suggested that downstream products of nitrate reduction (ammonium or Gln) might act as signaling molecules (see below). A significant regulatory gene, ANR1, was isolated while searching for nitrate-induced clones. Repression of ANR1 in antisense transgenics was shown to impair systemic nitrate repression of lateral root growth and localized nitrate stimulation of lateral root growth (26). The isolation on ANR1 was significant, as it succeeded to combine a nutrient (nitrate) signaling property with a morphological response.

AMMONIUM AND DERIVED AMINO ACIDS AS SIGNALS

By contrast to nitrate, evidence for a role of ammonium or a derived amino acid as a signaling molecule is still rather poor. The first evidence that ammonium or a derived metabolite could serve as signals in plants came from studies on the regulation of ammonium transport. In 1953, N starvation was shown to induce ammonium uptake in N-starved *Chlorella*, and similar studies were conducted in wheat in 1962 (9). In the ensuing decades, ammonium and/or its assimilation products (Glu/Gln) have each been implicated as negative regulators of nitrate and ammonium uptake in plants (5,7). A molecular basis for this regulation came with the cloning of ammonium transporter (AMT) genes, where it was shown ammonium treatment repressed AMT1 expression (24). More recently, evidence has been presented that Gln, a product of ammonia assimilation, may affect the repression of AMT1-mediated ammonium transport in Arabidopsis roots (16). These studies support the notion that N-assimilation products (Gln or Glu) might act as signals whose levels are sensed as an indicator for a high internal N status. Along these lines, putative sensors of Glu in plants, Glu receptor genes, have been identified in Arabidopsis (14). Further identification of components of putative amino acid sensing systems will profit from both forward and reverse genetic approaches.

N:SSENSING IN PLANTS

N and C metabolism are tightly linked in almost every biochemical pathway in the plant. As such, it is not surprising that C metabolites regulate genes involved in N acquisition and metabolism. Early studies on NR in 1976, showed that NR activity could be affected by Glc/Suc (3,4). Those observations were supported by later experiments that showed sugars induce NR mRNA in dark-adapted, green seedlings (1). The notion that C and N may have antagonistic relationships as signaling molecules was reported byVincentz et al., who showed that light induction of NR activity and mRNA levels could be mimicked by C metabolites and that N-metabolites caused repression of NR induction in tobacco (23). Despite the fact that gene regulation by C:N status has been demonstrated for a number of N-metabolic genes (21), the mechanistic basis for this regulation remains to be revealed.

FUTURE PROSPECTS FOR THE IDENTIFICATION OF N-SIGNALING COMPONENTS

The isolation of mutants defective in components of the N or C:N signaling pathways as well as the use of insertion mutants in transporter genes are very promising tools to identify sensing mechanisms in the future. The success of these approaches will very much depend on the development of adequate screening tests to make a sensing response visible or measurable. The completion of the Arabidopsis genome sequencing project and the development of genomic technologies has aided in the identification of components in N-signaling systems. For example, in 2000 Wang et al. used micro-array analysis to identify the induction by nitrate of putative regulatory genes (e.g. MYB transcription factors, protein kinases, etc.), metabolic enzymes, and novel gene products (25).

C METABOLITES AS SIGNALS

Early evidence of C metabolite signaling was provided in the 1960s by experiments linking the rate of photosynthesis with assimilate partitioning (for review, see 15). These initial observations were followed up with many experiments in the 1970s that examined the relationship between photosynthetic capacity in source tissue with carbohydrate use in sinks (for review, see 8). Gifford and Evans’ analysis of those results led to the conclusion that sink tissue plays a pivotal role in controlling assimilate partitioning. However, despite this conclusion, there were many contradictory observations in the literature and the identity of a potential integrating signal (such as turgor, a metabolite or plant growth hormone) was not known. Moreover, multiple examples of allosteric regulation of enzyme activities by key metabolites added another layer of complexity to understanding assimilate partitioning as a globally integrated system.

The first evidence that C metabolites play a direct role in regulating photosynthetic activity at the tran-
scriptional level was Sheen’s demonstration that sugars down-regulate photosynthetic gene expression in protoplasts isolated from maize mesophyll (19). In those experiments, seven photosynthetic genes were coordinately repressed by Glc and Suc. Subsequent experiments with a variety of sugar analogs suggested hexokinase plays a key role in transducing a hexose-dependent signal in the maize protoplast system and that conclusion was then supported with genetic results using hexokinase antisense plants (10). Although earlier publications provided evidence of links between carbohydrate status and changes in growth or gene expression, Sheen’s results were the first to demonstrate a direct effect of C metabolites on the expression of photosynthetic genes. Evidence that these observations parallel changes in photosynthetic activity in intact leaf tissue was shown in leaf girdling experiments that documented carbohydrate-dependent decreases in photosynthetic gene expression and the rate of photosynthesis (12).

THERE ARE MULTIPLE PATHWAYS OF C-METABOLITE REGULATION

Carbohydrate-mediated changes in gene expression have been demonstrated for a variety of physiological and developmental processes (11,20). In general, these responses fall into two broad categories: (a) those that increase gene expression under carbohydrate rich conditions and (b) those that increase expression in depleted conditions (Dr. Koch’s “feast and famine” responses, 11). Carbohydrate-modulated response pathways are also differentiated by tissue and developmental specificity and the identity of the signaling molecule. For example, evidence for Suc-mediated changes in gene expression, not mimicked by hexoses, has been demonstrated for a Suc symporter in sugar beet leaf tissue whose message and transport activity are down-regulated when Suc accumulates in the leaf (2). Even hexose signaling may be transduced by a hexokinase-independent system linked to a membrane bound hexose transporter (11,20).

METABOLITE REGULATION OF GENE EXPRESSION IS ONE COMPONENT OF A WEB OF INTERLINKED REGULATORY NETWORKS

In addition to the C:N cross-talk discussed above, genetic analysis of C-metabolite signaling has recently shown that it is also linked to ethylene (27) and abscisic acid response pathways (13). Genetic analysis of gin1, a Glc-insensitive mutant in Arabidopsis, showed that this gene product acts downstream of the hexokinase in the Glc signaling pathway. Significantly, gin1 insensitivity to Glc repression of cotyledon and shoot development was phenocopied by ethylene precursor treatment of wild-type plants and etr1-1, an ethylene insensitive mutant, was shown to be Glc hypersensitive. Epistasis analysis placed GIN1 downstream of the ethylene receptor, ETR1. Taken together, these results suggest GlN1 may integrate antagonistic signals from these two pathways in controlling plant development (27). Analysis of additional Glc-insensitive mutants has also identified cross-talk with abscisic acid signaling (13) and, based on paclobutrazol-insensitive seed germination, gibberellin (S. Gibson, personal communication). Given the central role of C metabolism in plant cell biology, additional examples of regulatory cross-talk are likely to be uncovered.

Our understanding of metabolite regulation of gene expression has grown by quantum leaps over the last 10 years. As with many areas of biological inquiry, many of these advances can be attributed to the development of genetics and recombinant DNA technology as potent tools in physiological research. We are on the threshold of the next revolution in biology as genomics leads us into a new century. The emerging tools of genomics and bio-informatics will allow us to identify the interacting pathways that control gene expression in response to changes in N and C status, which should give us new insight into how plants modulate growth and development in response to fluctuating environmental conditions.

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LITERATURE CITED