Primary N-assimilation into Amino Acids in Arabidopsis

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Primary N-assimilation into Amino Acids in Arabidopsis

Gloria M. Coruzzi

INTRODUCTION

The assimilation of inorganic nitrogen into organic form has marked effects on plant productivity, biomass, and crop yield (Hageman and Lambert, 1988; Lawlor et al., 1989). As such, a tremendous amount of biochemical and physiological studies have been performed on nitrogen assimilatory enzymes from a variety of plant species, especially crops and legumes. Summaries of such biochemical studies can be found in several comprehensive reviews (Miflin and Lea, 1976, 1977; Miflin, 1980; Miflin and Lea, 1980, 1982; Miflin and Cuillimore, 1984; Poulton et al., 1989). Although these biochemical studies have provided a solid groundwork, a complete picture of the N-assimilation process and its regulation in a single plant is still lacking for a number of reasons. The existence of multiple isoenzymes for each step in nitrogen assimilation has confounded biochemical purification as have the technical difficulties of isoenzyme purification and organelle isolation. As the mechanisms controlling intra- and intercellular transport of inorganic and organic nitrogen in plants are still under investigation, it is impossible to predict the in vivo function of nitrogen assimilatory enzymes localized in distinct cells or subcellular compartments based on in vitro biochemistry. Plant mutants have provided a mechanism to dissect the process of N-assimilation in vivo (Lea and Forde, 1994) (Lam et al., 1996). The aim of this chapter is to specifically highlight and update examples where molecular, genetic, and biochemical analyses of N-assimilation genes and mutants in Arabidopsis have begun to define the in vivo roles of individual isoenzymes in plant nitrogen assimilation and to uncover the mechanisms regulating this process.

In all higher plants, inorganic nitrogen is first reduced to ammonia prior to its incorporation into organic form (Lea, 1993). For a review of the regulation of Nitrate assimilation and reduction in Arabidopsis see (Crawford and Forde, 2002). Ammonia is assimilated into organic form as glutamine and glutamate, which serve as the nitrogen donors in the biosynthesis of essentially all amino acids, nucleic acids, and other nitrogen-containing compounds such as chlorophyll (Lea, 1993). The individual isoenzymes of GS, GOGAT or GDH have been proposed to play roles in three major ammonium assimilation processes: (i) primary nitrogen assimilation; (ii) reassimilation of photorespiratory ammonia; and (iii) reassimilation of “recycled” nitrogen. For a review of these processes see (Stewart et al., 1980; Lea, 1993). Glutamine and glutamate can then be used to form aspartate and asparagine, and these four amino acids are used to translocate organic nitrogen from sources to sinks (Lea and Miflin, 1980; Peoples and Gifford, 1993). The enzymes involved in the primary assimilation of ammonium into these four N-transport amino acids Glu/Gln and Asp/Asn are shown in Fig. 1 and include: glutamine synthetase (GS), glutamate synthase (GOGAT), aspartate aminotransferase (AAT) and asparagine synthetase (AS). While most studies of nitrogen metabolism have previously been performed in legumes and crop species, HPLC analyses of Arabidopsis has demonstrated that these same four amino acids can account for 60-64% of the total free amino acids present in Arabidopsis leaves and are also transported in the vascular tissues (Fig. 2) (Schultz, 1994; Lam et al., 1995). Thus, Arabidopsis appears to be a suitable model plant for the study of nitrogen assimilation into primary amino acids and the results should have impact on less genetically tractable crop plants. It is noteworthy that each enzyme for GS, GOGAT, AspAT and AS exists as multiple isoenzymes, encoded by multiple genes, even in Arabidopsis (Fig. 1). Table 1 lists the enzymes, the genes, and available mutants for these N-assimilatory enzymes in Arabidopsis. Below, we review how studies of the genes and mutants for these isoenzymes in Arabidopsis have helped to illuminate the role of specific genes in this N-assimilation pathway.

Glutamine Synthetase (Gs, E.C.6.3.1.2)
GS, which has a very high affinity for ammonia (Km 3-5 µM), catalyzes the ATP-dependent conversion of glutamate into glutamine by incorporating a molecule of ammonia (Lea et al., 1990). GS is an octomeric holoenzyme, and two classes of GS isoenzymes were originally identified by ion-exchange chromatography: cytosolic GS1 and chloroplastic GS2 (Lea et al., 1990). The distinct physiological roles of chloroplast GS2 and cytosolic GS1 has been suggested primarily based on their organ-specific distribution. For example, as chloroplastic GS2 is the predominant isoenzyme in leaves, it has been proposed to function in primary assimilation of ammonia reduced from nitrate in chloroplasts and/or in the reassimilation of photorespiratory ammonia (Miflin and Lea, 1980). As cytosolic GS1 is the predominant isoenzyme in roots, it has been proposed to function in root nitrogen assimilation, although root plastid GS2 has also been implicated in this process (Miflin, 1974). The expression and localization of GS1 in vascular bundles of number of species further supports the notion that cytosolic GS1 functions to generate glutamine for intercellular nitrogen transport (Edwards and Coruzzi, 1990; Carvalho et al., 1992; Kamachi et al., 1992). This data, along with molecular-genetic studies described below in Arabidopsis have helped to assign in vivo functions to each GS isoenzyme.

Arabidopsis like all other higher plants contains a single nuclear gene for chloroplast GS2 (GLN2) and multiple genes for cytosolic GS1 (GLN1) (Peterman and Goodman, 1991; Oliveira and Coruzzi, 1999). Gene expression studies in Arabidopsis have shown that GLN2 is expressed predominantly in leaves, and its expression is regulated by light via phytochrome (Peterman and Goodman, 1991; Oliveira and Coruzzi, 1999). By contrast, the GLN1 genes (GLN1.1-1.3) encoding cytosolic GS1 isoenzymes are expressed at higher levels in roots (Oliveira and Coruzzi, 1999) (Peterman and Goodman, 1991). These organ-specific expression patterns suggest that GLN2 functions in leaf specific processes such as primary N-assimilation, and photospiration. By contrast, the cytosolic GS1 isoenzymes are likely to function in primary N-assimilation in roots, as N is exported from roots via xylem as Gln (Fig. 2C). Gene expression studies also showed regulation of GS mRNA levels by light, carbon and nitrogen metabolites (Oliveira and Coruzzi, 1999). In particular, GS expression is induced by light or sucrose and repressed by applications of Glu and Gln (Fig. 3A). These gene regulation patterns mimic changes in levels of GS enzyme activity (Oliveira and Coruzzi, 1999) (Fig. 3B). This analysis suggests that N-assimilation into Gln is active when levels of carbon backbones are high (in the light), and is repressed when levels of Gln are high (Oliveira and Coruzzi, 1999; Oliveira et al., 2001). This negative regulation of GS expression by levels of Glu/Gln is reminiscent of the Ntr system of GS in bacteria (Magasanik, 1994). In fact, a component of the Ntr system in bacteria (PtrII) has been identified in Arabidopsis, and there is some evidence that it may be involved in sensing C:N ratios, and in regulating N-assimilation in

Figure 1. Nitrogen Assimilatory Pathway

Figure 2. Amino Acid Levels in Light-Grown and Dark-Adapted Wild-Type Arabidopsis Plants. Amino acids levels in Arabidopsis plants grown in light (empty boxes) or subsequently dark adapted for 24 hr (filled boxes). The standard three letter code is used for all amino acids. (A) Average total free amino acid content. Each sample represents the average of three different plants (two leaves/plant). (B) Average free amino acid content in phloem exudates of three independent plants (one leaf/plant). (C) Average free amino acid content of xylem sap collected from cut hypocotyls of three independent plants. Data are from Schultz (1994). From: Hon-Ming Lam, Karen Coschigano, Carolyn Schultz, Rosana Melo-Oliveira, Gabrielle Tjaden, Igor Oliveira, Nora Ngai, Ming-Hsiun Hsieh, and Gloria Coruzzi (1995). Use of Arabidopsis Mutants and Genes to Study Amide Amino Acid Biosynthesis. Plant Cell 7, 887-898.
Table 1: N-assimilation-Enzymes, Genes, Mutants.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Enzyme</th>
<th>Gene Name</th>
<th>Gene ID#</th>
<th>Organelle</th>
<th>Mutant</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamine</td>
<td>GS</td>
<td>GLN 2</td>
<td>At5g35630</td>
<td>Chloroplastic</td>
<td></td>
<td>Peterman and Goodman (1991)</td>
</tr>
<tr>
<td></td>
<td>Glutamine synthetase</td>
<td>GLN 1.1</td>
<td>At5g37600</td>
<td>Cytosolic</td>
<td></td>
<td>Olivera et al. (2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GLN 1.2</td>
<td>At1g66200</td>
<td>Cytosolic</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GLN 1.3</td>
<td>At5g17820</td>
<td>Cytosolic</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fd-GOGAT</td>
<td>GLU 1</td>
<td>At5g04140</td>
<td>Chloroplastic</td>
<td>gls 1</td>
<td>Somerville and Ogren (1980)</td>
</tr>
<tr>
<td></td>
<td>glutamate synthase</td>
<td>GLU 2</td>
<td>At2g41220</td>
<td>Chloroplastic</td>
<td></td>
<td>Cordigano et al. (1998)</td>
</tr>
<tr>
<td>Glutamate</td>
<td>NADH-GOGAT</td>
<td>GLT 1</td>
<td>At5g53460</td>
<td>Chloroplastic</td>
<td>glt 1T</td>
<td>Lancien et al. (2002)</td>
</tr>
<tr>
<td></td>
<td>glutamate synthase</td>
<td>ODH 1</td>
<td>At5g18170</td>
<td>Mitochondrial</td>
<td>gdh 1-1</td>
<td>Meo-Oliveira et al. (1996)</td>
</tr>
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<td></td>
<td></td>
<td>ODH 2</td>
<td>At5g07440</td>
<td>Mitochondrial</td>
<td></td>
<td>Turano et al. (1997)</td>
</tr>
<tr>
<td>Aspartate</td>
<td>AAT</td>
<td>ASP 1</td>
<td>At2g30970</td>
<td>Mitochondrial</td>
<td></td>
<td>Schultz et al. (1995, 1998)</td>
</tr>
<tr>
<td></td>
<td>aspartate aminotransferase</td>
<td>ASP 2</td>
<td>At5g19550</td>
<td>Cytosolic</td>
<td>aat 2</td>
<td>Miresak and Coruzzi (2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ASP 3</td>
<td>At5g11520</td>
<td>Peroxisomal</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>ASP 4</td>
<td>At1g62800</td>
<td>Cytosolic</td>
<td></td>
<td>Schultz et al. (1995, 1998)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ASP 5</td>
<td>At4g31990</td>
<td>Chloroplastic</td>
<td>aat 3</td>
<td>Wilke et al. (1995)</td>
</tr>
<tr>
<td>Asparagine</td>
<td>ASN</td>
<td>ASN 1</td>
<td>At5g47340</td>
<td>Cytosolic</td>
<td></td>
<td>Lam et al. (1994, 1998, 2003)</td>
</tr>
<tr>
<td></td>
<td>asparagine synthetase</td>
<td>ASN 2</td>
<td>At5g65010</td>
<td>Cytosolic</td>
<td></td>
<td>Lam et al. (1998)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ASN 3</td>
<td>At5g10240</td>
<td>Cytosolic</td>
<td></td>
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</tr>
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</table>

*All mutant strains are available through ABRC.*

Regarding mutant screens in *Arabidopsis*, thus far no GS mutants have been reported. This is surprising, as GS mutants have been uncovered in photorespiratory mutants screens in other species (barley) (Wallsgrove et al., 1987). While identical screens for plant mutants unable to survive in photorespiratory conditions were conducted in *Arabidopsis* (Somerville and Ogren, 1980) mutants specifically defective in GS2 were identified only in the barley screen (Wallsgrove et al., 1987). A dramatic result of this mutant study in barley is the finding that a GS2 isoenzyme located in the chloroplast is essential for the reassimilation of photorespiratory ammonia that is released in mitochondria. Paradoxically, the barley GS2 mutants were unable to reassimilate photorespiratory ammonia released in the mitochondria even though they contained normal levels of cytosolic GS1 (Wallsgrove et al., 1987). This paradox has been resolved by studies on the cell-specific expression patterns of genes for chloroplastic GS2 and cytosolic GS1. Studies of GS-promoter-GUS fusions revealed that chloroplastic GS2 and cytosolic GS1 of pea are expressed in non-overlapping cell types in transgenic tobacco (Edwards et al., 1990). Chloroplastic GS2 is expressed predominantly in leaf mesophyll cells, where photorespiration occurs, while cytosolic GS1 is expressed exclusively in the phloem (Forde et al., 1989; Edwards et al., 1990). These promoter-GUS fusion results were later confirmed by studies of the native cytosolic GS1 proteins in rice and tobacco (Carvalho et al., 1992; Kamachi et al., 1992). In those studies, antibodies specific to cytosolic GS1 were used in *in situ* immunolocalization experiments to show that all cytosolic GS1 proteins are expressed solely in vascular tissues. Thus, this vascular-specific expression pattern may explain why cytosolic GS1 cannot compensate for the loss of chloroplastic GS2 in mesophyll cells of the barley GS2 mutants.

One piece of the GS isoenzyme puzzle that is outstanding is fact that the screens for photorespiratory mutants in *Arabidopsis* failed to uncover any mutants defective in GS, either chloroplastic GS2 or cytosolic GS1. There are several possible explanations for this finding: (i) The *Arabidopsis* photorespiratory screen was not saturating. This is unlikely as multiple alleles for many enzymes in the photorespiratory pathway were isolated in that screen, including 58 mutants affecting Fd-GOGAT (Somerville and Ogren, 1980; Artus, 1988). (ii) Both chloroplastic GS2 and cytosolic GS1 are expressed in mesophyll cells, so that a mutation in one gene is masked. (iii) There is more than one gene for chloroplastic GS2 in *Arabidopsis*. This is not possible as the complete sequence of the *Arabidopsis* genome (Bevan et al., 2001) has revealed a single gene for chloroplastic GS2. (iv) A mutation in chloroplastic or cytosolic GS is lethal in *Arabidopsis*, preventing the isolation of mutants.
Figure 3. C:N Regulation of GS mRNA levels and GS Activity.

(A) Amino acids and carbon reciprocally regulate GS mRNA accumulation and enzyme levels. Arabidopsis plants were grown semihydroponically and dark-adapted. The dark-adapted Arabidopsis plants were transferred in the dark to fresh low-nitrogen MS medium with no carbon supplementation (Con, lane 1), to fresh low-nitrogen MS medium with 3% Suc (lane 2), or to fresh low-nitrogen MS medium with 3% Suc in addition of either 10 mM Asp (lane 3), 10 mM Asn (lane 4), 10 mM Glu (lane 5), or 10 mM Gln (lane 6). After transfer, the plants were further incubated for 12 h in the dark.

(B) Same as A except that an aliquot of each sample was collected for determination of total GS activity. A representative experiment of two repetitions is shown (results are GS activity per milligram of total protein ± SE of three independent determinations). From: Igor C. Oliveira and Gloria M. Coruzzi (1999). Carbon and Amino Acid Reciprocally Modulate the Expression of Glutamine Synthetase in Arabidopsis. Plant Physiology 121, 301-309.

Glutamate Synthase/ (Nadh-Gogat: E.C.1.4.1.14; Fd-Gogat: E.C.1.4.7.1)

Glutamate synthase (GOGAT) functions in a cycle with glutamine synthetase (GS), catalyzing the transfer of the amide group from glutamine (generated by GS) to 2-oxoglutarate to form two molecules of glutamate (Fig. 1).

Table 2. Relative GOGAT activity levels in wild-type and Fd-GOGAT mutants (gls1).

<table>
<thead>
<tr>
<th></th>
<th>Fd-GOGAT</th>
<th>NADH-GOGAT</th>
<th>Total GOGAT</th>
</tr>
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<tbody>
<tr>
<td>WT</td>
<td>95%</td>
<td>5%</td>
<td>100%</td>
</tr>
<tr>
<td>gls1</td>
<td>5%</td>
<td>5%</td>
<td>10%</td>
</tr>
</tbody>
</table>

In higher plants, there are two forms of GOGAT, a Fd- and an NADH-dependent GOGAT, each of which is chloroplast localized (Lea, 1993). Fd-GOGAT is uniquely found in photosynthetic organisms and biochemical studies in Arabidopsis have shown that Fd-GOGAT is the major isoenzyme in leaves accounting for 95% of the GOGAT activity, while NADH-GOGAT activity is a minor component in leaves accounting for 5% (Somerville and Ogren, 1980) (Table 2). These organ-specific distribution patterns were used to infer in vivo function. For Fd-GOGAT, the high levels in leaves suggested that it plays a role in N-assimilation processes in photosynthetic tissues such as primary N-assimilation and/or photorespiration. By contrast, biochemical studies on other species showed that NADH-GOGAT is located primarily in plastids of non-photosynthetic tissues such as roots (Matoh and Takahashi, 1982; Suzuki and Gadal, 1984) where it is proposed to function in primary assimilation or reassimilation of ammonia released during amino acid catabolism (Miflin and Lea, 1980). The molecular and genetic studies of GOGAT genes and mutants in Arabidopsis outlined below have helped to clarify the relative in vivo roles of Fd- vs. NADH-GOGAT in primary N-assimilation vs. the reassimilation of photorespiratory ammonium.

Arabidopsis mutants in Fd-GOGAT were uncovered in 1980 during a screen for photorespiratory mutants (Somerville and Ogren, 1980). In the three Arabidopsis gluS (aka gls) mutants initially characterized, leaf Fd-GOGAT activity was reduced to less than 5% of wild type levels, whereas NADH-GOGAT - which contributes about 5% of the total GOGAT activities in normal conditions - remained unchanged (Somerville and Ogren, 1980) (Table 2). The gluS mutants had a conditional phenotype: they were chlorotic when grown in air (photorespiratory conditions), but could be rescued when grown in conditions under which photorespiration was suppressed (1% CO2) (Fig. 4). This finding suggested that Fd-GOGAT plays a major role in the reassimilation of photorespiratory ammonium released in mitochondria. This gluS mutant revealed a number of surprises that could not have been determined based on traditional biochemical studies. 1. It showed that photorespiratory ammonium released in the mitochondria is reassimilated primarily in the chloroplast by Fd-GOGAT. Thus, despite the fact that glutamate dehydrogenase (GDH) is located in the mitochondria, these studies on the gluS mutants suggested GDH plays a minor role, if any, in the reassimi-
Primary N-assimilation into amino acids in Arabidopsis

Figure 4. Photorespiratory Phenotype of gls Mutants Defective in Fd-GOGAT Activity. In (A) - (E), Arabidopsis plants were grown in air; in (F) - (J), plants were grown in 2% CO2. Genotypes: (A) and (F) Arabidopsis wild-type Columbia. (B) and (G) Photorespiratory gls mutant CS103. (C) and (H) NA60. (D) and (I) CS37. (E) and (J) CS340. From: Karen T. Coschigano, Rosana Melo-Oliveira, Jackie Lam, and Gloria Coruzzi (1998). Arabidopsis gls Mutants and Distinct Fd-GOGAT Genes: Implications for Photorespiration and Primary Nitrogen Assimilation. Plant Cell 10, 741-752.

Figure 6. A gls Mutant Displays a Defect in the Assimilation of Inorganic Nitrogen under Nonphotorespiratory Conditions. (A) and (B) Plants grown in air and plants in 1% CO$_2$, respectively. Wild-type Columbia (WT) and a gls mutant (NA60) were grown for 12 days on nitrogen-free MS media supplemented with either low levels of inorganic nitrogen (2 mM ammonium and 4 mM nitrate; open bars) or high levels of inorganic nitrogen (20 mM ammonium and 40 mM nitrate; hatched bars). Mean values of total chlorophyll measurements $\pm$ SE are shown. (C) Two-way ANOVA of (1) the wild type (WT) versus a gls mutant in air and CO$_2$ (2) low versus high nitrogen conditions in air and CO$_2$, and (3) interactions of 1 and 2 as given above. Statistically significant results are indicated by an asterisk. From: Karen T. Coschigano, Rosana Melo-Oliveira, Jackie Lam, and Gloria Coruzzi (1998). *Arabidopsis gls Mutants and Distinct Fd-GOGAT Genes: Implications for Photorespiration and Primary Nitrogen Assimilation. Plant Cell 10, 741-752.*

At first glance to be dispensable for non-photorespiratory roles, such as in primary nitrogen assimilation. This conclusion was paradoxical as most primary assimilation occurs in leaves, where Fd-GOGAT activity predominates (95% of total GOGAT activity) and NADH-GOGAT is a minor component (5% of total GOGAT activity) (Somerville and Ogren, 1980). How could Fd-GOGAT, the major GOGAT activity in leaves be dispensable for primary nitrogen assimilation? To address this question, Coschigano et al measured levels of chlorophyll as an indicator of N-status (Delgado et al., 1994) in wild-type and gluS mutant plants grown under non-photorespiratory conditions (Fig. 6). These studies suggest that the primary gluS mutants in fact have defects in N-assimilation (Coschigano et al., 1998). However, the gluS mutants are viable, suggesting that the normal low level expression of GLU2 in the mutants (and potentially NADH-GOGAT) is sufficient to support primary N-assimilation.

In contrast to Fd-GOGAT, NADH-GOGAT appears to be present primarily in non-green tissues such as roots. Arabidopsis has a single gene for NADH-GOGAT, GLT1, and recently, a T-DNA knock out mutant in this gene has been identified (Lancien et al., 2002). The GLT1 gene is expressed at low constitutive levels in leaves, and at higher levels in roots. The T-DNA mutant in the GLT1 gene (glt1-T) was null, and had no detectable levels of NADH GOGAT (Lancien et al., 2002). The glt1-T mutant was thus used to study the in vivo role of NADH GOGAT by scoring the mutants for defects related to N-assimilation, compared to wild-type and to a mutant in Fd-GOGAT (gls113) (Lancien et al., 2002). This analysis showed that the glt1-T mutant shows defects in fresh weight and chlorophyll accumulation, and glutamate production specifically when the plants are grown under non-photorespiratory conditions. These findings suggest that NADH-GOGAT plays a significant role in primary nitrogen assimilation in plants grown under non-photorespiratory conditions. The models for the respective roles of NADH vs. Fd-GOGAT are shown in Fig. 7.

Glutamate Dehydrogenase (Nadh-Gdh: E.C.1.4.1.2; Nadph-Gdh: E.C.1.4.1.4)

Glutamate dehydrogenase (GDH) is an enzyme that can catalyze forward and reverse biochemical reactions: the
ammonia into 2-oxoglutarate into glutamate (biosynthetic) or the deamination of glutamate into ammonia and 2-oxoglutarate (catabolic) (Fig. 1) (Lea et al., 1990). Two major forms of GDH have been reported, an NADH-dependent form (NADH-GDH) that is found in the mitochondria (Day et al., 1988; Loulakakis and Roubelakis-Angelakis, 1990), and an NADPH-dependent form (NADPH-GDH) localized to the chloroplast (Lea and Thurman, 1972). GDH is a hexameric enzyme (Srivastava and Singh, 1987). In Arabidopsis, the GDH enzymes can be resolved into seven isoenzymes by enzyme activity staining of non-denaturing gel electrophoresis (Cammaerts and Jacobs, 1983; Melo-Oliveira et al., 1996) (Fig. 8). These seven GDH activity bands are the result of the random association of two types of subunits into a hexameric complex. The most anodal homohexamer (GDH1) is thought to catalyze the anabolic reaction, while the cathodal most homohexamer (GDH2) is thought to be primarily catabolic (Cammaerts and Jacobs, 1985). Under this model, the heterohexamers of GDH1 and GDH2 would have intermediate catabolic and anabolic activities depending on the proportion of GDH1 versus GDH2 subunits. It has been proposed that two non-allelic genes are responsible for the synthesis of the GDH1 and GDH2 subunits (Cammaerts and Jacobs, 1985), and the discovery of two GDH genes: GDH1 and GDH2, and gdh1-1 mutant of Arabidopsis supports this conclusion, as discussed below (Melo-Oliveira et al., 1996; Turano et al., 1997). GDH isoenzymes have also been used to study the effect of nitrogen on GDH in Arabidopsis (Jacobs and Jokas, 1978).

Although GDH exists in plant tissues at high levels, there is an ongoing debate as to its physiological role in higher plants. Originally, GDH was proposed to be the primary route for the assimilation of ammonia in plants. However, this biosynthetic role of GDH has been challenged by the discovery of an alternative pathway for ammonia assimilation via the GS/GOGAT cycle (Miflin and Lea, 1976). Moreover, the fact that the GDH enzyme has a high Km for ammonia argues against a role in primary nitrogen assimilation (Stewart et al., 1980). Studies have shown that GDH enzyme activity can be induced in plants exposed to high levels of ammonia, and as such GDH has been proposed to be important specifically for ammonia-detoxification purposes. Mitochondrial GDH has also been proposed to be involved in the assimilation of high levels of photosynthetic ammonia released in mitochondria (Yamaya and Oaks, 1987). However, the isolation of photorespiratory mutants defective in chloroplastic GS2 (in barley) (Wallsgrove et al., 1987) or Fd-GOGAT (in barley and in Arabidopsis) (Somerville and Ogren, 1980; Kendall et al., 1986; Blackwell et al., 1987) have argued against the importance of GDH in photorespiration (Wallsgrove et al., 1987). Furthermore, treatment of plants with the GS inhibitor MSO prevents the incorporation of ammonia into glutamate and glutamine, even though both GDH activity and ammonia levels remain high (Lea et al., 1990). Altogether these results argue against a biosynthetic role for GDH. Instead a catabolic role for GDH has been invoked. Biochemical evidence for such a role is the fact that GDH activity is induced during germination and senescence, two periods where amino acid catabolism occurs (Stewart et al., 1980; Lea et al., 1990). To date, however, biochemical studies have failed to uncover the true in vivo role for GDH. The Arabidopsis gdh mutants described below have helped to define an in vivo role for GDH in plants.

Arabidopsis contains two genes for GDH: GDH1 and GDH2 (Melo-Oliveira et al., 1996; Turano et al., 1997). The predicted protein sequences suggest that they each encode NADH-dependent enzymes that are likely to be associated with the mitochondria. Gene expression studies have shown that each gene is subject to regulation by light and metabolites (Turano et al., 1997). Arabidopsis mutants deficient in GDH were identified in the M2 generation of EMS- and NMU-mutagenized Arabidopsis in a brute force screen using a GDH activity stain on crude leaf protein extracts following electrophoresis on native gels (Melo-Oliveira et al., 1996). A single Arabidopsis GDH mutant, gdh1-1, has been identified which has an altered pattern of GDH activity: it possesses a single GDH2 holoenzyme and is missing the GDH1 holoenzyme as well as the GDH1:GDH2 heterohexamers (Fig. 8) (Melo-Oliveira et al., 1996). The Arabidopsis gdh1-1 mutant displays an impaired growth phenotype compared to wild-type, specifically when plants are grown under conditions of high inorganic nitrogen (Fig. 9). This conditional phenotype suggests a non-redundant role for GDH in the assimilation of ammonia under conditions of inorganic nitrogen excess. A similar GDH-deficient mutant has been previously described in Zea mays, a C4 plant, which also appears to

Figure 8. GDH Activity in Wild-Type Arabidopsis and gdh 1-1 Mutant. Crude leaf protein extracts were made from rosette leaves of 21-day-old Arabidopsis plants, separated by electrophoresis on a nondenaturing polyacrylamide gel, and stained for GDH activity. Lanes 1 and 7, extract of wild-type Arabidopsis (Columbia). The seven holoenzymes result from the formation of two homohexamers (GDH1 and GDH2), and five heterohexamers of GDH are indicated on the right (GDH1/GDH2). M, individuals from a selfed gdh 1-1 mutant display only the GDH2 homohexamer (lanes 2-6); From: Rosana Melo-Oliveira, Igor Cunha Oliveira, and Gloria M. Coruzzi (1998). Arabidopsis mutant analysis and gene regulation define a nonredundant role for glutamate dehydrogenase in nitrogen assimilation. Proc. Natl. Acad. Sci. 93, 4718-4723.
The growth phenotype of the gdh1-1 mutant was assessed by measuring the vertical root length of wild-type Arabidopsis versus the gdh1-1 mutant seedlings. Wild-type (wt) and M3 seeds of the gdh1-1 mutant were sown side-by-side on ammonia-free/nitrate-free MS media containing vitamins and 3% sucrose supplemented with either: (A) no organic nitrogen (0 mM ammonia, 0 mM nitrate); (B) intermediate levels of inorganic nitrogen (2 mM ammonia, 4 mM nitrate); or (C) high levels of inorganic nitrogen (20 mM ammonia, 40 mM nitrate). Plants grown on MS media supplemented with 3% sucrose containing high levels of inorganic nitrogen (20 mM ammonia, 40 mM nitrate) without the vitamin supplement (*) were also included. Plates were incubated vertically for 12 days and grown under a normal day/night cycle.


Aspartate Aminotransferase (AspAT: E.C. 2.6.1.1)

Aspartate aminotransferase (AspAT) is a pyridoxal phosphate-dependent enzyme that catalyzes the reversible transfer of an amino group from glutamate to oxaloacetate to generate aspartate and 2-oxoglutarate (Fig. 1). The AspAT holoenzyme is a homodimer consisting of two subunits (Lea, 1993). In addition to its role in aspartate synthesis and catabolism, AspAT plays an important role in C3 plants, as part of the malate-aspartate shuttle which allows the transfer of reducing equivalents from mitochondria and chloroplasts into the cytoplasm (Ireland and Joy, 1985). Accordingly, biochemical studies show that AspAT exists as distinct isoenzymes found in different subcellular locations such as the cytosol, mitochondria, chloroplasts, glyoxysomes or peroxisomes. For examples see: (Weeden and Gottlieb, 1980; Wadsworth et al., 1993; Schultz and Coruzzi, 1995; Taniguchi et al., 1995). The subcellular compartmentation of AspAT isoenzymes suggests that the different forms of AspAT might serve distinct roles in plant metabolism. The isolation of Arabidopsis mutants in genes for distinct AspAT isoenzymes has helped to elucidate the role of these isoenzymes in plant N-assimilation.

Arabidopsis contains a family of five genes encoding distinct AspAT isoenzymes, ASP1-5 (Schultz and Coruzzi, 1995; Wilkie et al., 1995). ASP1 encodes mitochondrial AAT1, ASP2 and ASP4 encode cytosolic isoenzymes, ASP5 encodes chloroplastic AAT3, and ASP3 encodes a putative peroxisomal isoenzyme (Fig. 10) (Schultz and Coruzzi, 1995; Wilkie et al., 1995; Schultz et al., 1998). AAT isoenzymes of Arabidopsis were identified by native gel analysis. Three major isoenzymes were detected in crude leaf extracts, and identified by subcellular fractionation (Schultz and Coruzzi, 1995; Schultz et al., 1998). The two major isoenzymes are AAT2 (cytosolic) and AAT3 (chloroplastic) (Fig. 10). Mitochondrial AAT1 was also detected when the gels were overloaded. This native gel assay was used in a brute force screen to identify plant mutants deficient in one of the two major AAT isoenzymes; cytosolic AAT2 and chloroplastic AAT3.
deficiencies in Asp and Asn in siliques, suggesting that redundant function compared to the other 4 ASP genes.

Studies indicate that cytosolic AAT2 plays a non-
acid pools in the aat mutant plants, the
mutations on the growth phenotypes and the free amino
and Coruzzi, 2002). By analyzing the effects of these
sequenced in the aat mutants (Schultz et al., 1998; Miesak
mutants were mapped and the defective ASP genes
chloroplastic AAT3 (Fig. 11). The ASP genes and aat
defective in cytosolic AAT2, and aat3 mutants defective in
DNA lines uncovered two genetic loci: aat2 mutants
defective in cytosolic AAT2, and aat3 mutants defective in
chloroplastic AAT3 (Fig. 11). The ASP genes and aat
mutants were mapped and the defective ASP genes
sequenced in the aat mutants (Schultz et al., 1998; Miesak
and Coruzzi, 2002). By analyzing the effects of these
mutations on the growth phenotypes and the free amino
acid pools in the aat mutant plants, the in vivo importance
of each AAT isoenzyme was determined. Initial analyses
show that a mutation in the cytosolic ASP2 gene results in
a retarded growth phenotype and a decrease in the pools
of free aspartate (Schultz et al., 1998). Moreover, the aat2
mutants also showed dramatic decreases in asparagine in
dark-adapted plants (Fig. 12). These studies indicate that
cytosolic AAT2 is responsible for the synthesis of pools of
aspartate in the light that are used for asparagine
synthesis in the dark. Further analysis of additional aat2 alleles, including a T-DNA mutant in ASP2 revealed
additional information on the cytosolic AAT2 isoenzyme
(Miesak and Coruzzi, 2002). For example, in an attempt to
determine whether the growth deficiency of aat2 was due
to an aspartate deficiency, aat2 mutants were supplied
with exogenous aspartate. Surprisingly, the aat2 mutants
exhibited an aspartate sensitivity (compared to wild-type)
suggesting that the aat2 mutation led not only to an
inability to synthesize aspartate, but also an inability to
catabolize aspartate (Miesak and Coruzzi, 2002). These
studies indicate that cytosolic AAT2 plays a non-
redundant function compared to the other 4 ASP genes.
Further HPLC studies on siliques of aat2 mutants revealed
deficiencies in Asp and Asn in siliques, suggesting that

cytosolic AAT2 plays an important role in N-transport to
siliques (Miesak and Coruzzi, 2002).

Asparagine Synthetase (AS: E.C.6.3.5.4)

Asparagine was the first amino acid discovered, as it was
crystallized from asparagus almost 200 years ago
(Vauquelin and Robiquet, 1806). Despite this historical
placement, the mechanism of asparagine biosynthesis in
plants has been elucidated in plants only recently. While
three possible routes for asparagine synthesis have been
proposed (Sieciechowicz et al., 1988), the glutamine-
dependent asparagine synthetase enzyme is now
generally accepted as the major route for asparagine
biosynthesis in plants (Lea et al., 1990). In an ATP-
dependent reaction, AS catalyzes the transfer of an amino
group of glutamine to a molecule of aspartate to generate
a molecule of glutamate and asparagine (Fig. 1). Glutamine is the preferred substrate for nearly all of the
AS enzymes studied in higher plants (Sieciechowicz et al.,
1988). However, ammonia was also reported to be a
possible AS substrate, particularly in the case of maize
roots (Oaks and Ross, 1984). Traditional biochemical
studies of AS in higher plants were hampered by the fact
that AS is a very unstable enzyme in vitro and only
partially purified AS enzymes have been isolated from

Primary N-assimilation into amino acids in Arabidopsis
Figure 12. *aat2-2* mutants have specific reductions in levels of aspartate in light-grown plants and asparagine in dark-adapted plants. The relative proportions of aspartate and asparagine in the phloem exudates from wild-type Columbia (Col) and *aat2-2* mutant plants grown in light (unshaded boxes) or dark adapted (shaded boxes). Each sample is the average of a single leaf from three representative plants. Plants were grown in soil in a normal day/night cycle (16 hr light/8 hr dark) for 3 wk and either light adapted (unshaded box) or dark adapted (shaded box) for 24 hr. Error bars represent the standard error of the mean. From: Carolyn J. Schultz, Meier Hsu, Barbara Miesak and Gloria Coruzzi (1998). *Arabidopsis* Mutants Define an *in Vivo* Role for Isoenzymes of Aspartate Aminotransferase in Plant Nitrogen Assimilation. Genetics 149; 491-499.

Various plant species (Lea and Miflin, 1980). Compared to glutamine, asparagine is relatively inert and carries more nitrogens per carbon. Thus, asparagine is used to store and transport nitrogen in many higher plant species including legumes, crop plants and also in *Arabidopsis*. While no higher plant mutants in AS exist to date, molecular and reverse genetic studies on ASN genes in *Arabidopsis* have begun to provide the first insights into the regulation of asparagine biosynthesis in plants.

*Arabidopsis* contains three genes for asparagine synthetase, ASN1, ASN2, and ASN3. ASN1 was cloned by homology to pea AS1 (Lam et al., 1994), while ASN2 and ASN3 were cloned functionally by complementation of a yeast asparagine auxotroph (Lam et al., 1998). The AS polypeptides encoded in each of these ASN cDNA clones contains a purF-type glutamine-binding domain (Richards and Schuster, 1992), suggesting that glutamine is the preferred substrate. Phylogenetic grouping suggests that ASN1 forms a separate clade from the ASN2 and ASN3 genes of *Arabidopsis*, suggesting the possibility that these enzymes may play distinct roles *in vivo* (Lam et al., 1998). The ASN2 & 3 genes group with monocot AS enzymes. Studies in maize suggest this form of AS encodes in form of the enzyme that uses Gln or ammonia as a substrate *in vitro* (Oaks and Ross, 1984).

The ASN genes also differ in expression patterns. ASN1 appears to be the major expressed gene in *Arabidopsis* seedlings (Lam et al., 1998). Expression of ASN3 is very low, and ASN2 expression while higher, is distinct from ASN1 suggesting that they serve distinct functions. ASN1 is expressed at highest levels in dark-adapted plants, and light inhibits its transcription (Fig. 13) and (Lam et al., 1994; Lam et al., 1998). By contrast, ASN2 is expressed at higher levels in the light (Fig. 13). The light repression of ASN1 and the light induction of ASN2 occur with 30 minutes and are kinetically reciprocal, suggesting distinct functions for the encoded enzymes (Lam et al., 1998). There are several pieces of evidence suggesting that ASN1 controls the major levels of free asparagine synthesized in *Arabidopsis*. 1. Levels of ASN1 mRNA parallel changes in free levels of asparagine (Fig. 13), and 2. Transgenic manipulation of ASN1 (overexpression or antisense) affect levels of asparagine in plants (Lam et al., 2003).

Light also plays a major role in regulating asparagine levels in *Arabidopsis*, and this appears to be controlled at...
Asparate Aminotransferase in Plant Nitrogen Assimilation.

Arabidopsis

Schultz, Meier Hsu, Barbara Miesak and Gloria Coruzzi (1998).

phytochrome, and in part by light-induced changes in
13). This light repression of ASN1 is mediated in part by
in light-grown plants and high in dark-adapted plants (Fig.
the level of transcription of the ASN1 gene. Levels of
asparagine are low in light grown plants, and high in dark
adapted plants. Similarly, levels of ASN1 mRNA are low
in light-grown plants and high in dark-adapted plants (Fig.
13). This light repression of ASN1 is mediated in part by
phytochrome, and in part by light-induced changes in
levels of photosynthate (Lam et al., 1994). More recently
we have shown that blue light is also involved in
repressing ASN1 expression (Thum et al., 2003). Studies
have shown that sucrose (or glucose) can mimic the
repressive effects of light on ASN1 expression when
supplied to dark-adapted plants (Lam et al., 1998). Finally,
why do plants use light and carbon to repress ASN1
transcription to affect asparagine biosynthesis? Because
asparagine carries more nitrogen per carbon compared
to glutamine, asparagine is used to transport nitrogen
when levels of carbon are low. Hence, ASN1 is
transcriptionally active in the dark, when levels of carbon
are low, and transcription is repressed in the light, when
carbon levels are high. Understanding the mechanisms
by which light and carbon transcriptionally repress ASN1
is significant, because this transcriptional mechanism controls asparagine biosynthesis in Arabidopsis.

Figure 14. N-assimilation into Amino Acids is regulated by light. Cytosolic AAT2 controls the synthesis of aspartate in the light, which is converted to asparagine in the dark. A model is depicted for the metabolic flow of nitrogen assimilation into the nitrogen-transport amino acids glutamate, glutamine, aspartate, and asparagine in the light and dark. In the light, inorganic nitrogen is assimilated initially into glutamate and glutamine by the combined actions of the plastid enzymes: chloroplastic glutamine synthetase (GS2, encoded by GLN2), and ferredoxin-dependent glutamate synthase (Fd-GOGAT, encoded by GLUT; Oliveira et al. 1997; Coschigano et al. 1998). The conversion of glutamate into aspartate in the light is controlled by cytosolic AAT2. In the dark, this pool of aspartate is converted into asparagine by asparagine synthetase (ASN1) (Lam et al., 1994, 1995). From: Carolyn J. Schultz, Meier Hsu, Barbara Miesak and Gloria Coruzzi (1998). Arabidopsis Mutants Define an in Vivo Role for Isoenzymes of Aspartate Aminotransferase in Plant Nitrogen Assimilation. Genetics 149; 491-499.

Figure 15. C:N Regulation of GLN2/ASN1 mRNA levels. Light and metabolites cause a reciprocal effect on Arabidopsis GLN2 and ASN1 gene expression. The mRNA levels of ASN1 are repressed to nearly undetectable levels in light-grown plants (lane 8) and are strongly enhanced in dark-adapted plants (lane 9). By contrast, the low, undetectable levels of GLN2 mRNA from plants grown in the dark (lane 2) are highly elevated in light-grown plants (lane 1). Sucrose can partially mimic the effects of light by causing the induction of GLN2 and repressing the expression of ASN1 mRNA in the dark (lane 10). In the dark, the light-mimicking effects of sucrose can be antagonized by treatment with amino acids (AA) (GLN2, lane 4 to 7 and ASN1, lanes 11 to 13) with each AA affecting the expression of GLN2 and ASN1 to different extents. The differential effects of the AA on both GLN2 and ASN1 mRNA levels may be explained by the possibility that each AA exerts its effects through different but partially overlapping pathways. However, one cannot rule out differences due to rate of uptake or metabolism. PHY, phytochrome; GLN2, Arabidopsis GS2 (glutamate synthetase) gene; ASN1, Arabidopsis gene AS1 (asparagine synthetase) gene. From: I.C. Oliveira, E. Brenner, J. Chiu, M.-H. Hsieh, A. Kouranov, H.-M. Lam, M.J.Shin and G. Coruzzi (2001). Metabolic and light regulation of metabolism in plants: lessons from the study of a single biochemical pathway. Brazilian Journal of Medical and Biological Research. 34: 567-575.

Light and Metabolic Control of Nitrogen Assimilation into amino acids

A significant amount of evidence has been accumulated to show that the genes regulating nitrogen assimilation into amino acids is subject to control at the transcriptional level. The signals affecting the transcription of N-assimilatory genes are light, carbon metabolites, and nitrogen metabolites. Below is a brief review of these three forms of regulation, as well as evidence for interactions between these signals. While effects of light on N-assimilation have been studied largely at the transcriptional level, they are also reflected at the level of amino acids. For example, the repression of ASN1 transcription by light (Lam et al., 1998), results in the specific accumulation of asparagine in dark-adapted plants (Lam et al., 1995). By contrast, Gln levels are higher in light-grown plants (Lam et al., 1995), and this is reflective of the induction of GLN2 transcription by light (Oliveira and Coruzzi, 1999). In Arabidopsis, the reciprocal control of GLN2 versus ASN1 by light at the mRNA level has been shown to reflect similar light-induced changes in the levels of glutamine and asparagine. Glutamine levels have shown that sucrose (or glucose) can mimic the effects of light through different but partially overlapping pathways. However, one cannot rule out differences due to rate of uptake or metabolism. PHY, phytochrome; GLN2, Arabidopsis GS2 (glutamate synthetase) gene; ASN1, Arabidopsis gene AS1 (asparagine synthetase) gene. From: I.C. Oliveira, E. Brenner, J. Chiu, M.-H. Hsieh, A. Kouranov, H.-M. Lam, M.J.Shin and G. Coruzzi (2001). Metabolic and light regulation of metabolism in plants: lessons from the study of a single biochemical pathway. Brazilian Journal of Medical and Biological Research. 34: 567-575.
It appears that light also exerts an effect on the plant photoreceptor phytochrome (Lam et al., 1994; Oliveira and Coruzzi, 1999) and by blue light (Thum et al., 2003). By contrast, the light repression of ASN1, can be at least partially mimic the inductive effects of light (Fig. 15). By supplying sucrose to dark adapted plants, the light repression of ASN1, can be at least partially mimic the effects of light. In the case of GLN2, a carbon source such as glucose or sucrose can at least partially mimic the inductive effects of light (Fig. 15).

The light control of N-assimilatory genes such as ASN1 and GLN2 in Arabidopsis has been shown to operate via the plant photoreceptor phytochrome (Lam et al., 1994; Oliveira and Coruzzi, 1999) and by blue light (Thum et al., 2003). It appears that light also exerts an effect on the expression of other genes in the N-assimilatory pathway. For example, both GLN2 and the GLU1 gene encoding Fd-GOGAT are both induced by light (Coschigano et al., 1998) (Fig. 14). As the GS/GOGAT cycle affects N-assimilation, the coregulation of these genes by light serves to coordinate their expression with the production of C-skeletons during photosynthesis. Conversely, light has been shown to repress genes encoding ASN1 and GDH (Lam et al., 1994; Turano and Fang, 1998) (Fig. 14).

This coordinate regulation by light of N-assimilatory genes appears not only to involve phytochrome and blue light photoreceptors, but also reflects a coordination of N-assimilation by carbon availability. It has been shown that a carbon source such as glucose or sucrose can at least partially mimic the effects of light. In the case of GLN2, sucrose supplied to dark-adapted plants can at least partially mimic the inductive effects of light (Fig. 15) (Oliveira and Coruzzi, 1999; Thum et al., 2003). By contrast, the light repression of ASN1, can be at least partially mimicked by supplying sucrose to dark adapted plants (Fig. 15) (Lam et al., 1994; Thum et al., 2003).

Studies on glucose control of gene expression in Arabidopsis suggest that the regulation of N-assimilation by carbon is mediated via a non hexokinase dependent pathway (Sheen et al., 1999). This regulation of nitrogen assimilatory genes by the cellular carbon status appears to coincide with the interrelationship between carbon and nitrogen metabolism in plants. On the one hand, carbon assimilation and nitrogen assimilation compete for reducing power and energy sources generated by photosynthesis. On the other hand, carbon metabolism provides the necessary carbon backbones for the biosynthetic process of nitrogen assimilation while the products, e.g. amino acids, are essential components of the photosynthetic apparatus. In addition to the control by carbon status in the cell, it has been proposed that the relative abundance of nitrogen pools also plays a significant role in regulating nitrogen assimilation (Coruzzi and Zhou, 2001). In fact, some reports claim that the cellular carbon to nitrogen ratio is a major player in the metabolic control of nitrogen assimilation (Coruzzi and Zhou, 2001; Foyer et al., 2003). Plant glutamate receptors uncovered by (Lam et al., 1998) have also been implicated in sensing changes in Glu and in C/N levels (Kang and Turano, 2003). In the case of N-assimilation gene expression, the notion that plants sense C:N ratio is supported by the data which shows that the carbon induction of GLN2 is reversed by the addition of organic N, while the carbon repression of ASN1 is relieved by the addition of organic N (Lam et al., 1994; Oliveira and Coruzzi, 1999) (Fig. 15). In more recent gene chip experiments, Crawford's group showed that nitrate treatments affected the transcription of a host of genes involved in nitrogen assimilation including nitrate and nitrite reduction, as well as genes involved in N-assimilation such as Fd-GOGAT and asparagine syntetase (Wang et al., 2000). Data using Arabidopsis mutants or GS inhibitors suggest that either inorganic N (nitrate) (Crawford and Glass, 1998; Stitt, 1999) and organic N (Gln) (Rawat et al., 1999) may serve as signals to report on N-status and control the expression of genes involved in nitrogen uptake (e.g. the ammonium transporter AMT1) and/or assimilation.

In addition to its regulation by factors such as light, carbon and nitrogen, the pathway of N-assimilation genes such as ASN1 is further affected transcriptionally by other forms of regulation including regulation by circadian control (Hamer et al., 2001). Thus, the control of N-assimilation in plants is subject to complex control mechanisms. Both forward and reverse genetic approaches have been initiated in Arabidopsis in order to uncover the molecular mechanisms by which plants regulate gene expression in response to C and N metabolites. These studies are in their infancy, and this section is prime for expansion in the subsequent updates of this chapter.

**Figure 16. Multiple Input Regulation of N-assimilation genes.** A simplified scheme depicting reciprocal regulation of GLN2 & ASN1 by Light, Carbon and Nitrogen. Also depicted are NR (nitrate reductase) and NIR (nitrite reductase).
Primary N-assimilation into amino acids in *Arabidopsis*

Figure 17. Boolean Circuits for Interaction of Light and Carbon (light grown). Boolean circuits model *ASN1*, *ASN2* and *GLN2* regulation by light and carbon in light-grown plants. A, B, and C Boolean circuits based on 16 experiments. A. *GLN2* regulation by white, blue, red or far-red light when compared against a base-case of no light, no carbon or no light, carbon. B. *ASN2* regulation by white, blue, red or far-red light when compared against a base-case of no light, no carbon or no light, carbon. C. *ASN1* regulation by white, blue, red or far-red light when compared against a base-case of no light, no carbon or no light, carbon. The inputs are white light (WL), blue light low fluence (BLF), blue light high fluence (BHF), red light low fluence (RLF), red light high fluence (RHF), far-red light low fluence (FRLF), far-red light high fluence (FRHF). Low fluence is 2 \( \mu \text{mol} \, \text{m}^{-2} \, \text{s}^{-1} \), high fluence is 100 \( \mu \text{mol} \, \text{m}^{-2} \, \text{s}^{-1} \). The arrow or barred lines indicate the function of the inputs as either inductive or repressive. Double arrows or double bars denote super-induction or super-repression, respectively. For a Boolean OR, if any one of the inputs is active, the output will also be active. Differences in the input for Boolean circuits when comparing ‘absence of carbon’ to ‘presence of carbon’ are shown as boxed inputs. From: Thum KE, Shasha DE, Lejay L, Coruzzi G (2003) Light- and Carbon Signaling Pathways. Modeling Circuits of Interactions. Plant Physiology 132: 440-452.

**PERSPECTIVES**

Our lab has recently begun to use a systems approach to model and integrate the intersection of multiple signals that regulate the expression of N-assimilatory genes (Shasha et al., 2001; Thum et al., 2003). In one approach, we have employed a math tool called Combinatorial Design to design a parsimonious set of experiments that cover all combinations of treatments with light, carbon, and nitrogen sources (Shasha et al., 2001). By covering such experimental spaces, we have been able to use Boolean logic to model how signals interact. In another recent study, we covered an experimental space that allowed us to model how light sources (red, blue, far red, white) interact with C sources (sucrose) in the regulation of genes in the N-assimilation pathway (Thum et al., 2003) (Fig. 17). Modeling the regulation of this pathway and incorporating metabolic data, is a first step to the creating of a virtual plant which may be used in a predictive mode to allow changes that will enhance N-assimilation in plants. Molecular and genetic analyses of N-assimilation have provided important tools to extend our knowledge of nitrogen assimilation that was originally based on biochemical studies. The cloned genes and *Arabidopsis* mutants in specific genes have helped to distinguish the physiological roles of specific nitrogen assimilatory isoenzyme. The mechanisms by which light and/or
metabolic status (carbon and nitrogen) regulate nitrogen assimilation is beginning to be dissected using cloned genes and using genetic approaches. For example, some potential candidate regulatory genes have already been identified. In addition, specific screens for mutants in this process can be conducted in a genetic tractable system such as Arabidopsis. A combined molecular and genetic study of the regulatory network by which a gene responds to the metabolic status will lead to a better understanding of the genetic cross-talk between different carbon and nitrogen metabolic pathways. Basic research studies in these areas in Arabidopsis may also make significant contributions that can be applied to the improvement of nitrogen usage efficiency and crop yield in less genetically tractable systems.

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Primary N-assimilation into amino acids in Arabidopsis


