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MACRONUTRIENT UTILIZATION BY PHOTOSYNTHETIC EUKARYOTES AND THE FABRIC OF INTERACTIONS

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■ **Abstract** Organisms acclimate to a continually fluctuating nutrient environment. Acclimation involves responses specific for the limiting nutrient as well as responses that are more general and occur when an organism experiences different stress conditions. Specific responses enable organisms to efficiently scavenge the limiting nutrient and may involve the induction of high-affinity transport systems and the synthesis of hydrolytic enzymes that facilitate the release of the nutrient from extracellular organic molecules or from internal reserves. General responses include changes in cell division rates and global alterations in metabolic activities. In photosynthetic organisms there must be precise regulation of photosynthetic activity since when severe nutrient limitation prevents continued cell growth, excitation of photosynthetic pigments could result in the formation of reactive oxygen species, which can severely damage structural and functional features of the cell. This review focuses on ways that photosynthetic eukaryotes assimilate the macronutrients nitrogen, sulfur, and phosphorus, and the mechanisms that govern assimilatory activities. Also discussed are molecular responses to macronutrient limitation and the elicitation of those responses through integration of environmental and cellular cues.

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INTRODUCTION

This review focuses on acquisition and assimilation of the macronutrients nitrogen (N), sulfur (S) and phosphorus (P) by plants and algae (primarily *Chlamydomonas reinhardtii*; designated Chlamydomonas throughout) and responses of these organisms to macronutrient deprivation. This version of the text has been abstracted, with a complete version of the text available at http://www.annualreviews.org/pubs/supmat/supmat.asp.

Organisms grown in medium limiting for a particular nutrient exhibit responses specific for the limiting nutrient and more general responses elicited by a variety of different stress conditions. Nutrient-specific responses include the biosynthesis of transport systems for the limiting nutrient and induction of hydrolytic activities that enable cells to access alternative sources of the limiting nutrient. Table 1 lists transporters of Chlamydomonas and vascular plants that function in the transport of ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-) and sulfate (SO_4^{2-}). Phosphate (Pi) transporters were excluded because they have not been extensively enough characterized. General responses to nutrient limitation include changes in the morphology of the organism (316, 333, 334), cessation of cell division (47), accumulation of polysaccharides (11, 276), reduced photosynthetic activities (47, 207, 325), and modification of metabolic processes to satisfy the demands of a reduced nutrient environment (61, 168, 291). Some responses may result from changes in metabolism that occur because depriving cells of nutrients retards growth and limits the utilization of reductant and chemical bond energy. A dramatic slowing of anabolic processes leads to a reduced need for ATP and NADPH generated by photosynthetic electron transport. Hence, even when nutrient-deprived plants and algae are grown in moderate or low light, photosynthetic electron carriers will tend to be fully reduced. The redox potential of the cell will increase as a consequence

of hyperreduction of the plastoquinone pool (and other photosynthetic electron carriers), although some of this redox pressure may be relieved by elevated starch synthesis. This increase in intracellular redox potential will have a global effect on cellular metabolism and gene expression (66, 308). Additionally, with a decreased demand for reductant during nutrient limitation, the cell would tend to accumulate high potential electrons and excited chlorophyll molecules that could interact with oxygen, creating reactive oxygen species. These species could cause extensive cell damage and also function as regulatory signals that alter metabolic activities.

Finally, responses to a changing nutrient environment are integrated with other environmental conditions and the developmental stage of the organism. Most results described in this review are from experimentation in which conditions were engineered to insure that levels of a specific element limited the growth of the organism, a more static condition than is found in nature.

NITROGEN

Nitrogen in the Environment

All organisms require an abundance of N for the synthesis of proteins and nucleic acids. N limits the growth of organisms in a number of different ecosystems and is especially critical in marine ecosystems. N (P or S) limitation can profoundly influence the morphology and physiology of plants. Plants deprived of N develop an elevated root:shoot ratio relative to unstarved plants with shortened lateral branches whereas an excess of NO_3^- inhibits root growth and leads to a decrease in the root:shoot ratio (260, 334). Internal levels of nitrogenous metabolites and/or the N:C ratio may serve as a systemic signal that influences root development (260).

Plants and algae actively transport and utilize both NH_4^+ and NO_3^-, although the concentration of NH_4^+ in the soil is generally much lower than that of NO_3^- (170). However, relative to the utilization of NO_3^- or NO_2^-, the assimilation of NH_4^+ is metabolically inexpensive since it does not have to be reduced prior to incorporation into amino acids and nitrogenous bases.

Ammonium Transport

Generally, administration of NH_4^+ to plants as a sole source of N leads to stunted growth. Diminution of shoot biomass is the consequence of a reduction in both the number and size of leaf cells (316). Co-addition of NH_4^+ and NO_3^- eliminate stunting, suggesting that the growth phenotype resulted from a lack of NO_3^- rather than NH_4^+ toxicity. The growth defect has also been correlated with a dramatic reduction in the level of cytokinins in the xylem and an increase in the level of abscissic acid (210, 316). These results suggest that NO_3^- strongly influences plant hormone levels and may be required for cytokinin biosynthesis or transport of cytokinins from the root, its site of synthesis, to leaves. In contrast, Chlamydomonas readily grows with NH_4^+ as its sole N source.

TABLE 1 Characteristics of ammonium, nitrate, nitrite and sulfate transporters

Species	Gene	Affinity	Localization	Response	Reference
Ammonium Transporters					
Arabidopsis	*AtAmt1;1*	High	Root	-N (up) Gln (down)	91, 233
			Leaf	Const.	
Arabidopsis	*AtAmt1;2*	High	Root	Nitrate (down)	91
Arabidopsis	*AtAmt1;3*	High	Root	Light (up) Sucrose (up)	91, 314
Arabidopsis	*AtAmt2*	N.D.	Root	-N (up)	273
			Leaf	Const.	
Tomato	*LeAmt1;1*	N.D.	Root (rh)	-N (up)	146, 315
			Leaf	Const.	
Tomato	*LeAmt1;2*	N.D.	Root (rh)	Nitrate (up) Ammonia (up)	315
			Leaf	Nitrate (up) Light (up) CO_2 (down)	
Tomato	*LeAmt1;3*	N.D.	Leaf	Nitrate (up) Light (down) CO_2 (down)	315

Group 1 Nitrate Transporters					
Arabidopsis	*AtNrt1;1*	Low/High	Root (ep, co, en)	Nitrate (up) Light (up) Low pH (up) Sucrose (up) -N (down)	77, 151, 300, 322
Brassica	*BnNrt1;2*	Low	Root	Nitrate (up)	337
Tomato	*LeNrt1;2*	N.D.	Root (rh)	Nitrate (up)	146
Arabidopsis	*AtNrt1;2*	Low	Root	Const.	39, 157
Rice	*OsNrt1;1*	Low	Root (ep, rh)	Const.	156
Tomato	*LeNrt1;1*	N.D.	Root	Const.	146
Group 2 Nitrate Transporters					
Arabidopsis	*AtNrt2;1*	High	Root	-N (up) Nitrate (up) Light (up) Sucrose (up) Ammonia (down) Gln (down)	77, 151, 322, 340
Arabidopsis	*AtNrt2;2*	N.D.	Root	N.D.	340
Tobacco	*NpNrt2;1*	N.D.	Root (ep, en)	Nitrate (up) Ammonia(down) Gln (down)	135, 220

(*Continued*)

TABLE 1 (*Continued*)

Species	Gene	Affinity	Localization	Response	Reference
Soybean	*GmNrt2*	N.D.	Root	-N (up) Nitrate (up) Ammonia (down)	1
Barley	*HvNrt2;1*	N.D.	Root	Nitrate (up)	297
Barley	*HvNrt2;2*	N.D.	Root	Nitrate (up)	297
Algal Nitrate and Nitrite Transporters					
Chlamydomonas	*Nrt2;1*	High (nitrate/nitrite)		Nitrate (up) Ammonia (down)	89, 217
Chlamydomonas	*Nrt2;2*	High (Nitrate)		Nitrate(up) Ammonia (down)	89, 217
Chlamydomonas	*Nrt2;3*	High (nitrite) Low (nitrate)		Nitrite (up) CO_2 (up) Ammonia (down)	238
Chlamydomonas	*Nar1*	High (nitrite)	Chloroplast	Nitrate (up) Ammonia (down)	239 (*a*)
Group 1 Sulfate Transporters					
Arabidopsis	*Sultr1;1*	High	Root (ep, co, rh)	-S (up)	284
Arabidopsis	*Sultr1;2*	High	Root (co)	-S (up)	(*b*)
Barley	*Hvst1*	High	Root	-S (up) GSH (down)	271, 310
Stylosanthes	*Shst1*	High	Root	-S (up)	270
Stylosanthes	*Shst2*	High	Root	-S (up)	270
Maize	*Zmst1*	N.D.	Root	-S (up) Cys (down)	21

Group 2 Sulfate Transporters					
Arabidopsis	*Sultr2;1*	Low	Root (pc, xp)	-S (up) GSH (down)	144, 284, 285
			Leaf (ph, xp)	-S (down)	
Arabidopsis	*Sultr2;2*	Low	Root (ph)	const.	284
			Leaf (vbs)	-S (up)	
Stylosanthes	*Shst3*	Low	Root	-S (up)	270
			Leaf	-S (down)	
Group 3 Sulfate Transporters					
Arabidopsis	*Sultr3;1*	N.D.	Leaf	Const.	284
Arabidopsis	*Sultr3;2*	N.D.	Leaf	Const.	284
Arabidopsis	*Sultr3;3*	N.D.	Leaf	Const.	284
Chloroplast and Algal Sulfate Transporters					
Arabidopsis	*Sultr4;1*	N.D.	Chloroplast	-S (up)	283, 284
Chlamydomonas		N.D.	N.D.	-S (up)	(*c*)

(*a*) A putative chloroplast nitrite transporter from cucumber that is not related to Chlamydomonas *Nar1* is in the database (accession no. Z69370). (*b*) Accession no. AB042322 (H Takahashi, unpublished data). (*c*) J Davies (personal communication).

There are several additional sequences encoding ammonium, nitrate, and sulfate transporters in the Arabidopsis genome; *AtAmt1;4* (accession no. AL035353), *AtAmt1;5* (accession no. AP000382); *AtNrt2;3* and *AtNrt2;4* (accession no. AB015472); *Sultr1;3* (accession no. AC069252), *Sultr3;4* (accession no. AB012247), *Sultr4;2* (accession no. AP002047).

up, up-regulation; down, down-regulation; const, constitutive expression; N.D., not determined. co, cortex; en, endodermis; ep, epidermis; pc, pericycle; ph, phloem; rh, root hair; vbs, vascular bundle sheath; xp, xylem parenchyma.

Use of Ammonium by Chlamydomonas Chlamydomonas has at least two NH_4^+ carriers (84); the gene for neither has been cloned. Mutants defective for NH_4^+ transport were selected for resistance to methylammonium and the lesions mapped to two nuclear loci. One locus defines a constitutively expressed, low-affinity NH_4^+ transporter with a high maximum uptake velocity whereas the other defines an NH_4^+-repressible, high-affinity NH_4^+ transporter with a low maximum uptake velocity. The low-affinity system functions under N-replete conditions whereas the high-affinity system is important when the concentration of NH_4^+ in the environment becomes limiting.

Use of Ammonium by Plants Plants also have several NH_4^+ transporters. Six potential isoforms of NH_4^+ transporters have been identified in Arabidopsis (91, 192, 314, 315). The first gene encoding a plant high-affinity NH_4^+ transporter ($K_m = 0.5\ \mu M$) was designated *AtAmt1;1* (192). *AtAmt1;1* mRNA accumulates in Arabidopsis leaves, roots, and seedlings (91), increases in abundance during N starvation, and declines as the glutamine concentration in roots rises (91, 192, 233). These characteristics suggest that AtAmt1;1 facilitates efficient NH_4^+ transport when Arabidopsis experiences low NH_4^+ conditions. The uptake of NH_4^+ by roots correlates with an increase in the level of *AtAmt1;1* mRNA and a decrease in the concentration of glutamine (233). Furthermore, when the conversion of NH_4^+ to glutamine is blocked by administration of methionine sulfoximine, high levels of NH_4^+ are unable to suppress accumulation of *AtAmt1;1* mRNA. Therefore, a metabolite derived from NH_4^+, and not NH_4^+ itself, serves to control *AtAmt1;1* mRNA levels. NH_4^+ may also have a direct effect on uptake activity. There are strong interactions between NH_4^+ assimilation and the generation of carbon (C) skeletons, which are critical for NH_4^+ assimilation.

In contrast to AtAmt1;1, the AtAmt1;2 and AtAmt1;3 transporters are root specific and have low affinities for NH_4^+ ($K_m = 30$–$35\ \mu M$) (91). Two other putative NH_4^+ transporter genes present on the Arabidopsis genome are *AtAmt1;4* and *AtAmt1;5*. The tissue specificity and N dependence of expression of these genes have not been characterized. A sixth putative NH_4^+ transporter is encoded by *AtAmt2;1*. This gene is expressed in both roots and leaves and is unique in that it encodes a protein that is only distantly related to the other plant NH_4^+ transporters (273).

Genes encoding NH_4^+ transporters have also been isolated and characterized in tomato. Two of these genes, *LeAmt1;1* and *LeAmt1;2*, are preferentially expressed in root hairs (146, 315). The mRNA level of *LeAmt1;1* dramatically increases when tomato plants are starved for N, similar to the expression pattern of *AtAmt1;1*, and like AtAmt1;1, the LeAmt1;1 transporter may be critical for the uptake of NH_4^+ by roots when the soil is depleted for NH_4^+. In contrast, the level of *LeAmt1;2* mRNA is highest when plants are experiencing N-replete growth (315).

The rate of influx of NH_4^+ into Arabidopsis roots shows a diurnal rhythm with a maximum that occurs toward the end of the light period (91). There is a sharp decline in influx soon after the dark period begins. The *AtAmt1;1*, *AtAmt1;2*, and

AtAmt1;3 genes all show diurnal patterns of expression, although peak levels of *AtAmt1;3* mRNA best correspond to the peak of NH_4^+ uptake (91). The influx of NH_4^+ toward the end of the light period may be influenced by increased availability of sugars (91, 314), suggesting a link between NH_4^+ uptake, expression of *AtAmt1;3*, and C metabolism.

Nitrate and Nitrite Transport NO_3^- is the major source of inorganic N in the soil and serves as a substrate for N assimilation. The concentration of NO_3^- in the cytoplasm of a plant cell is maintained at a relatively constant level (179), although considerable quantities can be stored in vacuoles and only mobilized when N becomes limiting (304). NO_3^- can also be transported out of roots into the rhizosphere by a passive efflux system that is saturable and both NO_3^- selective (99) and inducible (7). Nearly all studies on NO_3^- transport concern the movement of NO_3^- from the soil solution into cells or the plant body.

Nitrate and Nitrite Transport by Chlamydomonas Chlamydomonas synthesizes several transport systems that are specifically involved in acquisition of NO_3^- and NO_2^-. At least six genes encoding polypeptides that function in NO_3^-/NO_2^- assimilation are clustered on the Chlamydomonas genome (218, 219). This locus includes two regions for systems (System I and II) that transport NO_3^- and NO_2^- into cells. There are at least two additional genes encoding NO_3^-/NO_2^- transport systems (Systems III and IV) at different chromosomal locations. The accumulation of transcripts from these loci is repressed by NH_4^+, whereas the transcripts are most abundant when cells are exposed to NO_3^-. System I is encoded by the *Nrt2;1/Nar2* genes. This system catalyzes high-affinity transport of both NO_3^- (High Affinity Nitrate Transport or HANT) and NO_2^- (High Affinity Nitrite Transport or HANiT). System II is encoded by *Nrt2;2/Nar2* and exhibits monospecific HANT activity. Although the *Nar2* gene product is required for the activity of both of these systems, its function is not known. It may be a structural element that associates with the Nrt2;1 and Nrt2;2 polypeptides (89, 217), a hypothesis supported by the finding that electrogenic NO_3^- transport into *Xenopus* oocytes occurs only when the oocytes harbor both the Nrt2;1 and Nar2 polypeptides (336). System III, encoded by *Nrt2;3*, exhibits both HANiT and low-affinity NO_3^- transport or [LANT] activities (238). The *Nrt2;3* gene is clustered with *Nar5*, a gene of unknown function that, like the transporter genes, is regulated by the N status of the environment. System IV exhibits both HANT and HANiT activities and is probably encoded by *Nrt2;4* (238). *Nar1*, a separate gene, encodes an integral membrane protein involved in the transport of NO_2^- into chloroplasts (239), the site of NO_2^- reduction.

Nitrate and Nitrite Transport by Plants Energy-dependent transporters in vascular plants move NO_3^- across the plasma membrane of root epidermal and cortical cells. Subsequently, NO_3^- is reduced, stored in the vacuole, or transported symplastically across the Casparian strip through the endodermal cell layer to reach

the stele (39). Inside the vascular tissue, NO_3^- is taken up by xylem parenchyma cells and loaded into xylem vessels, where it is routed to the leaves. Loading of NO_3^- into the xylem requires an efflux system that transports the anion out of xylem parenchyma cells. Following transport, NO_3^- is unloaded from the xylem and loaded into leaf mesophyll cells. Most reduction of NO_3^- to NO_2^- is thought to occur in the cytoplasm of leaf cells, while further reduction and assimilation occurs primarily in leaf cell chloroplasts. However, a considerable amount of NO_3^- assimilation can occur in plant roots, especially the roots of woody plants. Following reduction of NO_3^- to NH_4^+, GS catalyzes its incorporation into glutamine and the ferredoxin-dependent glutamate synthetase or glutamine:2-oxoglutarate aminotransferase (GOGAT) catalyzes the transamination of an amino group from glutamine to 2-oxoglutarate to form two molecules of glutamate. Glutamate is used for the synthesis of aspartate and alanine, and glutamate and aspartate then serve as amino donors for the biosynthesis of other amino acids and various N-containing compounds.

NO_3^- uptake by plant roots is driven by a proton motive force (39) and probably mediated by $2H^+/1NO_3^-$ symport (175). Low- and high-affinity NO_3^- uptake systems are associated with plant roots. Low-affinity NO_3^- transport systems (LATS; $K_m > 0.5$ mM) are constitutively expressed and serve as the major transport activity when the NO_3^- concentration in the environment is in the millimolar range. Low-affinity NO_3^- transporters have been placed in the NRT1 class (82). High-affinity NO_3^- transporters (HATS; 200 μM $> K_m >$ 5 μM) operate at micromolar concentrations of NO_3^-. The high-affinity NO_3^- transporters are in the NRT2 class. The HATS and LATS polypeptides are predicted to be integral to membranes with 12 transmembrane helices separated into two groups of six helices by a hydrophilic region; they are members of the major facilitator superfamily of transport proteins (205). HATS have been divided into two categories, cHATS and iHATS, that respond differently to environmental NO_3^- concentrations. cHATS are constitutively expressed at a high level, although they can be upregulated in the presence of NO_3^- (5, 138). High-level expression of the iHATs requires NO_3^- (6, 137).

Several genes on plant genomes encode NO_3^- transporters (82); barley has 7-10 putative NO_3^- transporters (297). NO_3^- transporters have been identified by analyses of NO_3^- transport mutants (300, 320), functional complementation of yeast transport mutants (299), homologies of deduced protein sequences to known NO_3^- transporters (146), patterns of expression of putative transport genes with respect to N conditions, and activity of specific transporters in *Xenopus* oocytes (156, 180, 336). Mutants defective for NO_3^- transport and reduction have been generated by selecting for strains resistant to ClO_3^- (reviewed in 38); this selection identified the *chl1* mutant of Arabidopsis (300). A T-DNA tagged allele of *chl1* facilitated the cloning of the gene, designated *AtNrt1;1*. *AtNrt1;1* is NO_3^--inducible and expressed in the epidermis, cortex, and endodermis of Arabidopsis roots (115, 300). The *AtNrt1;1* product is a LATS (115, 296) that, under certain conditions, exhibits HATS activity (157, 322), suggesting that AtNrt1;1 is a dual function transporter

operating at both low and high substrate levels. NO_3^- uptake experiments using the *chl1* mutant have suggested the existence of an additional constitutive LATS activity that is likely encoded by *AtNrt1;2* (157). Expression studies in *Xenopus* oocytes have confirmed that *AtNrt1;2* encodes a LATS.

Genes that are putative *AtNrt1* homologs have been cloned from a number of organisms. *OsNrt1;1*, the *AtNrt1;2* homolog from rice, encodes a LATS component that is constitutively expressed in root epidermal cells and root hairs (156). *BnNrt1;2* is an *AtNrt1;1* homolog from *Brassica napus* that displays NO_3^--induced transcript accumulation and LATS activity upon expression in *Xenopus* oocytes (337). In tomato, the *LeNrt1;2* gene may encode a polypeptide that functions similarly to *AtNrt1;1* since it is preferentially expressed in root hairs and up-regulated by NO_3^- (146). In contrast, *LeNrt1;1* encodes a transporter that is constitutively expressed and not exclusively localized to root hairs (146).

The members of the plant *Nrt2* gene family encode high-affinity NO_3^- transporters of the cHAT or iHAT type (82). Expression of genes encoding iHATs is often susceptible to negative feedback regulation through products of N assimilation (128). Key metabolites involved in this control are the amino acids glutamine and asparagine (187, 203). At least four *Nrt2* genes have been identified on the Arabidopsis genome. *AtNrt2;1* and *AtNrt2;2* are contiguous in a tail-to-tail configuration at the top of chromosome 1 (340) whereas *AtNrt2;3* and *AtNrt2;4* are 4 kbp apart in a head-to-tail configuration at the top of chromosome V (GenBank Accession number AB015472) (82). The *Chl8* gene of Arabidopsis probably encodes a cHAT (320), although there is no sequence information that clearly establishes it as a member of the NRT2 family. Antisense suppression of *AtNrt2;1* expression has provided direct evidence that this gene encodes a high-affinity NO_3^- transporter (82). Based on spatial distribution of transcripts of *NpNrt2;1*, a homolog of *AtNrt2;1* in *Nicotiana plumbaginifolia*, the gene is expressed in root epidermal and endodermal cells (135). The physiological functions of the different *Nrt2* genes of Arabidopsis are unknown.

Regulation of Transport NO_3^- assimilation is controlled by both N and C metabolites. Addition of NH_4^+ to plants suppresses the assimilation of NO_3^- (82, 275, 276). Tobacco transformants with elevated expression of nitrate reductase (NR), which can lead to an increase in the glutamine concentration in the cell, have depressed rates of NO_3^- uptake relative to wild-type cells (93). This is strong evidence that NO_3^- uptake is controlled by downstream products of NO_3^- assimilation. Furthermore, either the addition of exogenous sugars or conditions that elevate CO_2 fixation strongly affect NO_3^- uptake (see below).

NO_3^- stimulates the accumulation of *AtNrt1;1* and *AtNrt2;1* mRNA (77, 151). An Arabidopsis mutant lacking NR accumulates high levels of both NO_3^- and *AtNrt1;1* and *AtNrt2;1* transcripts. This is probably a consequence of an inability of the mutant to synthesize reduced N metabolites that feedback to suppress expression of the NO_3^- transport genes. Accumulation of NH_4^+ and glutamine causes repression of the *AtNrt2;1* in Arabidopsis (340) and its homologs in *N.*

plumbaginifolia (220) and soybean (1). An increase in root *AtNrt2;1* mRNA abundance correlates with the rate of high-affinity NO_3^- uptake in plants exposed to low N conditions. Induction of *AtNrt2;1* mRNA by N limitation may be a consequence of release from feedback control by reduced N metabolites. Expression of both *AtNrt1;1* and *AtNrt2;1* genes are diurnally regulated with expression peaking just prior to the dark period and declining as plants enter the dark period (151). Exogenous sucrose prevents the decline in mRNA during the dark period (151), suggesting that C metabolite levels are critical for controlling NO_3^- uptake and that the circadian pattern of expression of the NO_3^- transport genes may be a consequence of changes in the cellular concentrations of C and N metabolites.

Transport of Other Nitrogen Sources

In addition to assimilating NH_4^+, NO_3^-, and NO_2^-, Chlamydomonas can utilize N from urea, certain nitrogenous bases, and amino acids. Purines can support the growth of Chlamydomonas in the absences of other N sources (212). Chlamydomonas also has transporters specific for urea and arginine (129). Although Chlamydomonas cannot rapidly take up other amino acids, it synthesizes an extracellular L-amino acid oxidase in response to N limitation (211, 301) that catalyzes the deamination of amino acids and the release of NH_4^+.

Plant roots possess active uptake systems for amino acids. These systems may supply plants with N in soils that contain high concentrations of organic matter. Genes encoding these amino acid permeases (designated *Aap*) in Arabidopsis have been cloned (78). Most of these transporters have broad substrate specificities (78, 81) and the tissue specificity of some may reflect a function in moving amino acids between plant tissue types (78, 81). Aap3 is exclusively in Arabidopsis roots (81), and it probably facilitates the uptake and retrieval of amino acids from the soil. The precise roles of the amino acid permeases in plant nutrition and development are still relatively unexplored. Other transporters that move small oligopeptides across membranes are also present in plants (237), but their role in N metabolism is not clear.

Nitrate and Nitrite Reduction

The conversion of NO_3^- to NO_2^- is performed by NR, and of NO_2^- to NH_4^+ by nitrite reductase (NiR). NR is cytosolic, mainly located in root epidermal and cortical cells (70) and leaf mesophyll cells (307) and highly regulated (69, 116, 216). NiR is chloroplast localized and encoded in the nuclear genome (80).

Most reducing equivalents used for the reduction of NO_3^- to NH_4^+ are derived directly from photosynthetic electron transport. Reduced ferredoxin (Fd) is the electron donor that fuels the catalytic activity of NiR. In nonphotosynthetic tissue, NADPH derived from the oxidative pentose phosphate pathway (121, 235) can be used to generate NH_4^+ from NO_3^-. Reducing equivalents are transferred from NADPH to Fd via Fd-NADPH oxidoreductase (FNR). NO_3^- induces expression of Fd (174), FNR (2, 240), and the 6-phosphogluconate dehydrogenase genes; the latter is an integral component of the oxidative pentose phosphate pathway (235).

Chlamydomonas Nitrate and Nitrite Reductase The NO_3^-/NO_2^- assimilation gene cluster of Chlamydomonas contains the structural genes encoding NR and NiR. The *Nia1* gene encodes NR (74). Mutants have been isolated that are defective in the NR structural gene (74) or in genes required for producing a functional molybdopterin cofactor (reviewed in 72). NiR of Chlamydomonas, encoded by *Nii1*, has both a siroheme and $[Fe_4S_4]$ cluster that function as redox centers (25).

NH_4^+ and NO_3^- are key regulatory molecules that control the transcriptional activity of genes encoding the NO_3^-/NO_2^- transporters, NR and NiR. Several posttranscriptional processes also govern NO_3^-/NO_2^- assimilation. NH_4^+ addition to NO_3^--grown cells triggers a rapid decrease in NR activity, which reflects both enzyme inactivation (83) and inhibition of de novo synthesis (71, 101). Inactivated NR is more rapidly degraded than the active enzyme (83). Analyses of a Chlamydomonas strain expressing *Nia1* from the *cabII-1* gene promoter also suggest posttranscriptional control (188). Furthermore, constitutive expression of NR results in altered regulation of the NO_3^-/NO_2^- transporters, supporting the idea that N metabolites downstream of NR regulate the acquisition of NO_3^- and NO_2^- (188).

Light is another environmental cue involved in controlling NO_3^- assimilation and the uptake of a number of anions (3, 223). Blue light activates NR (3, 126), and in the green alga *Monorhapidium braunii* it is required for the biosynthesis of NiR (221). Recently it was shown that blue light is needed for the biosynthesis of the NO_2^- transport system in Chlamydomonas and that protein synthesis inhibitors block the stimulatory effect of blue light (222).

Several mutants of Chlamydomonas devoid of NR activity have been isolated. These mutants fall into three categories; (*a*) those defective in *Nia1*; (*b*) those that cannot synthesize a functional molybdopterin cofactor; and (*c*) those that are aberrant for *Nit2*. *Nit2* encodes a polypeptide that acts as a positive regulator for the NO_3^- assimilation genes (73). A *Nit2* gene of Chlamydomonas was identified in a mutant strain in which the gene was tagged by the transposon *Gulliver* (262). The *Nit2* transcript is high in N-free medium and downregulated by NH_4^+. Other loci involved in the control of NO_3^-/NO_2^- assimilation are *NRG1, NRG2* (214), and *FAR1* (335). Mutants in the *Nrg* and *Far1* genes are defective in NH_4^+ repression of NO_3^- transport.

Nitrate and Nitrite Reductase in Plants Arabidopsis has two genes that encode NR, *Nia1* and *Nia2* (323). These genes are responsive to light, levels of N metabolites, and hormones. Light can act to regulate expression of the *Nia* genes as a consequence of direct absorption by a photoreceptor or by altering the rate of photosynthesis and levels of C metabolites in the cell. However, there are significant differences in the way in which *Nia1* and *Nia2* are controlled with respect to the kinetics of induction by light and in their expression levels in the absence of NO_3^- and following administration of cytokinin (27, 155). *Nia1* and *Nia2* are both induced by light, but the induction of the latter is more rapid than that of the former. While both genes are positively regulated by NO_3^-, especially in the presence of

sucrose, the induction of the *Nia1* gene is stronger (155). Administration of cytokinins to Arabidopsis seedlings triggers an increase in the transcription of *Nia1* but not of *Nia2* (332).

Nia expression is controlled by the cellular concentrations of N metabolites. *Nia* transcripts are low when plants are exposed to high NH_4^+ or glutamine and are induced by NO_3^- (29, 30, 258). In plants maintained on a day-night cycle, *Nia* transcripts are high toward the end of the night, remain high as plants are exposed to light, and decline after 1 or 2 h in the light (NR protein levels remain high for approximately 4 h into the light period). The decline in mRNA continues throughout the entire light period but increases gradually during the subsequent dark period (87, 92, 259). The decline in NR protein levels during the second half of the light period exhibits a slight lag with respect to the decline in transcript levels, which suggests that the levels of NR protein are controlled, at least in part, by mRNA abundance. Furthermore, the protein remaining at the end of the light period is rapidly inactivated when the lights are extinguished; most of the decline in NR activity is not reversed until initiation of the next light period. Daily changes in N metabolite levels in plants maintained on a light-dark cycle correlate with the observed pattern of *Nia* expression. The decline in *Nia* mRNA during the light period parallels a decline in NO_3^- and an accumulation of glutamine in leaves (259). Conversely, the accumulation of *Nia* mRNA during the dark period coincides with an increase in NO_3^- and a decrease in glutamine. The level of *Nii* mRNA is coordinated in a similar manner (112).

As suggested above, posttranscriptional processes (in addition to transcriptional control) modulate NR activity (116, 117, 259). When leaves are placed in the dark, NR activity declines. Dark inactivation of NR is reversed in plants that are either starved for NO_3^- or that express NR at a reduced level (258). The inability to inactivate the enzyme in the dark in NO_3^--starved plants or in plants genetically modified for reduced NR or NiR synthesis (259, 306) correlates with decreased glutamine and NH_4^+ levels, suggesting a marked influence of N metabolites on posttranscriptional regulation of NR. In support of this view, NR activation is diminished in detached leaves that are fed glutamine (184, 259). Additionally, posttranslational control of NR activity is observed in plants that are constitutively expressing *Nia* from the CaMV 35S promoter (75, 311) and is dependent upon the amino terminus of the NR protein (196). Dark inactivation is a consequence of phosphorylation of serine-543 (9) catalyzed by a calcium-dependent or Snf1-related protein kinase (55, 56, 277). Phospho-NR activity is inhibited by binding 14-3-3 polypeptide (8, 183).

Sugar levels can strongly influence N assimilation. When Arabidopsis is maintained in the dark there is little expression of *Nia* (29) and other genes involved in N assimilation (275, 276), but administration of sucrose to dark-grown plants can mimic light induction (29). The mechanism by which sucrose causes activation of N assimilation genes is not understood, although it has been suggested that hexokinase function is required for sensing and responding to sugars (119). Furthermore, C skeletons, and more specifically organic acids, are required for the

assimilation of NH_4^+, and certain organic acids such as 2-oxoglutarate stimulate expression of genes involved in N acquisition and utilization. Conversely, high levels of available N favor elevated expression of genes encoding enzymes required for the synthesis of organic acids, including the genes for PEP carboxylase, pyruvate kinase, citrate synthase, and NADP-isocitrate dehydrogenase (258).

Light activation of N assimilation genes is only partly a consequence of increased sugar levels, and sugars do not mimic light with respect to the elevation of *Nii* mRNA in *N. plumbaginifolia* (312). Both *Nia* and *Nii* mRNA levels in etiolated seedlings increase in response to red (14, 177, 226) and blue (177) light, and phytochrome has been implicated in light control (14, 226). Furthermore, constitutive photomorphogenesis mutants that are defective in red light–mediated de-etiolation exhibited derepression of *Nia2* (but not *Nia1* and *Nii*) in the dark, along with many other light-regulated genes, including *RbcS* and *Lhc* (52). These results suggest that *Nia2*, *Lhc*, and *RbcS* expression is influenced by a similar light-dependent regulatory pathway and that a different pathway modulates *Nia1* and *Nii* activities.

The accumulation of active NR and NiR in plants is coordinated. Like *Nia* genes, *Nii* genes are regulated by the levels of N metabolite, sugars, and light (10, 190, 312). Light-induced accumulation of NiR activity in mustard seedlings required NO_3^- (266), and NR mutants have elevated levels of *Nii* mRNA and NiR activity. Furthermore, the expression of *Nii* genes exhibits a diurnal rhythm (312) that might reflect fluctuations in the levels of the C metabolites. Four *Nii* genes have been identified in tobacco. The *Nii1* and *Nii2* genes exhibit leaf- and root-specific expression, respectively (136). As with NR, posttranscriptional regulation is important for controlling levels of active NiR (41).

Glutamine Synthetase

The main route of NH_4^+ assimilation into organic compounds is through the GS/GOGAT system (141). GS catalyzes amination of glutamate to form glutamine and GOGAT facilitates reductive amination of 2-oxoglutarate using the amide of glutamine as the N donor. There are distinct GS isoenzymes in vascular plants. GS1 resides in the cytoplasm of leaf cells and cells of nonphotosynthetic tissue. GS2 is encoded by a single gene in most plants and is located in plastids (102, 167). The cytoplasmic and chloroplast isoforms of GS of Chlamydomonas are similar to the GS enzymes of vascular plants (28). The genes encoding different GS isoforms are also developmentally and temporally regulated (64, 154, 256).

Several plants harbor a number of different genes encoding GS1 (209, 317); many of these genes exhibit tissue-specific expression and are developmentally regulated. A specific *GS1* gene is expressed in nodules of leguminous plants where high levels of NH_4^+ may be generated and converted into organic molecules (110, 241). GS1 is also important during the senescence of leaves in which protein N must be remobilized and exported to plant sinks (208). Maize root GS1 is encoded by five distinct genes with different expression patterns within the root tissue (154). GS_{1-3} and GS_{1-4} are constitutive and expressed throughout

the root. GS_{1-2} is predominantly expressed in vascular tissue, while the transcripts from GS_{1-1} and GS_{1-5} are present in cortical tissue. GS1 of the root cortex is likely to be involved in the assimilation of external NH_4^+ (279). Furthermore, specific upregulation of GS_{1-1} and GS_{1-5} occurs upon administration of NH_4^+ whereas high NH_4^+ levels depress the activities of GS_{1-3} and GS_{1-4} (253). Interestingly, transgenic plants impaired for expression of *GS1* in phloem companion cells exhibited a marked reduction in proline levels under conditions in which the plants were forced to assimilate high levels of NH_4^+. The plants were also more sensitive to water stress conditions (23). These results suggest that the phloem-associated GS1 isoform may control proline synthesis for both N storage and as an osmoticum to protect plants from conditions that lead to desiccation.

The plastid-localized GS2 isoform is predominantly expressed in leaf mesophyll cells and functions in primary assimilation of N (64) and recapture of NH_4^+ generated by photorespiration (64, 176, 274). *GS2* mutants of barley cannot reassimilate NH_4^+ released from the photorespiratory pathway even though they synthesized cytosolic GS1 isoforms (319). Transgenic tobacco seedlings overexpressing *GS2* also exhibited reduced levels of free leaf NH_4^+ and an increase in the pool of some amino acids (including glutamine and glutamate), although protein levels did not increase. Finally, the transgenic seedlings exhibited an increase in biomass, suggesting that the levels of GS2 protein may be limiting to biomass production (178). Expression of *GS2* is regulated by several factors including light, sucrose concentrations, and the levels of NO_3^- and amino acids (40, 64, 199). Although addition of NO_3^- can stimulate *GS2* expression, there is also a dramatic induction of *GS2* mRNA by light; this induction is mediated in part by light-induced changes in C metabolite levels and in part by phytochrome (199). The *GS1* genes are also upregulated by light, but the increase in abundance is primarily a consequence of changes in C metabolite levels. In addition to influencing levels of GS transcripts, sucrose may modulate GS enzyme activity (176, 199). Amino acids such as glutamine, glutamate, asparagine, and aspartate antagonize the sucrose-stimulated increases in GS mRNA and enzyme activity. In general, it appears that metabolic regulation of GS expression in plants reflects the relative abundance of C skeletons and amino acids. These regulatory features are similar to those associated with both the uptake and reduction of NO_3^-. Furthermore, posttranscriptional processes control tobacco GS2 (178).

Glutamine:2-Oxoglutarate Aminotransferase

Chlamydomonas and vascular plants have two classes of the iron sulfur protein glutamate synthase or GOGAT. The reductant used by one form of GOGAT is NADH whereas the other uses reduced Fd (88, 169). The Fd-GOGAT is plastid-localized (147, 267) and often accounts for over 90% of GOGAT activity in leaves and over 60% in roots (274, 281).

Expression of the Fd-GOGAT genes is controlled by NO_3^- (252) and light (15, 251, 287). In Arabidopsis there are two distinct Fd-GOGAT genes, *Glu1* and

Glu2. *Glu1* mRNA is prevalent in leaves and increases in the light or upon administration of sucrose whereas *Glu2* mRNA is most abundant in roots and exhibits constitutive expression (36, 141). The *gls1* mutant of Arabidopsis contains less than 5% of the Fd-GOGAT activity. This mutant exhibits a conditional lethal phenotype, dying under conditions that enhance photorespiration (274). The *Glu1* gene and the *gls1* mutant map to the same region of chromosome 5 (*Glu2* maps to a different location) (36). Furthermore, although the *gls1* mutant is rescued from lethality under conditions of high CO_2 (e.g. 1% CO_2), it is still somewhat chlorotic relative to wild-type Arabidopsis plants (36). These results suggest that the leaf-specific *Glu1* Fd-GOGAT is important for the primary assimilation of NH_4^+ and recapture of NH_4^+ generated by photorespiration (36, 255). The root-specific Fd-GOGAT encoded by *Glu2* is likely to function in primary N assimilation and re-assimilation of catabolically generated NH_4^+.

There are also a number of distinct NADH-GOGAT isoforms. In rice, the root isoforms have been localized to the apical meristem, the central cylinder, and the primordia of secondary roots, and their levels strongly increase in epidermal and exodermal cells following administration of NH_4^+ (118). Furthermore, NADH-GOGAT and GS1 are coordinately regulated (107, 118). These enzymes, mostly in roots, function in primary assimilation of NH_4^+ generated from soil NO_3^- and NH_4^+ (197) and re-assimilation of NH_4^+ released during seed germination and amino acid catabolism (288). The NADH-GOGAT is also important for the assimilation of NH_4^+ produced as a consequence of N_2 fixation in root nodules (263, 303).

Additional Regulatory Considerations

Little is known about regulatory proteins that control N metabolism in plants. GS activity in bacteria is regulated at posttranscriptional/posttranslational levels by the PII protein (120). A nuclear-encoded, chloroplast-localized PII homolog, designated GLB1, was isolated from Arabidopsis (114). GLB1 lacks the conserved tyrosine-51 that is uridylylated in bacterial PII (this modification is important for controlling PII activity), but GLB1 mRNA increases in plants exposed to sucrose and decreases in plants exposed to glutamine, asparagine, and aspartate. These characteristics of GLB1 expression suggest that it plays a role in regulating N metabolism in plants.

The transcriptional regulator Nit2 controls expression of N assimilation genes in Chlamydomonas (262) and fungi (171). This protein binds to GATA elements in the promoters of N-regulated genes (327). The promoter of the spinach *Nii* gene contains GATA elements that could bind the zinc finger domain of the *Neurospora crassa* NIT2 protein (227). A cDNA encoding a GATA-1 zinc finger protein with homology to NIT2 has been identified in tobacco (43), but its role in the control of N metabolism is not known.

NO_3^- control of gene activity is not understood at a mechanistic level. Two distinct regulatory pathways appear to control the expression of the N and C assimilation genes. Induction of genes for N assimilation enzymes by NO_3^- may involve

calcium and protein phosphorylation. In contrast, induction of the *PEP* carboxylase gene (and other genes encoding enzymes of the C4 dicarboxylic acid cycle) by NO_3^- requires synthesis and accumulation of cytokinin in roots (278, 280). One regulatory element induced in maize by both NO_3^- and cytokinin is ZmRR1; this protein is similar to response regulators of two component regulatory systems (254). The Arabidopsis homologs, *ARR3 – ARR7*, show elevated transcript levels in response to NO_3^- and cytokinin treatments (286). More detailed biochemical investigations should reveal the roles of these regulatory elements in N metabolism and the relationships of cytokinin to this process.

SULFUR

Sulfur in the Environment

S is an essential element present in proteins, lipids, intermediary metabolites, molecules involved in photoprotection, electron carriers, and redox controllers. Sulfur can be limiting in the environment and may limit plant productivity in certain agricultural settings.

There is no specific S storage compound within cells, so plant growth is dependent on external S sources. The majority of S taken up from the soil solution by microorganisms and plants is the SO_4^{2-} anion. Assimilation of SO_4^{2-} involves reduction to sulfide and the subsequent synthesis of cysteine and methionine. SO_4^{2-} in soils is often covalently bonded to organic molecules. The two main classes of organic S compounds that occur in soils are SO_4^{2-} esters and sulfonates. Furthermore, plants and microorganisms release a variety of organic compounds containing S (e.g. proteins, lipids, polysaccharides) when they die.

Sulfate Utilization by Chlamydomonas

Arylsulfatase Many soil organisms access the S bonded to organic molecules through the synthesis and secretion of sulfatases and sulfonatases. Chlamydomonas synthesizes a prominent, extracellular arylsulfatase (Ars) in response to S limitation (264). This arylsulfatase has been purified to homogeneity and characterized, and the *Ars* gene has been cloned (50, 51). Expression of the *Ars* gene is positively controlled by the Sac1 protein during S starvation and repressed by the Sac3 protein during S-replete growth (47–49). The Ars protein is tightly associated with the Chlamydomonas cell wall and is synthesized as a preprotein with a signal sequence that is cleaved during export from the cell (51). The extracellular location of Ars allows it to hydrolyze soluble SO_4^{2-} esters in the medium, thereby releasing free SO_4^{2-} for assimilation by the cell. No *Ars* gene has been identified to date in vascular plants.

Sulfate Transport and Assimilation SO_4^{2-} is taken up by plants and microorganisms by specific transport systems and is either used for direct sulfation of

compounds or is assimilated following reduction and incorporation into cysteine (153, 247, 272). Eukaryotic organisms have multiple transport systems for SO_4^{2-} uptake. Generally, systems capable of high-affinity SO_4^{2-} uptake increase when S levels in the environment drop and the cells are deprived of S (32, 106, 145, 328).

When Chlamydomonas is starved for S there is a dramatic increase in the V_{max} and decrease in the K_m for SO_4^{2-} transport (328). Recently a gene for an inducible SO_4^{2-} transporter, probably with high affinity for its substrate, has been isolated from Chlamydomonas. This gene is activated during S-limited growth and controlled by Sac1, although it is not clear if it is located on the plasma membrane or chloroplast envelope (J Davies, personal communication).

Sulfate Utilization by Plants

Sulfate Transport Genes encoding high-affinity SO_4^{2-} transporters have been isolated from *Stylosanthes hamata* (270), *Arabidopsis thaliana* (284, 285), and barley (271, 310). Levels of transcripts for these transporters increase in plant roots following S starvation. Arabidopsis has multiple genes encoding SO_4^{2-} transporters; these genes have been designated *Sultr*, with the Sultr1 group representing high-affinity transporters and the Sultr2 group representing transporters with a lower affinity for SO_4^{2-}. Expression of *Sultr1;1* was noted in root hairs as well as in epidermal and cortical cells of roots (284) whereas *Sultr1;2* expression was primarily in the root cortex (Accession number AB042322; H Takahashi, unpublished data). These data suggest that high-affinity transporters are synthesized in the outer cell layers of roots and help maximize SO_4^{2-} uptake under starvation conditions.

Once taken up by roots, SO_4^{2-} is translocated symplastically through endodermal cells to the stele. Inside the stele, SO_4^{2-} is transferred to xylem parenchyma cells, either by moving symplastically or by re-absorption of SO_4^{2-} that leaks into the intracellular space. The SO_4^{2-} is exported from xylem parenchyma cells and loaded into xylem vessels for distribution to various plant tissues. Two low-affinity Arabidopsis transporters probably play roles in the internal translocation of SO_4^{2-} from roots to leaves. The Sultr2;1 low-affinity transporter ($K_m \sim 0.4$ mM) is located in the pericycle and xylem parenchyma cells of the root stele, and in phloem and xylem parenchyma cells of leaf veins (284, 285). Under conditions of S limitation, expression of *Sultr2;1* increases in roots and declines in leaves. This transporter may function to re-absorb SO_4^{2-} that leaks from vascular tissue into the intercellular space. Upregulation of *Sultr2;1* in roots during S starvation would partly contribute to maintaining efficient transfer of SO_4^{2-} to leaves. The Sultr2;2 transporter is expressed in the root phloem and leaf bundle sheath cells; it has an even lower affinity for SO_4^{2-} ($K_m > 1.2$ mM) (284) than Sultr2;1. This transporter is probably responsible for distributing SO_4^{2-} from the vascular bundle to palisade and mesophyll cells. *Sultr2;2* mRNA levels in leaves increase when plants experience low SO_4^{2-} condition, whereas leaf *Sultr2;1* mRNA declines under the same conditions. The coordinated expression

of these two low-affinity transporters may optimize the distribution of SO_4^{2-} in leaf tissue when the plants are limited for S.

Once inside the cells of leaves, SO_4^{2-} is either transported into chloroplasts for assimilation or stored in vacuoles. Sultr4;1 is a unique SO_4^{2-} transporter isoform that is localized to Arabidopsis chloroplasts (283). The mRNA for *Sultr4;1* accumulates to high levels in leaves of S-starved plants (285). This transporter is likely involved in the uptake of SO_4^{2-} by chloroplasts, the major site of reductive assimilation. Vacuolar influx and efflux of SO_4^{2-} requires specific transporters on the tonoplast membrane; the genes and proteins involved in vacuolar transport have not been identified.

Sulfate Reduction Once SO_4^{2-} enters cells it is activated by ATP sulfurylase to form adenosine 5′-phosphosulfate (APS). ATP sulfurylase isoforms in plants are located either in plastids or in the cytosol (166, 265). The cDNAs for these isoforms were first isolated from potato (130). In Arabidopsis there appear to be at least three plastidic and one putative cytosolic ATP sulfurylase (105, 152, 162). The APS generated by ATP sulfurylase can serve as a substrate for SO_4^{2-} reduction or can be phosphorylated by APS kinase to yield 3′-phosphoadenosine 5′-phosphosulfate (PAPS) (4, 149). PAPS is the substrate of various sulfotransferases to catalyze the sulfation of a range of metabolites including flavanols, choline, and glucosides (305).

The SO_4^{2-} of APS is reduced to sulfite by the plastid-localized APS sulfotransferase (243), also called APS reductase (104, 268). The reductant used by the enzyme is probably reduced glutathione (215); a domain of the enzyme resembles a glutathione-dependent reductase (18). mRNA for APS sulfotransferase accumulates during S starvation, suggesting that a key juncture for controlling assimilatory processes occurs at the point at which APS interacts with either APS kinase or APS sulfotransferase (104, 285). Interestingly, mRNAs encoding ATP sulfurylase and APS sulfotransferase accumulate upon exposure of plants to cadmium. This increase in expression may allow for the efficient synthesis of phytochelatin (148). The SO_3^{2-} generated in the APS sulfotransferase reaction is reduced to sulfide by plastid sulfite reductase. A gene encoding sulfite reductase has recently been identified (22). Electrons used for SO_3^{2-} reduction are donated by reduced ferredoxin (329).

Cysteine Synthesis Cysteine is synthesized from sulfide and O-acetylserine (OAS) in a reaction catalyzed by O-acetylserine(thiol)lyase, which is present in the cytosol, plastids, and mitochondria (166). cDNA clones encoding different isoforms of O-acetylserine(thiol)lyase have been isolated from various plants (96, 109, 249). The mRNA for the cytosolic isoform of the Arabidopsis enzyme was highest in trichomes under normal growth conditions (96), and it accumulated in leaves and shoots following salt stress (13). The induction of the gene under high salt conditions may allow for the synthesis of the osmoprotectant 3-dimethylsulfoniopropionate (131). Furthermore, overexpression of O-acetylserine(thiol)lyase in transgenic tobacco increased the tolerance of the

plant to H_2S gas and sulfite (248, 331), probably because these potentially toxic compounds could be more rapidly assimilated.

Serine acetyltransferase, which catalyzes the formation of OAS, is localized in the cytosol, chloroplasts, and mitochondria (245). The activity of cytosolic serine acetyltransferase is inhibited at micromolar concentrations of cysteine, suggesting stringent feedback control (194, 250). Furthermore, the level of plastid serine acetyltransferase mRNA increases upon S starvation (283). Serine acetyltransferase is associated with O-acetylserine(thiol)lyase in the cysteine synthase complex (20). The complex is inefficient in synthesizing cysteine (58) and free O-acetylserine(thiol)lyase appears to be responsible for cysteine synthesis. Overexpression of the serine acetyltransferase gene in tobacco chloroplasts increased both cysteine and GSH levels, and the plants were more tolerant to oxidative stress (19).

Methionine Synthesis Methionine is synthesized from cysteine and O-phosphohomoserine (OPH) through the three consecutive reactions catalyzed by cystathionine γ-synthase, cystathionine β-lyase, and methionine synthase (230). Cystathionine γ-synthase, localized to chloroplasts, catalyzes cystathionine formation (228, 229). Its activity is controlled by the availability of OPH and S-adenosylmethionine. OPH, which is generated from aspartate, can serve as substrates for both cystathionine γ-synthase and threonine synthase. The relative activities of these enzymes are controlled by the S status of cells. Threonine synthase is most active during S-replete growth when the levels of S-adenosylmethionine are high (42). However, when S-adenosylmethionine levels drop, threonine synthase activity declines and a greater proportion of the OPH is converted to methionine via cystathionine γ-synthase (229). The gene encoding cystathionine γ-synthase has been cloned from Arabidopsis (127). Antisense suppression of cystathionine γ-synthase levels was lethal in homozygous transgenic plants, indicating that cystathionine synthesis in chloroplasts is a dominant pathway (and perhaps the only pathway) for methionine synthesis (230). Interestingly, recent work with Arabidopsis has demonstrated that cystathionine γ-synthase mRNA is controlled at the level of stability and that this stability is governed by a sequence present in the first exon of the gene. This *cis*-acting destabilization of the mRNA is triggered either by methionine or a methionine metabolite (31).

In plants and bacteria, α,β-elimination of cystathionine by cystathionine β-lyase generates homocysteine; the enzyme activity is present in plant cell chloroplasts (57, 318). The gene encoding cystathionine β-lyase appears to be represented as a single copy on the Arabidopsis genome (232). The native recombinant cystathionine β-lyase is a tetramer of four identical 46-kDa subunits, each associated with one molecule of pyridoxal 5′-phosphate. The physicochemical properties of the plant enzyme are similar to those reported for bacterial enzymes (57, 231).

Methionine synthase catalyzes the methylation of homocysteine to generate methionine. Methionine synthase genes of plants and algae encode cobalamin-independent enzymes with no presequences characteristic of transit peptides

(85, 140). The level of methionine synthase mRNA increases several fold in Chlamydomonas gametes activated by fertilization (140), which may be important for adhesion-induced processes that accompany fertilization.

Glutathione Synthesis GSH and GSSH, reduced and oxidized forms of glutathione, respectively, are readily interchangeable. This tripepeptide (γ-Glu-Cys-Gly) is the dominant non-protein thiol in plants (236) and can play a role in regulating the uptake of SO_4^{2-} by plant roots (108, 143). It is also a substrate for GSH-S-transferases, which are important for detoxification of xenobiotics (142, 168), and is the precursor of phytochelatins, peptides that enable plant cells to cope with heavy metals in the environment (98, 261). GSH is an abundant antioxidant in cells and supports redox buffering (139). The synthesis of GSH occurs in plastids by a two-step reaction catalyzed by γ-glutamylcysteine synthetase and GSH synthetase; genes encoding both have been isolated from Arabidopsis (234).

Exposure of plants to cadmium induces phytochelatin synthesis. This heavy metal chelator is synthesized from GSH by phytochelatin synthase and consists of repetitions of the γ-glutamylcysteine dipeptide that terminates with a glycine (33, 34). Mutants defective in phytochelatin synthesis are sensitive to heavy metals (35) whereas overexpression of γ-glutamylcysteine synthetase or GSH synthetase in *Brassica juncea* allowed increased cadmium tolerance (338, 339).

Regulation

In general, high concentrations of cysteine and glutathione repress S assimilation activities while S starvation results in increased activities of key enzymes in the assimilatory pathway. Administration of high concentrations of cysteine and glutathione to plant roots leads to lowered steady-state levels of mRNAs for the SO_4^{2-} transporter, ATP sulfurylase, and APS sulfotransferase (21, 144). Glutathione, which is readily transported through the phloem sap, may be a signaling molecule that represses the activity of genes encoding key enzymes in the S assimilation pathway (144). Plant roots subjected to exogenous OAS exhibit an increase in accumulation of mRNA encoding the SO_4^{2-} transporter (271) and APS sulfotransferase (133). Limited evidence derived from studies of plant systems suggests that, as in bacteria, a number of the S starvation responses are linked to the concentration of OAS.

Recently, specific proteins involved in the regulation of Chlamydomonas to S deprivation have been identified. Most responses of Chlamydomonas to S limitation require the Sac1 protein. A *sac1* mutant of Chlamydomonas exhibited abnormal SO_4^{2-} uptake and was unable to synthesize extracellular arylsulfatase, as well as other extracellular proteins, in response to S deprivation. The inability of the *sac1* strain to properly respond to S limitation is reflected in the rapid decline in viability of mutant cells following exposure to S deprivation (47). This decline in viability reflects an inability of the mutant to modify metabolic processes, such as photosynthesis, as the alga experiences sulfur limitation.

Interestingly, the *Sac1* gene product is predicted to be a polypeptide with similarity to ion transporters (46; J Davies & AR Grossman, unpublished). The deduced polypeptide sequence of Sac1 and the phenotype of the *sac1* mutant display some similarities with the sequence of Snf3 of yeast and the phenotype of the *snf3* mutant, respectively. Snf3 is a yeast regulatory protein that governs the expression of genes involved in hexose utilization (201, 202). The similarity between Sac1 and Snf3 raises the possibility that polypeptides whose original function was to bind and transport various substrates into cells may have evolved into regulatory elements.

Another mutant, *sac3*, was selected for low-level constitutive Ars activity. In addition, SO_4^{2-} transport could not be activated to the same extent as in wild-type cells (49). These results suggest that *Sac3* encodes a negative regulator of *Ars* gene activity and a positive regulator of gene(s) encoding the SO_4^{2-} transport system. *Sac3* encodes a putative serine-threonine kinase; the exact mechanism by which this kinase modulates the transcription of genes in both a positive and negative sense requires additional analyses.

PHOSPHATE

Phosphate in the Environment

The prevalent form of available P in the environment is Pi, which is a major component of nucleic acids, phospholipids, and intermediary metabolites. P occurs both in the intracellular and extracellular environment as the free Pi anion, precipitated Pi salts, Pi esters such as phytates, and more rarely as phosphonates; much of the P in soil is not readily available to plants. Crop yields are often limited by Pi availability and, as a consequence, high levels of Pi are important components of commercial fertilizers. A considerable proportion of this supplementary Pi is leached from agricultural fields and deposited into nearby lakes and rivers, triggering rapid algal blooms that cause eutrophication and fish kills.

Phosphatases and Phosphate Transport in Chlamydomonas

Phosphatases Yeast has served as a model system for elucidating how P conditions modulate gene expression, primarily through the analysis of phosphatase activities (see the complete version of the text at http://www.annualreviews.org/pubs/supmat/supmat.asp). Less is known for Chlamydomonas and vascular plants. Chlamydomonas does synthesize a number of phosphatases during P limitation. Two Chlamydomonas acid phosphatases are constitutively expressed, although their levels increase when the cells are limited for P (172, 173). There is also a neutral phosphatase and two alkaline phosphatases that accumulate upon P starvation (163, 164). Mutants that exhibit aberrant expression of the various phosphatases have been isolated (164, 165, 172). Subcellular locations of some phosphatases have been determined; one of the acid phosphatases is in the vacuole, the second

may be associated with the cell wall, while the neutral and alkaline phosphatases are extracellular and probably associated with the cell wall (173, 206).

Quisel et al (224) have identified and characterized phosphatases that accumulate in the periplasmic space upon exposure of Chlamydomonas to P limitation. The majority of periplasmic phosphatase activity behaves like a 5′ nucleotidase and has a monomeric molecular mass of approximately 190 kDa. A second periplasmic phosphatase (10% of the total) has a molecular mass of approximately 73 kDa and can slowly hydrolyze IMP and AMP. The third periplasmic species (<5% of the total) can be resolved from the other two activities by column chromotography (224), although the polypeptide responsible for this activity has not been identified. Other genes that are activated during P starvation encode enolase, pyruvate-formate lyase, and two ribosomal proteins; some of the genes may also be induced under other growth-limiting conditions (63).

Phosphate Transport Pi transport into Chlamydomonas cells has recently been characterized (269). Upon P starvation the V_{max} for Pi transport increases by a factor of 10 to 20. Furthermore, both a low- and high-affinity kinetic component can be distinguished. The K_m for low-affinity transport was between 10 and 20 μM Pi and that of the high-affinity transporter was between 0.1 and 0.5 μM. Low-affinity transport dominates when cells are maintained on complete medium. After 24 h of P starvation, Pi uptake is essentially all via the high-affinity system.

Phosphatatases and Phosphate Transport in Plants

Several visual changes occur when plants are limited for P. Older leaves experiencing P limitation exhibit an overall slowing of growth, increased root-to-shoot ratio, decreased production of lateral roots, increased length and density of root hairs, and they accumulate anthocyanin (170). Biochemical changes observed in plants starved for P include an increase in the fixed C storage reserves and elevated levels of lipids and phenolics and the production of phosphatases (76, 94, 95). Roots of some P-starved plants exude organic acids such as malate and citrate (113). Increased rhizosphere acidity may mobilize soil Pi, facilitating uptake in the immediate vicinity of the root.

Phosphatases and Rnases Phosphate starvation causes increased accumulation of intracellular and/or extracellular acid phosphatase (APase). This response has been noted for many plants (e.g. 62, 298; see the expanded article for additional references at http://www.annualreviews.org/pubs/supmat/supmat.asp). These phosphatases probably serve the same purpose in plants as they do in bacteria, yeasts, and algae: They increase the level of available Pi by hydrolyzing Pi-esters in the soil. Intracellular phosphatases may also increase during P limitation and help mobilize internal Pi stores.

Phosphatases have been characterized to some extent from different plant species (95, 103, 193, 204). Goldstein et al (94) demonstrated that P starvation

resulted in an increase in the APase activity secreted from tomato seedlings and that the activity was associated with a 57-kDa extracellular protein. Duff et al (59, 60) resolved two acid phosphatases (APases) from *B. nigra*. One of these enzymes was vacuolar and had significant specificity for PEP (59). Little is known about genes encoding plant APases. An APase gene from nematode-resistant tomato (324) has been isolated; the sequence of this APase is unlike that of any other characterized from nonplant tissue. However, it shows some sequence similarity to soybean vegetative storage proteins (53, 246), which appear to be capable of hydrolyzing Pi from several different substrates, but are most effective on short chain polyphosphates (53).

In addition to the production of phosphatases, plants produce other hydrolytic enzymes that help scavenge Pi from intracellular or extracellular sources. In tomato, four RNases induced upon P starvation have been characterized (122, 160, 161). Three of these RNases are vacuolar and probably function in the release of Pi from cellular RNA. Nürnberger et al (195) identified a periplasmic RNase of tomato that was specifically synthesized during P limitation and presumed to be important for releasing ribonucleotides from RNA in the soil.

RNase genes of Arabidopsis have also been characterized (reviewed in 97). The genes encoding S-like ribonucleases, including RNS1 and RNS2, are strongly induced in Arabidopsis during P starvation. These RNases are part of the T-2 ribonuclease family, which includes members that also function in gametophytic self-incompatibility in plants (191). Recently, antisense constructs were used to suppress expression of RNS1 and RNS2 of Arabidopsis (12). The transgenic plants with reduced levels of RNases showed increased anthocyanin accumulation, which is often induced by Pi stress.

Phosphate Transport Several studies have demonstrated that P limitation can elevate the V_{max} for Pi uptake via an increase in high-affinity transport (see http://www.annualreviews.org/pubs/supmat/supmat.asp). The uptake of Pi by plants is metabolically driven, and the Pi concentration in the xylem sap can be 400 times that of the soil solution. The main form of Pi transported into plants is $H_2PO_4^-$, which becomes prominent in acid soils. Only recently have a number of different genes encoding Pi transport proteins of plants been isolated and characterized (see 225). Like the NO_3^- and SO_4^{2-} transporters, the Pi transporters have 12 membrane-spanning helices. Transcripts for high-affinity Pi transporters often preferentially accumulate in root tissue (44, 159), and the genes are often induced when plants are starved for P (150, 159, 185, 186). Recently, antibodies were used to demonstrate that in addition to the LePT1 (high-affinity transporter in tomato) transcript, the LePT1 protein rapidly increases in the plasma membrane of tomato root cells following P starvation (186). High-affinity Pi transporters have also been shown to be present in leaves, tubers, stems, and flowers (150, 159); they may function to distribute Pi within the plant tissue. This is supported by the phenotype of the *pho1* mutant, which is defective in loading Pi into the xylem (213), and the *pho2* mutant, which may have aberrant phloem transport (54). Pi transporters are

also associated with plant mitochondria (282) and plastids (79), and specific plant Pi transporters may interface with mycorrhizal fungi and facilitate the transfer of nutrients from the fungus to the plant (242).

There are reports of six high-affinity Pi transporters (AtPT1-AtPT6) in Arabidopsis (159, 182, 272), and sequence information suggests the existence of an additional three (*AtPT7–AtPT9*; BAC F9H16.16, Accession numbers AC015450, AC007551) (225). None of the nine *PT* genes are allelic with genes altered in the *pho1* and *pho2* mutants (225). Recently, a gene encoding a low-affinity transporter, *Pht2;1*, was also isolated from Arabidopsis (45). This transporter appears to be a $Pi:H^+$ symporter with 12 membrane-spanning helices. It is constitutively expressed in Arabidopsis leaves and may function in the allocation of Pi within shoots.

The distinct location of the individual Pi transporters in plants reflects the need of the plant to balance Pi levels within its tissue and suggests that control of transport activities may be complex and strongly linked to physiological conditions. This Pi homeostasis is also controlled by Pi efflux from the cell and the flux of Pi in and out of the vacuole; these processes maintain a relatively constant concentration of Pi within the cytoplasm (181). The transporters on tonoplast membranes have yet to be identified.

Phosphorus Limitation and Metabolism

P limitation causes modification of various metabolic pathways. In P-starved Arabidopsis the levels of phospholipids decline whereas levels of sulfolipids increase; this increase was also reflected by an increase in expression of at least one gene important for sulfolipid biosynthesis (68). Furthermore, the *pho1* mutant (213) of Arabidopsis also showed decreased levels of phospholipids and increased levels of sulfolipids. These data suggest that one compensatory response for phospholipid deficiency is elevation of sulfolipid production, perhaps to help preserve the anionic character of plant thylakoid membranes (68).

There are also significant modifications with respect to activities associated with the glycolytic pathway. PEP phosphatase (the PEP-specific, vacuolar APase), which catalyzes the formation of pyruvate and Pi from PEP, increases in specific activity during P deprivation in both *Brassica nigra* (60) and *Selenastrum minutum* (90, 290); it generates pyruvate, effectively substituting for pyruvate kinase. Although the reaction is metabolically wasteful (ATP is generated by the pyruvate kinase reaction but not by PEP phosphatase), it would eliminate the need for phosphorylated adenylates, which would be limiting during P deprivation, in the conversion of PEP to pyruvate. This may increase the capacity of the cell to synthesize key glycolytic intermediates during P-limited growth.

Other enzymes have also been identified that might serve bypass functions described. PEP carboxylase, which catalyzes the carboxylation of PEP to form malate and Pi, also increases in *B. nigra* during P limitation (61). If coupled with malate dehydrogenase and the NAD malic enzyme, it offers an alternate route

for the formation of pyruvate. The levels of phosphofructophosphatase (PFP) and the nonphosphorylating, NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (nonphosphorylating NADP-G3PDH) were elevated 20-fold during Pi-deprivation, whereas the level of the phosphorylation-dependent NAD glyceraldehyde-3-phosphate dehydrogenase (phosphorylating NAD-G3PDH) decreased by a factor of six (61, 289). The PFP reaction can substitute for the phosphofructokinase reaction while the nonphosphorylating NADP-G3PDH reaction can substitute for the coupled phosphorylating NAD-G3PDH/phosphoglycerate kinase reactions. Both PFP and nonphosphorylating NADP-G3PDH reduce the Pi pressure by circumventing the need for adenylates and Pi as substrates in glycolysis. Some of these reactions (PFP, PEP phosphatase, and PEPC) may also promote Pi-recycling by releasing Pi from P-esters, and add to the flexibility of the plant's metabolic machinery.

Although many bypass reactions offer some conservation of P and promote recycling of Pi, it is uncertain whether they significantly affect Pi availability for plant metabolism. Furthermore, some bypass reactions still require pyrophosphate as the high-energy Pi donor. An alternative interpretation of the data might be that during P deprivation the cells must lower the pool of adenylates (perhaps to limit certain metabolic processes). The decrease in adenylates may severely slow the flux of metabolites through glycolysis. To sustain glycolysis at a rate needed for cell maintenance, specific steps of the glycolytic pathway are changed, making the pathway less sensitive to decreased adenylate levels.

Regulation

Control of the P deprivation responses may reflect a variety of regulatory mechanisms that operate at different levels. It is likely to include cellular responses, responses elicited by long-distance communication between different tissue types, and responses triggered by changes in metabolite pools, redox levels, and growth rates. For most responses, it is likely that the internal Pi supply is the dominant regulatory factor. This is supported by results of split root experiments in which one half of the tomato root system was placed in P-sufficient medium and the other in medium devoid of P. Transcripts encoding the Pi transporters LePT1 and LePT2 remained low (not induced) on the side of the root exposed to medium devoid of Pi (158), suggesting that internal pools of Pi control acclimation responses.

Recently, a regulatory element involved in acclimation to P limitation has been isolated. The Chlamydomonas mutant *psr1* (phosphate starvation response) (326) is unable to induce the specific responses to P starvation. The mutant was complemented and the *Psr1* gene identified (326). The Psr1 protein contains domains characteristic of transcription regulators; the region from residues 187 to 245 has similarity to Cca1, an Arabidopsis transcription factor responsible for phytochrome-mediated regulation of the light-harvesting genes (321). This DNA binding domain is distantly related to the DNA binding domain of myb proteins.

Psr1 also contains three stretches rich in glutamine and a helix-loop-helix structure that are characteristic of eukaryotic transcription factors.

During P limitation, the levels of both *Psr1* transcript and protein increase. Immunocytochemical experiments have localized the Psr1 polypeptide to the nucleus (326); the level of Psr1 in the nucleus paralleled the increase in whole-cell Psr1 polypeptide during P-limited growth. The sequence of Psr1, its location in the cell, and the phenotype of the *psr1* mutant all suggest that the Psr1 protein functions as a regulator of cellular transcription that enables Chlamydomonas to acclimate to P-limited growth.

Interestingly, although Psr1 resembles proteins deduced from plant cDNA sequences, it does not resemble any protein encoded by the yeast genome. This raises the possibility that Pi metabolism in Chlamydomonas and possibly in vascular plants is regulated in a different way from that of nonphotosynthetic eukaryotes.

GENERAL RESPONSES TO NUTRIENT LIMITATION

Cell Division

Nutrient deprivation prevents cell cycle progression, a phenomenon examined in some detail in yeast. Both the cAMP-dependent (26, 294) and MAP kinase (37, 123) pathways control cell division during nutrient-limited growth. Specific regulators of S metabolism (292) are also involved in controlling cell cycle progression by modulating the degradation of components that influence the activity of cyclin-dependent kinases (125).

Cell Cycle/Cell Division/Elongation Little is known about cell cycle control in eukaryotic algae or vascular plants, although a few intriguing observations have been reported. A *mat3* mutant of Chlamydomonas, which is aberrant for cell cycle control, is defective in a gene encoding a MAP kinase (J Uhman & U Goodenough, personal communication). Characterization of the root meristem-less *rml1* mutant suggests that cell division in Arabidopsis is partly associated with the control of S metabolism (309). The *rml1* mutant is unable to establish an active postembryonic meristem in the root apex and exhibits no cell division in roots (cell division continues in shoots). The *Rml1* gene encodes γ-glutamylcysteine synthetase, which catalyzes the first step of GSH synthesis. Addition of GSH rescues the phenotype of the mutant, suggesting a link between root meristem function and GSH levels.

Photosynthesis

Regulation of photosynthetic electron transport is a critical aspect of tailoring the metabolism of the cell to nutrient availability. Starvation of Chlamydomonas for S or P results in a decrease in O_2 evolution that correlates with a decline in electron flow through photosystem II (325). This decline in electron transport

is a consequence of both a loss of reaction center activity and the formation of Q_B nonreducing centers (100), which are more resistant to photoinhibition and may function to dissipate excess absorbed light energy (189). Nutrient-limited Chlamydomonas cells also dissipate energy by nonphotchemical quenching of chlorophyll fluorescence (325), and are mostly in state 2, in which the majority of the antennae complexes of the photosynthetic apparatus are directing energy to photosystem I (86, 302). This results in a decreased yield of O_2 evolution at subsaturating light levels, which can be beneficial to nutrient-deprived organisms because it decreases the production of NADPH, favors ATP generation through cyclic electron flow, and allows cells to more effectively dissipate excess absorbed excitation energy.

Photosynthetic electron transport also declines when Chlamydomonas is deprived of N (207), although much of this reduction is due to the loss of the cytochrome b_6f complex. Furthermore, N-limited Chlamydomonas cells tend to be in state 2, resulting in a reduced ratio of photosystem II to photosystem I activity at subsaturating light levels (17). Similar to S- and P-starved Chlamydomonas cells, N-limited *Dunaliella tertiolectra*, and *Thalassiosira weissflogii* exhibit diminished photosytem II activity and retardation of electron flow from Q_A to Q_B.

Overall, these results suggest that profound changes in functional aspects of the photosynthetic apparatus occur when algae and plants are starved for nutrients. Effectors that generate these changes are not known although they may be sensitive to redox levels, oxidative stress, and/or changes in the pool of cellular metabolites. It will be important to determine the ways in which these signals interface with the metabolic machinery of the cell and how such signals might elicit senescence and cell death, modulate various developmental processes, and trigger changes in rates of turnover and synthesis of proteins, nucleic acids, and lipids.

CONCLUDING REMARKS

Many lessons are being learned from the analysis of nutrient utilization and the acclimation of plants to nutrient deprivation. The process of nutrient acquisition and assimilation is dynamic and is continually modulated with changing environmental conditions. Internal and external cues determine the details of nutrient management, and such cues may be perceived directly by the plant or indirectly sensed as changes in cellular redox conditions and/or metabolite pools. Phytochrome and blue light photoreceptors participate in controlling nutrient level-responsive genes, while C metabolites generated by photosynthesis clearly integrate into regulatory circuits that control N utilization.

Only now are we beginning to identify regulatory elements that govern specific responses of photosynthetic organisms to their environment. The identification of such regulatory elements in simple algal systems is stimulating reverse genetic approaches that may unmask regulators in vascular plants. However, the different cell and tissue types that occur in a vascular plant will necessitate specific allocation

of nutrients to different plant organs and tissues, and this allocation will be controlled by both the developmental stage of the plant (including an influence of plant growth regulators) and environmental conditions. The control of nutrient allocation will require strategies for systemic communication among plant tissues and controlled synthesis of activities tailored to function in specific tissue/cell types and that respond to particular regulatory signals.

Researchers have begun to define processes critical for acquiring, assimilating, and distributing nutrients within plants. However, we will only develop a holistic understanding of the effects of nutrient conditions on plants by elucidating the integration of nutrient availability with other environmental cues, and with both the growth potential and the developmental stage of the plant.

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