Nitrogen Nutrition Special Issue

SPECIAL ISSUE PAPER

Glutamate in plants: metabolism, regulation, and signalling

Brian G. Forde* and Peter J. Lea
Lancaster Environment Centre, Department of Biological Sciences, Lancaster University, Lancaster LA1 4YQ, UK

Received 9 March 2007; Revised 30 April 2007; Accepted 2 May 2007

Abstract

Glutamate occupies a central position in amino acid metabolism in plants. The acidic amino acid is formed by the action of glutamate synthase, utilizing glutamine and 2-oxoglutarate. However, glutamate is also the substrate for the synthesis of glutamine from ammonia, catalysed by glutamine synthetase. The \( \alpha \)-amino group of glutamate may be transferred to other amino acids by the action of a wide range of multispecific aminotransferases. In addition, both the carbon skeleton and \( \alpha \)-amino group of glutamate form the basis for the synthesis of \( \gamma \)-aminobutyric acid, arginine, and proline. Finally, glutamate may be deaminated by glutamate dehydrogenase to form ammonia and 2-oxoglutarate. The possibility that the cellular concentrations of glutamate within the plant are homeostatically regulated by the combined action of these pathways is examined. Evidence that the well-known signalling properties of glutamate in animals may also extend to the plant kingdom is reviewed. The existence in plants of glutamate-activated ion channels and their possible relationship to the GLR gene family that is homologous to ionotropic glutamate receptors (iGluRs) in animals are discussed. Glutamate signalling is examined from an evolutionary perspective, and the roles it might play in plants, both in endogenous signalling pathways and in determining the capacity of the root to respond to sources of organic N in the soil, are considered.

Key words: Enzymes, GLR genes, glutamate, homeostasis, ionotropic glutamate receptors, metabolism, root architecture, signalling, synthesis.

Introduction

In their stimulating article ‘Glutamate: an amino acid of particular distinction’, Young and Ajami (2000) reflect that perhaps God’s inordinate fondness may have been for glutamate, and not for beetles as the evolutionary biologist (and atheist) JBS Haldane is famously quoted as suggesting. The authors had in mind both the ubiquity of the amino acid in Nature and its remarkably diverse biological roles as a metabolite, an energy-yielding substrate, a nutrient, a structural determinant in proteins, and even a signalling molecule. They noted that glutamate has a number of chemical properties, including its reactivity, that make it particularly well suited to its multiplicity of functions.

Many aspects of the importance of glutamate in plants have been appreciated for decades, but others are only now coming to light. The purpose of this focus paper is to review key aspects of the place of glutamate in plant biology, focusing on its metabolic roles, the question of whether its cellular concentration is homeostatically regulated, and the evidence surrounding its possible role as a signalling molecule in plants.

Glutamate metabolism

As can be seen from Fig. 1, there is little doubt that glutamate is a central molecule in amino acid metabolism in higher plants. The \( \alpha \)-amino group of glutamate is directly involved in both the assimilation and dissimilation of ammonia and is transferred to all other amino acids. In addition, both the carbon skeleton and \( \alpha \)-amino group form the basis for the synthesis of \( \gamma \)-aminobutyric acid (GABA), arginine, and proline. It should also be noted that glutamate is the precursor for chlorophyll synthesis in
developing leaves (Yaronskaya et al., 2006). The biochemistry and molecular biology of glutamate metabolism have been reviewed previously (Lea and Miflin, 2003; Suzuki and Knaff, 2005), and this paper provides an overview of the enzymes involved in the metabolism of glutamate as shown in Fig. 1, focusing on advances that have been made in the past few years.

**Glutamine synthetase–glutamate synthase**

The key enzyme involved in the *de novo* synthesis of glutamate is glutamate synthase, also known as glutamine:2-oxoglutarate amidotransferase (GOGAT). The reaction is a reductant-driven transfer of the amide amino group of glutamine to 2-oxoglutarate to yield two molecules of glutamate. The enzyme in plants is present in two distinct forms, one that uses reduced ferredoxin (Fd) as the electron donor (EC 1.4.7.1) and one that uses NADH as the electron donor (EC 1.4.1.14). The Fd-dependent enzyme is normally present in high activities in the chloroplasts of photosynthetic tissues, where it is able to utilize light energy directly as a supply of reductant. The NADH-dependent enzyme, which is also present in plastids, is located predominantly in non-photosynthesizing cells, where reductant is supplied by the pentose phosphate pathway (Bowsher et al., 2007). The Fd- and NADH-dependent forms would appear to be expressed differently in separate plant tissues. However, the situation is now more complex, as evidence is accumulating to suggest that in most plants there are two genes that encode each form of glutamate synthase. Two recent reviews have covered the early history of the discovery of the two enzymes, their structure, and gene regulation (Lea and Miflin, 2003; Suzuki and Knaff, 2005). In this volume, Tabuchi et al. (2007) and Cánovas et al. (2007) discuss the role of glutamate synthase in rice and conifers. Clear evidence of perturbations in amino acid metabolism have been demonstrated in plants that have reduced amounts of either form of enzyme activity, caused by mutation or gene knockout (Somerville and Ogren, 1980; Blackwell et al., 1988; Leegood et al., 1995; Ferrario-Méry et al., 2002a, b; Lancien et al., 2002).

The nitrogen-containing substrate for the glutamate synthase reaction is provided in the form of glutamine. This itself is synthesized in the ATP-dependent combination of glutamate and ammonia catalysed by glutamine synthetase (GS; EC 6.3.1.2). The ammonia may have been generated by direct primary nitrate assimilation, or from secondary metabolism such as photorespiration (Leegood et al., 1995). GS activity is located in both the cytoplasm and chloroplasts/plastids in most higher plants, except conifers. The enzyme proteins can be readily separated by standard chromatographic localization and western blotting techniques into cytoplasmic (GS1) and plastidic (GS2) forms. However, this distinction is not as simple as was first thought, as although only one gene has been shown to encode the plastidic form, a small family of up to five genes is now known to encode the cytoplasmic form. Recent information on the regulation of the genes encoding GS in conifers (Cánovas et al., 2007), maize (Hirel et al., 2007), and rice (Tabuchi et al., 2007) is included in this volume. Limited success in the improvement of growth rates has been demonstrated following the overexpression of GS1 genes in *Lotus corniculatus*, poplar trees, tobacco, and maize (Martin et al., 2006; Tabuchi et al., 2007). Early mutants of barley lacking chloroplastic GS2 exhibited a severe phenotype and were only able to grow under non-photorespiratory conditions (Blackwell et al., 1988; Leegood et al., 1995). However, more recently, GS1 knockout lines have been isolated and characterized in both rice and maize. The growth rate, spikelet number, and weight were considerably reduced in a homozygous knockout line of OsGS1;1 in rice (Tabuchi et al., 2005). In contrast, maize mutants with knockouts of the gln1-3 and gln1-4 genes appeared to grow normally until grain filling (Martin et al., 2006). However, there was a reduction of kernel size in gln1-4
and of kernel number in gln1-3 mutants. In the gln1-3/1-4 double mutant, a cumulative effect of the two mutations was observed (Martin et al., 2006). The data from these two experiments clearly show that individual GS1 proteins have a non-redundant role in plant metabolism.

**Glutamate dehydrogenase**

The two enzymes involved in glutamate synthesis discussed previously catalyse irreversible reactions. A third enzyme, glutamate dehydrogenase (GDH; EC 1.4.1.2), catalyses a reversible amination/deamination reaction, which could lead to either the synthesis or the catabolism of glutamate. During the last 33 years, the role of GDH in glutamate metabolism in plants has been the subject of continued controversy (Oaks and Hirel, 1985; Dubois et al., 2003; Tercé-Laforgue et al., 2004b). However, following recent investigations into the regulation of the genes encoding the enzyme protein, the presence of overexpressing and antisense lines and the use of nuclear magnetic resonance (NMR) and gas chromatography–mass spectrometry (GC-MS) techniques, the role is becoming clearer. Very recently, Mascalux-Daubresse et al. (2006) have reconfirmed that in both young and old tobacco leaves, glutamate is synthesized via the combined action of GS and glutamate synthase, whilst GDH is responsible for the deamination of glutamate.

GDH is located in the mitochondria, and on occasion the cytoplasm, within the phloem companion cells of shoots (Tercé-Laforgue et al., 2004a; Fontaine et al., 2006). GDH extracted from most plant species can be readily separated into seven isoenzymic forms following native gel electrophoresis (Thurman et al., 1965; Loulakakis and Roubelakis-Angelakis, 1996). The reason for this is that GDH comprises two distinct subunits (α and β) that are able to assemble, apparently at random, into enzymatically active hexamers. The relative proportion of the α- and β-subunits and hence the isoenzyme pattern observed varies with plant organ and nitrogen source (Loulakakis and Roubelakis-Angelakis, 1991; Turano et al., 1997). The α-subunit is encoded by GDHA in Nicotiana plumbaginifolia and GDH2 in Arabidopsis, and the β-subunit by GDHB in N. plumbaginifolia and GDH1 in Arabidopsis (Purnell et al., 2005).

Antisense and mutant lines of both genes in tobacco and Arabidopsis have been constructed, but a full metabolic analysis of the plants has not yet been carried out (Fontaine et al., 2006). Tobacco lines overexpressing the GDH β-subunit exhibited a greater capacity to catabolize glutamate but were not able to assimilate ammonia, when GS was inhibited (Purnell and Botella, 2007). GDH activity has long been known to increase during senescence and following the application of a range of stresses. It has recently been shown that high NaCl induces the formation of reactive oxygen species, which in turn induces the synthesis of the α-subunit of GDH in tobacco and grapevine. When GS was inhibited, there was evidence of incorporation of ammonia via GDH into [15N]glutamate and [15N]proline in the presence of high salt (Skopelitis et al., 2006).

**Glutamate as a substrate for aminotransferase reactions**

Following the formation of glutamate, the α-amino group can be transferred to a wide variety of 2-oxo acid acceptors to form amino acids, and similarly the α-amino group can be transferred back to form glutamate when 2-oxoglutarate and other amino acids are available. The reactions are carried out by reversible pyridoxal-5′-phosphate-containing enzymes termed aminotransferases (EC 2.6.1.x), also known as transaminases. It is probably the reversibility of these enzyme reactions that accounts for the relative stability of the glutamate concentration found in plants, which will be discussed in a later section. Studies on the substrate specificity of aminotransferases in the past have been confused by the use of impure plant extracts; however, there is now substantial evidence that aminotransferases can exhibit wide preferences for both the amino acid and oxo acid substrate. Following the publication of the genome sequence of Arabidopsis thaliana, it was calculated that there were 44 genes that encoded aminotransferases (Liepman and Olsen, 2004). Such a large number of enzymes is clearly beyond the scope of this article, and for this reason only a few reviews and key papers will be discussed.

The α-amino group of glutamate may be transferred to oxaloacetate to form aspartate by aspartate aminotransferase (EC 2.6.1.1). Aspartate is a precursor of asparagine (Lea et al., 2007) and the aspartate family of amino acids, lysine, threonine, methionine, and isoleucine (Azevedo et al., 2006). Aspartate aminotransferase also plays a key role in C₄ photosynthesis (Edwards et al., 2004). In Arabidopsis there are five distinct forms of aspartate aminotransferase that are located in the cytosol, mitochondria, plastids, and peroxisomes (Wadsworth, 1997; Liepman and Olsen, 2004). Very recently, a plastid-localized aspartate aminotransferase has been identified that is unrelated to other plant forms of the enzyme, but is similar to a prokaryotic enzyme (De la Torre et al., 2006).

The α-amino group of glutamate may also be transferred to pyruvate to form alanine by the action of alanine aminotransferase (EC 2.6.1.2). Alanine synthesis has been shown to play a key role in the response to hypoxia/anoxia (Ricout et al., 2006) and in C₄ photosynthesis (Edwards et al., 2004). Four genes encoding alanine aminotransferases have been identified in Arabidopsis (Liepman and Olsen, 2004) and Medicago truncatula (Ricout et al., 2006), with the enzymes being located in the cytosol, mitochondria, and peroxisomes. The transfer of amino groups to glyoxylate to form glycine in the peroxisomes in the photorespiratory nitrogen cycle (Keys,
2.7.2.8), the activity of which was inhibited by arginine synthesis was at
studies indicated that the major control point of arginine metabolism is in the
peroxisome. Recombinant GGT1 was shown to have glutamate:glyoxylye, alanine:glyoxylye, glutamate:pyru
tate, and alanine:2-oxoglutarate activities (Liepman and Olsen, 2004; Reumann and Weber, 2006). Aminotransferases involved in the metabolism of GABA, proline, and arginine will be discussed in later sections. At least six aminotransferases are involved in the synthesis and metabolism of branched chain amino acids (Binder et al., 2007) and two in the formation of histidinol phosphate in the synthesis of histidine (Mo et al., 2006). Very recently, a totally new aminotransferase capable of converting tetrahydrodipicolinate to LL-diaminopimelate, bridging three enzyme reactions, has been identified in the lysine synthetic pathway of Arabidopsis (Hudson et al., 2006).

GABA, arginine, and proline

Glutamate may be converted to GABA by the irreversible action of glutamate decarboxylase (GAD; EC 4.1.1.15) in the cytoplasm. GAD was initially shown to have a low pH optimum of <6.0. However, it is now known that the enzyme protein has a Ca\(^{2+}\)/calmodulin-binding site at the C-terminus (Zik et al., 2006), which allows the enzyme to be stimulated in the presence of Ca\(^{2+}\)/calmodulin at pH values >7.0. GABA accumulates in higher plants following the onset of a variety of stresses such as acidification, oxygen deficiency, low temperature, heat shock, mechanical stimulation, pathogen attack, and drought (Shelp et al., 1999; Bouché and Fromm, 2004; Bown et al., 2006). Shelp et al. (2006) have presented evidence that GABA is involved in communication between plants and animals, fungi, bacteria, and other plants. Very recently, Lancien and Roberts (2006) have also shown that GABA can down-regulate the expression of genes encoding 14-3-3 proteins in a calcium-, ethylene-, and abscisic acid-dependent manner.

Glutamate is the precursor of arginine and is metabolized via acetylated derivatives to ornithine, citrulline, and arginosuccinate in a nine-step process (Slocum, 2005). Arginine has a high N:C ratio (4:6), and along with asparagine (2:4) acts as a major nitrogen storage compound in higher plants, where it occurs in both the protein and soluble form. Arginine plays a key metabolic role in seed maturation and germination, phloem and xylem transport, particularly in conifer trees, and accumulates under stress and deficiency conditions (Lea et al., 2007). Arginine and ornithine may also act as precursors of polyamines, which can play an important role in the response of plants to stress (Alcázar et al., 2006). Early studies indicated that the major control point of arginine synthesis was at N-acetylglutamate kinase (NAGK; EC 2.7.2.8), the activity of which was inhibited by arginine and activated by N-acetylglutamate. There is now strong evidence that this control is mediated through the binding of PI(4,5)P\(_2\), a 2-oxoglutarate- and amino acid-sensing protein (Lam et al., 2006), which is able to relieve the inhibition of NAGK by the end-product arginine (Sugiyama et al., 2004; Chen et al., 2006; Ferrario-Méry et al., 2006).

Glutamate can act as the precursor of proline in a pathway that requires three enzyme-catalysed reactions and a spontaneous chemical reaction. Proline is a cyclic amino acid that has been shown to accumulate following a wide range of abiotic stresses such as that induced by drought, salinity, low and high temperatures, and heavy metals (Munn, 2005; Sharma and Dietz, 2006). There is also evidence that proline is a major component of the nitrogen transport stream in both the xylem and phloem (Brugière et al., 1999). The bifunctional enzyme protein \(\Delta^1\)-pyrroline-5-carboxylate synthetase (P5CS) catalyses two reactions (EC 2.7.2.11 and EC 1.2.1.41), the first of which, the phosphorylation of glutamate, is subject to feedback inhibition by proline (Deauney and Verma, 1993; Strizhov et al., 1997; Hong et al., 2000; Armengaud et al., 2004).

Glutamate concentrations in plant tissues

Methods for identifying and quantifying amino acids have improved dramatically over the last 50 years, from paper chromatography to ion exchange chromatography (Steward and Durzan, 1965), HPLC (Lindroth and Mopper, 1979; Geigenberger et al., 1996), NMR (Mesnard and Ratcliffe, 2005), GC-MS (Roessner et al., 2000), and LC-MS (Jander et al., 2004), with the recent addition of principal component analysis (PCA) (Fritz et al., 2006a). It should be remembered that the plant extracts used will have been derived from a range of intracellular organelles as well as extracellular material (Lunn, 2007), although the differences in amino acid concentrations between these organelles may not be very large (Riens et al., 1991). In addition, there are known to be >40 different cell types in plants, each of which may have a distinct transcriptome, proteome, and metabolome (Ohtsu et al., 2007). A vast number of papers have determined the soluble amino acid contents of higher plants under various conditions, including altered nitrogen nutrition, developmental changes, and, more recently, in mutants and transgenic lines. It would be impossible to cover even a fraction of the relevant publications, so only a small number of recent key papers will be discussed.

Light/dark diurnal cycle

In their paper entitled ‘Steps towards an integrated view of nitrogen metabolism’, Stitt et al. (2002) proposed that ‘glutamate plays a pivotal role in a sensitive feedback mechanism that regulates ammonium assimilation’. As
supporting evidence for this statement they drew our attention to the fact that in tobacco leaves, glutamate concentrations remained remarkably constant between 3 μmol g⁻¹ FW and 4 μmol g⁻¹ FW throughout the day, irrespective of the growth conditions, whilst glutamine concentrations varied considerably between 5 μmol g⁻¹ FW and 15 μmol g⁻¹ FW (Geiger et al., 1998). Similarly in tobacco plants grown continuously in 2 mM nitrate, the leaf glutamate concentration remained constant around 4 μmol g⁻¹ FW, whilst the glutamine concentration varied between 5 μmol g⁻¹ FW and 15 μmol g⁻¹ FW during the light/dark cycle (Matt et al., 2001a, b). Masclaux-Daubresse et al. (2002) confirmed that there were only small oscillations in the glutamate content of older source leaves of tobacco during the diurnal cycle (1100–1400 nmol mg⁻¹ chlorophyll), but that in younger sink leaves the glutamate concentration increased during the light period to 1600 nmol mg⁻¹ chlorophyll and decreased to 900 nmol mg⁻¹ chlorophyll in the dark. Similarly, in potato leaves, glutamate showed very little diurnal variation (1.0–1.3 μmol g⁻¹ FW), by comparison with alanine, aspartate, glutamate, and glycine (1–3 μmol g⁻¹ FW) (Urbanczyk-Wochniak et al., 2005). In a later study on Arabidopsis, Gibon et al. (2006) noted that a large proportion of the 137 metabolites measured in the rosettes exhibited marked diurnal changes during a 12/12 h light/dark cycle. These smooth oscillations in metabolite concentration included all of the amino acids, with the notable exception of glutamate, which showed only very small fluctuations.

Fritz et al. (2006a) re-examined data obtained previously that detailed the amino acid concentrations during a diurnal light/dark cycle in wild-type, nitrate reductase-deficient (Fritz et al., 2006b), and Rubisco-deficient (Matt et al., 2002) lines of tobacco, grown at two concentrations of nitrate and varying light intensities. The data set was analysed using PCA and regression analysis, and by normalizing the level of each individual amino acid to the growing root and shoot. The major forms of the transported amino acids are usually those again often as the amides and arginine (Hörtensteiner and Feller, 2002; Lea et al., 2007). Masclaux et al. (2000) analysed a number of metabolites in a wide range of different aged leaves on a mature vegetative tobacco plant. The total amino acid content was highest in the youngest leaves, but was reduced by 70% in the oldest leaves. The proportion of glutamate of the total (25%) remained constant, whilst that of GABA increased and that of proline decreased. Diaz et al. (2005) analysed the soluble amino acids in the leaves of different recombinant inbred lines of Arabidopsis during senescence. For all the lines, glutamate increased just after the onset of senescence from 20% to 40% of the total amino acid pool, but then decreased to between 10% and 20%, the rate of decrease of glutamate varying between the lines but correlating with senescence. In darkened Arabidopsis leaves, the asparagine concentration increased 20-fold over 6 d from 1 nmol mg⁻¹ FW to 10 nmol mg⁻¹ FW, whilst glutamate remained constant at 2 nmol mg⁻¹ FW (Lin and Wu, 2004). Some of the largest increases in glutamate concentration have been demonstrated during the ripening of tomato fruits (Gallardo et al., 1993; Baxter et al., 2005). In the leaves of tomato, the glutamate concentration was 2.32 μmol g⁻¹ FW, whilst in green fruit it was 40.07 μmol g⁻¹ FW, rising to 282.1 μmol g⁻¹ FW in red fruit (Roessner-Tunali et al., 2003). Carrari et al. (2006)
speculated that as glutamate was a precursor of chlorophyll, this accumulation towards the end of ripening could be due to the cessation of chlorophyll biosynthesis.

**Nitrogen nutrition**

The availability and uptake of nitrogen is considered as the major factor affecting growth (Lea and Azevedo, 2006). Increased nitrate has a beneficial effect and stimulates the synthesis of amino acids and protein, whilst excess ammonium ions can be toxic and can promote the formation of amides (Britto and Kronzucker, 2002).

Geiger et al. (1999) grew tobacco at three concentrations of ammonium nitrate and three of potassium nitrate at normal and elevated CO₂. Small increases in glutamate concentration in the leaves were determined at higher nitrogen inputs, which were further enhanced at elevated CO₂. Far greater increases in alanine, arginine, aspartate, glutamine, and serine were detected. However, at 3 mM ammonium nitrate, there was a 4-fold increase in glutamine, and serine were detected. However, at 3 mM nitrate, glutamine and glutamate increased by factors of 9 and 14, respectively, when compared with the wild type, which were maintained during both the light and dark period. However, the glutamate concentration in the deregulated plant was only greater by a maximum of 2-fold compared with the wild type (32 μmol g⁻¹ DW to 16 μmol g⁻¹ DW) during the light period and was not different during the dark period (Lea et al., 2006).

Nitrate-deficient Arabidopsis seedlings grown in sterile culture contained 2–3-fold higher 2-oxoglutarate (250 nmol g⁻¹ FW), 50-fold lower glutamine (0.25 μmol g⁻¹ FW), and 6-fold lower glutamate concentrations (1.0 μmol g⁻¹ FW) compared with seedlings grown in full nutrient medium. Three hours after the addition of 3 mM nitrate, glutamine and glutamate increased by 6-fold and 2-fold, respectively, whilst 2-oxoglutarate decreased by approximately 40% (Scheible et al., 2004). In a similar series of experiments involving carbon starvation of Arabidopsis seedlings, followed by the resupply of 15 mM sucrose, Osuna et al. (2007) demonstrated, using a false colour chart, that whilst alanine, arginine, aspartate, glycine, and serine exhibited major changes, glutamate remained constant.

**Feeding of metabolites**

One way of investigating how glutamate concentrations can vary is to feed metabolites into plant tissues from the outside. The feeding of high concentrations, 150 mM GABA and proline, or 100 mM glutamine and nitrate, to
leaf discs of tobacco had little effect on the endogenous glutamate concentration, which remained around 30 nmol mg\(^{-1}\) DW. Only the feeding of 100 mM glutamate caused an increase of 3–4-fold in glutamate, whilst glutamine increased up to 30-fold to 302 nmol mg\(^{-1}\) DW, and at the same time there was an induction of GS1 and GDH (Masclaux-Daubresse et al., 2005). The feeding of 20 mM glutamate through the petioles to tobacco leaves caused a small increase in the glutamate concentration from 2.6 \(\mu\)mol mg\(^{-1}\) FW to 3.7 \(\mu\)mol mg\(^{-1}\) FW and there was a decrease in nitrate reductase activity, whilst 40 mM 2-oxoglutarate caused a 5-fold increase in glutamate from 2.0 \(\mu\)mol g\(^{-1}\) FW to 9.8 \(\mu\)mol g\(^{-1}\) FW (Fritz et al., 2006a). Similar results obtained by a different research group showed that the feeding of 40 mM glutamate to tobacco had little effect on the glutamate pool, although 2-oxoglutarate increased 6–9-fold; in contrast, the feeding of 40 mM 2-oxoglutarate increased glutamate by >3-fold from 2 \(\mu\)mol g\(^{-1}\) FW to 7 \(\mu\)mol g\(^{-1}\) FW (Schneidereit et al., 2006). Thus it would appear that whilst even the supply of very high concentrations of amino acids has little effect on the glutamate concentrations in leaves, it is possible to manipulate the glutamate pools by feeding 2-oxoglutarate, indicating the important role that the supply of 2-oxoglutarate, probably through the action of isocitrate dehydrogenase, plays in glutamate synthesis (Hodges, 2002; Abiko et al., 2005).

**Use of inhibitors, mutants, and antisense/knockout lines**

The pathways involved in glutamate metabolism in vivo can be identified by preventing the action of key enzymes. Early work on establishing fluctuations in the amino acid pools of plants was carried out using inhibitors of GS (Fentem et al., 1982; Rhodes et al., 1986), glutamate synthase (Baron et al., 1994), and aminotransferases (Brunk and Rhodes, 1988). However, doubt was always cast on the specificity of such inhibitors and the possibility raised that other enzymes may have been inadvertently affected. The first isolation of a mutant of Arabidopsis deficient in Fd-glutamate synthase (Somerville and Ogren, 1980) led the way to the characterization of a range of mutants which were impaired in the photosynthetic nitrogen cycle (Keys, 2006). The mutants of Arabidopsis and barley deficient in Fd-glutamate synthase were characterized by an accumulation of glutamine and a reduction in the concentration of glutamate following transfer from elevated CO\(_2\) to normal air (Blackwell et al., 1988; Leegood et al., 1995). Leaves of mutants of barley deficient in chloroplastic GS accumulated high concentrations of ammonia, whilst glutamate decreased from 3.5 \(\mu\)mol g\(^{-1}\) FW to 0.5 \(\mu\)mol g\(^{-1}\) FW in 2 h following transfer from elevated CO\(_2\) to normal air (Blackwell et al., 1988).

When antisense lines of tobacco containing only 15% Fd-glutamate synthase activity were exposed to air, there was a 20-fold increase in glutamine, ammonia, and 2-oxoglutarate, whilst the glutamate concentration only fell by a maximum of 40% to 10 nmol mg\(^{-1}\) DW towards the end of the light period (Ferrario-Méry et al., 2002a, b). The leaves of antisense lines of tobacco deficient in a chloroplast 2-oxoglutarate/malate translocator, when exposed to air in the light contained considerably reduced glutamate, aspartate, serine, and proline, and elevated glutamine and 2-oxoglutarate concentrations (Schneidereit et al., 2006). Interestingly, the glutamate concentration in NADH-glutamate synthase knockout lines of Arabidopsis was unaffected when the plants were grown in air, but was reduced from 3 pmol mg\(^{-1}\) FW to <1 pmol mg\(^{-1}\) FW in plants grown in high CO\(_2\) (Lancien et al., 2002).

The glutamate content of the roots and leaves of rice is low when compared with glutamine and asparagine, and only small increases were detected in a homozygous OsGS1-1 knockout line deficient in GS1 (Tabuchi et al., 2005). In maize knockout lines of the gln1-3 and gln1-4 GS1 genes, the concentration of glutamate was increased in the mature leaves (where no phenotype was observed), but was slightly reduced in the phloem (79 \(\mu\)mol \(\mu\)l\(^{-1}\) to 56 \(\mu\)mol \(\mu\)l\(^{-1}\)) and xylem (5.6 \(\mu\)mol \(\mu\)l\(^{-1}\) to 4.6 \(\mu\)mol \(\mu\)l\(^{-1}\)) (Martin et al., 2006). In GGAT knockout lines of Arabidopsis, glutamate increased from 3.8 nmol mg\(^{-1}\) FW to a maximum of 5.8 nmol mg\(^{-1}\) FW, with similar increases in glutamine and aspartate being detected. However, in GGAT-overexpressing lines, there was little change in glutamate, glutamine, and aspartate, but serine increased from 1 nmol mg\(^{-1}\) FW to 20 nmol mg\(^{-1}\) FW and glycine from 0.2 nmol mg\(^{-1}\) FW to 2 nmol mg\(^{-1}\) FW (Igarashi et al., 2006). The glutamate concentration was unaffected in GDH antisense lines of tobacco (Purnell et al., 2005) and GAD insertion lines of Arabidopsis (Bouché et al., 2004).

**Is there glutamate homeostasis in plants?**

The experiments described above, whilst admittedly selective, do provide some evidence that the glutamate concentration of plant leaves is maintained relatively constant during a diurnal cycle. Considering the flux of nitrogen through the photosynthetic pathway, this is no mean feat (Reumann and Weber, 2006). At different stages of development, there can be a huge variation in soluble amino acid content, but glutamate changes less than the other amino acids, in particular glutamine. Although the glutamate concentration can change in response to major alterations in nitrogen nutrition or when certain key enzymes have been deactivated, this variation is again not normally as great as that measured for other amino acids. Amino acids when supplied externally are rapidly metabolized, with only 2-oxoglutarate causing a significant accumulation of glutamate.

The next question is how are the glutamate concentrations held within reasonable limits, whilst concurrently other amino acids can change dramatically? The answer
Glutamate as a phylogenetically conserved signal molecule

L-Glutamate is now well established as an important signalling molecule in the mammalian central nervous system (CNS), but for many years this was a matter of controversy. Speculation about a possible signalling role for L-glutamate began in the early 1950s, but it was not until well into the 1970s that its true status as the major excitatory neurotransmitter in the CNS became widely accepted (Watkins and Jane, 2006). Central to this story was the development by Watkins and colleagues of specific L-glutamate agonists and antagonists that could be used to identify and characterize the neuronal glutamate receptors (mGluRs) to distinguish them from the iGluRs. Indeed the broad classification of glutamate receptors. The mGluRs in general seem to play a modulatory role in synaptic activity and to be involved in effects that are slower and longer lasting than those mediated by iGluRs (Watkins, 2000). The mGluRs belong to Family C of the seven transmembrane family of G-protein-coupled receptors (GPCRs), along with the GABA_B receptors.

In the past decade, evidence has unexpectedly emerged from different directions that glutamate signalling may also occur in higher plants (reviewed in Chiu et al., 1999; Davenport, 2002; White et al., 2002; Filleur et al., 2005; Lam et al., 2006). In the following sections, recent progress towards establishing the role and the molecular basis for glutamate signalling in plants is discussed.

Molecular evidence: existence of a family of iGluR-like genes in plants

The first important clue to the occurrence of glutamate signalling in plants was the discovery in 1998 that Arabidopsis possesses a family of AtGLR genes homologous to mammalian iGluRs (Lam et al., 1998). Completion of the Arabidopsis genome sequence revealed that there are 20 AtGLR genes (compared with only 11 in humans), which can be grouped into three clades (Chiu et al., 2002). The regions of homology between the plant GLR sequences and their animal counterparts span all the important domains, including the four transmembrane segments (M1–M4) and the S1 and S2 regions that form the agonist-binding domain (Chiu et al., 1999, 2002; Davenport, 2002) (Fig. 2). Phylogenetic analysis suggests that the divergence of animal iGluRs and the AtGLRs occurred before the divergence of the different animal iGluR classes (NMDA, AMPA-kainate, and Delta) (Chiu et al., 1999). The majority of the AtGLR proteins are predicted to be targeted to the secretory pathway (Davenport, 2002), and a recent report has confirmed that a radish GLR homologue is localized to the plasma membrane (Kang et al., 2006).

It has been established that all 20 AtGLR genes are transcribed, and in some cases there is evidence for mRNA splice variants, adding to the total number of potential AtGLR gene products (Chiu et al., 2002). Studies of the expression patterns of the AtGLR family have shown that their transcripts are widely distributed throughout the plant and that all 20 are expressed in roots (Chiu et al., 2002). Clade II genes appear to show a preference for root expression, with five of the nine genes in this clade being expressed specifically in the roots of 8-week-old plants (Chiu et al., 2002).

Despite the overall similarities in their domain organization, the plant GLRs are sufficiently divergent in sequence from the iGluRs that it is extremely difficult to deduce either their channel activity or their agonist-binding properties (Lacombe et al., 2001). As has been noted (Davenport, 2002), the plant GLRs have an unusual sequence in their putative pore region, suggesting that if they do have channel activity it either has novel ion selectivity or a novel mechanism of ion selectivity is involved. Attempts to model the agonist-binding sites of
the AtGLRs led to the suggestion that only AtGLR1.1 would bind glutamate and that glycine would be the natural ligand for the other members of the gene family (Dubos et al., 2003). Experimental support for this hypothesis came from evidence that glycine was able to elicit transient increases in \([\text{Ca}^{2+}]_{\text{cyt}}\) in Arabidopsis seedlings in a manner similar to glutamate (Dubos et al., 2003). However, as discussed below, the relationship between these \([\text{Ca}^{2+}]_{\text{cyt}}\) spikes and the AtGLRs has not yet been unequivocally established. Furthermore, there is the additional possibility that glycine is not directly activating \([\text{Ca}^{2+}]_{\text{cyt}}\) channels but is having an indirect effect by triggering the efflux of glutamate (or a glutamate-like ligand) from the plant cells (Frauli et al., 2006; Qi et al., 2006; see also below).

Mammalian and plant members of the iGluR/GLR family have two domains that are homologous to bacterial periplasmic binding proteins: one acts as the agonist-binding site and belongs to the lysine/arginine/ornithine-binding protein (LAOBP)-like superfamily, while the other is in the N-terminal proximal region of the extended N-terminal domain (NTD) and belongs to the leucine/isoleucine/valine-binding protein (LIVBP)-like superfamily (Fig. 2). The LIVBP domain is not found in a prokaryotic member of the iGluR family, the GluR0 glutamate-gated K\(^+\) channel, nor in the kainate-binding iGluRs from lower vertebrates (Oswald, 2004), but is related to the LIVBP domain that serves as the agonist-binding domain in members of Family C of the GPCRs (which includes mGluRs, GABA\(_B\) receptors, and Ca\(^{2+}\) receptors). Functions ascribed to the LIVBP domain in iGluRs include roles in binding allosteric modulators (such as Zn\(^{2+}\), redox agents, and polyamines) and in facilitating the oligomeric assembly of subunits (Oswald, 2004). The LIVBP domain in the plant GLRs is particularly intriguing because it is more closely related to the LIVBP domain in the Family C GPCRs than to the LIVBP domain in mammalian GLRs (Turano et al., 2001; Acher and Bertrand, 2004). To explain this, it has been proposed that, early in the evolutionary history of the iGluR/GLR family and prior to the divergence of plants and animals, a recombination event occurred that fused the LIVBP domain of the ancestral iGluR/GLR to the seven transmembrane domains of a GPCR to create the ancestral Family C GPCR (Turano et al., 2001).

Crystal structure analysis of the LIVBP domain of rat mGluR1 has identified a number of key residues (Ser165, Thr188, Asp208, Tyr236, and Asp318) that participate in the binding of the \(\alpha\)-carboxylic and \(\alpha\)-amino groups of glutamate (Kunishima et al., 2000). It has been noted that these five residues are conserved in the N-terminal region of AtGLR3.5 (GLR6) (Taniura et al., 2006), and

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**Fig. 2.** Schematic representation of the 3D structures of eukaryotic glutamate receptors and the bacterial proteins with which they share homologous domains. Two types of Venus fly trap (VFT) domain occur in members of the iGluR/GLR family: the agonist-binding LAOBP domain (formed by the S1 and S2 regions of the primary sequence) and the LIVBP domain in the extended N-terminal region, which can act as an allosteric modulatory domain. The bacterial glutamate receptor (GluR0) lacks the C-terminal and LIVBP domains. M1–M4 refer to the transmembrane domains in the GluR0/iGluR/GLR family (modified from Acher and Bertrand, 2004).
alignment of the LIVBP domains of all 20 AtGLRs confirms that AtGLR3.5 is the only member of the family in which this is the case (BG Forde unpublished data). This indicates that glutamate (or a structurally related ligand) may have a role in regulating the function of AtGLR3.5 (and of any heteromeric complexes of which AtGLR3.5 is a component). It is noteworthy that the five glutamate-binding residues are also conserved in the LIVBP domain of two rice GLR genes belonging to clade III, Os06g46670 and Os04g49570 (OsGLR3.1). This suggests that the ligand-binding properties of this domain are conserved between monocots and dicots, and that glutamate potentially also has an important role in modulating the activity of these members of the rice GLR family.

Cytological and electrophysiological evidence for iGluR-like channel activity

While mammalian iGluRs have been characterized using heterologous expression systems such as *Xenopus* oocytes, attempts to do the same with plant GLR genes have so far been unsuccessful (Li *et al.*, 2006; Lai-Hua Liu, personal communication). It is possible that the GLR subunits only function as heteromers, as is the case with NMDA receptors (Davenport, 2002), or there might be a requirement for specific interactions with auxiliary proteins, such as the transmembrane AMPA receptor regulatory proteins (TARPs) that modify the kinetics of AMPA receptors (Mayer, 2005).

The lack of success with heterologous expression systems means that it has not yet been possible to establish unequivocally the ligand-binding properties or the ion channel specificity of any plant GLR. Nevertheless, there is evidence for the existence of glutamate-gated ion channel activity in plants that has properties consistent with those of iGluR-related proteins. Dennison and Spalding (2000) were able to show that a rapid membrane depolarization which is triggered in root tip cells by external glutamate is accompanied by a spike in cytosolic Ca^{2+}. Current evidence suggests that these glutamate-activated channels have similarities to excitatory amino acid-activated channels in animal cells, being voltage insensitive and permeable to cations, notably Na^{+} and Ca^{2+} (Dubos *et al.*, 2003; Demidchik *et al.*, 2004).

Until recently, the only evidence tentatively linking these cytological and physiological observations to the GLR gene products was pharmacological, based on the use of agonists and antagonists of mammalian iGluRs (see below). However, two recent papers have provided tantalizing evidence that at least some GLRs might have functional properties similar to their animal homologues (Kang *et al.*, 2006; Qi *et al.*, 2006). In one study, a radish GLR cDNA (RsGluR) was overexpressed in transgenic *Arabidopsis* seedlings (Kang *et al.*, 2006). Glutamate treatment of the root cells of the transgenic lines was found to trigger enhanced Ca^{2+} influx compared with the controls, as expected if RsGluR is a glutamate-gated Ca^{2+} channel. However, since it remains possible that the ectopic expression of the RsGluR gene has somehow enhanced the activity of an endogenous glutamate-activated channel, these results fall short of providing formal proof that RsGluR encodes a glutamate-gated Ca^{2+} channel.

In a second paper, two T-DNA insertion mutants in the *AtGLR3.3* gene were identified and were shown to be defective in both the fast electrical response to glutamate and the associated Ca^{2+} response (Qi *et al.*, 2006). While these results clearly link the *AtGLR3.3* gene to these responses, there are some puzzling aspects to the mutant phenotype. First, since *AtGLR3.3* is just one of 20 *AtGLR* genes expressed in roots (Chiu *et al.*, 2002), it is not clear why the phenotype of the mutants was so strong (e.g. the Ca^{2+} spike triggered by 1 mM glutamate was completely absent). Secondly, all 20 proteinogenic amino acids were tested and five were indistinguishable from glutamate in their effects on both the mutants and the wild type. The five that behaved like glutamate were glycine, alanine, asparagine, cysteine, and serine, a structurally diverse group that, apart from glutamate and glycine, are not known agonists of iGluRs. It is difficult to envisage how all five amino acids could be agonists of AtGLR3.3. As suggested by the authors, a possible explanation is that treating the roots with any of the six effective amino acids triggered the efflux of the true AtGLR3.3 ligand, so that what was observed was a secondary response. Significantly, a similar conclusion was reached in a recent study of the effect of all classical L-amino acids on the eight different mGluR receptors expressed in human embryonic kidney 293 (HEK293) cells (Frauli *et al.*, 2006). Initial observations had indicated that some mGluR receptor subtypes in these cells could be activated by cysteine, aspartate, and asparagine, as well as glutamate. Although these effects could each be blocked by specific mGluR antagonists, the authors were able to show that glutamate was the only true agonist and that the activating effects of the other three amino acids resulted from their ability to trigger the release of endogenous glutamate from the cells (Frauli *et al.*, 2006). These results underline the difficulties inherent in basing the identification of agonists of glutamate-activated channels solely on the results of *in vivo* experiments where the response may entail unsuspected levels of complexity.

Pharmacological evidence for iGluR-like channels in plants

Several well-known agonists and antagonists of mammalian iGluRs have been used as tools to obtain supporting evidence for the existence of functional iGluR-like proteins in plants. β-Methylamino-L-alanine (BMAA), a cycad-derived agonist of mammalian iGluRs, was...
shown to inhibit *Arabidopsis* root growth and cotyledon opening, and to stimulate elongation of light-grown hypocotyls, in a glutamate- (and glutamine-) reversible manner (Brenner *et al*., 2000). The antagonist most commonly used is 6,7-dinitroquinoxaline-2,3-dione (DNQX), a specific antagonist of mammalian AMPA- and kainate-type iGluRs. A role for plant iGluR-like proteins in light signalling was surmised from experiments showing that DNQX inhibited light-induced hypocotyl shortening and the reduction of chlorophyll accumulation (Lam *et al*., 1998). In other studies, DNQX was able to block glutamate- (and glycine-) mediated increases in cytosolic [Ca$^{2+}$] in cotyledons (Dubos *et al*., 2003). However, DNQX was ineffective at inhibiting glutamate-elicted changes in cytosolic [Ca$^{2+}$] in root tips, suggesting that glutamate-gated channels in root tips are insensitive to this compound (Dubos *et al*., 2003). It is important to note that DNQX and other antagonists that have been used in plants, such as 2-amino-5-phosphonopentanoate (Sivaguru *et al*., 2003), were developed and characterized for use with mammalian rather than plant iGluRs. Given the major structural differences that appear to exist between mammalian and plant GLR proteins, as already noted, it is necessary to interpret the effects of these compounds with caution until their physiological targets in plant cells have been confirmed.

**Forward and reverse genetics approaches to elucidating the function of GLR genes**

A number of studies have adopted a reverse genetics approach to investigate the role of specific AtGLR genes. Phenotypic analysis of antisense *AtGLR1.1* lines has implicated this gene in the regulation of C/N metabolism (Kang and Turano, 2003) and in the modulation of abscisic acid biosynthesis and signalling (Kang and Turano, 2003; Kang *et al*., 2004). The phenotype of transgenic plants overexpressing *AtGLR3.2* (formerly *AtGluR2*) suggested a role in Ca$^{2+}$ allocation within the plant (Kim *et al*., 2001). Microarray analysis of *Arabidopsis* lines overexpressing a radish GLR gene (RsGluR) revealed the up-regulation of jasmonic acid-responsive genes and jasmonic acid-biosynthetic genes (Kang *et al*., 2006). The transgenic lines also had enhanced resistance to a fungal pathogen, suggesting a possible connection between GLR function and plant defence responses.

The only GLR gene so far to emerge from a forward genetics screen was recently identified in rice by Li *et al*., (2006). In a collection of T-DNA insertion lines they found a short root mutant that was subsequently shown to have an insertion in the *OsGLR3.1* gene. The *OsGLR3.1* knockout was associated with disruption of root cap development, a reduction in the size of the quiescent centre, a decline in radial expansion, cessation of root meristematic activity, and, ultimately, cell death in the root apex (Li *et al*., 2006). The authors suggested that *OsGLR3.1* may maintain and co-ordinate the normal function of the root apical meristem, drawing parallels with the way in which iGluRs have been implicated in regulating the balance between cell proliferation and cell death in early CNS development (Hardingham and Bading, 2003).

### Are there other candidates for the role of glutamate sensor in plants?

Based on current data, and in the absence of homologues of the mGluR family in plants, members of the GLR family must currently be considered the strongest candidates for the role of glutamate receptors. However, amino acid-sensing mechanisms occur in many forms (Conigreve *et al*., 2000; Hyde *et al*., 2003; Kimball and Jefferson, 2005), and one should keep an open mind to the possibility that there are glutamate-sensing systems of other kinds. In particular, amino acid sensing is a property associated with some amino acid transport proteins, which in many cases are well placed to act as receptors by virtue of their location in the plasma membrane. In yeast, for example, the Ssy1p protein is a member of the AAP (amino acid permease family) with a role in sensing extracellular amino acid availability (Iraqui *et al*., 1999). Ssy1p has been reported to have the interesting property of enabling the yeast cell to respond to changes in the relative concentrations of intra- and extracellular amino acids (Wu *et al*., 2006).

Members of the excitatory amino acid transporter (EAAT) family in animals function as glutamate-gated anion channels, an activity that is distinct from their ability to transport glutamate (Fairman *et al*., 1995). The interaction of glutamate with the EAAT1 transporter appears to be involved in regulating the morphology of glial cells, as well as in up-regulating glutamate transport itself (Hyde *et al*., 2003). Although homologues of the EAAT1 transporters appear to be absent in plants, *Arabidopsis* has at least 50 amino acid transporters of various types, most of which are largely uncharacterized (Liu and Bush, 2006).

### What is glutamate signalling doing in plants?

**An evolutionary perspective**

The existence of a large family of plant GLR genes, together with the presence of glutamate-activated channel activity in roots and shoots, strongly suggests that glutamate signalling in plants is a reality. When glutamate signalling was thought of as something strictly associated with neurons and nervous systems, the notion of its existence in plants seemed paradoxical (Lam *et al*.,
1998). However, in the past decade, it has emerged that glutamate signalling in animals is not restricted to the nervous system, and indeed that it also occurs in primitive metazoans that lack a nervous system.

Examples where functional glutamate receptors (mGluRs and iGluRs) have been detected in non-neuronal mammalian tissues include bone cells, the pineal gland, and the pancreas (Gill and Pulido, 2001; Hinoi et al., 2004; Moriyama and Yamamoto, 2004). The same tissues also express glutamate/aspartate transporters (GLASTs), which are required in synapses for signal termination, and vesicular glutamate transporters (VGLUTs), which are responsible for the active transport and storage of L-glutamate in synaptic vesicles. These findings have led to the conclusion that glutamatergic signalling is a general and ubiquitous system for intercellular communication in animals (Hinoi et al., 2004; Moriyama and Yamamoto, 2004). Amongst the diverse functions ascribed to glutamate signalling in non-neuronal cells are included the regulation of insulin secretion (Storto et al., 2006), the control of cellular differentiation in osteoblasts (Hinoi et al., 2003), and the modulation of tumour cell proliferation (Kalariti et al., 2005).

That glutamate signalling has early evolutionary origins, before the divergence of plants and animals, was an idea first proposed by Chiu et al. (1999). Support for this idea has come from studies on the marine sponge *Geodia cydonium* and the cellular slime mould *Dictyostelium*. Isolated *Geodia* cells were shown to be responsive to glutamate and to agonists and antagonists of mGluRs, and a gene related to the mGluR/GABAR-type receptors was identified (Perovic et al., 1999). Marine sponges are regarded as belonging to the most primitive metazoan phylum (Muller, 2001), indicating that sophisticated glutamate signalling systems existed in the earliest metazoans. The slime mould *Dictyostelium* is an even more primitive unicellular eukaryote that appears to have split from the animal–fungal lineage after the divergence of plants and animals (Eichinger et al., 2005). The formation of spores on the fruiting body of this social amoeba is induced by a GABA signal, and glutamate acts as a competitive inhibitor of this process (Anjard and Loomis, 2006). *Dictyostelium* possesses a mGluR-like gene (*DdmGluPR* or *GrlE*) (Taniura et al., 2006), and disrupting this gene abolishes the responses to GABA and glutamate, suggesting that both amino acids can bind to the DdmGluPR receptor (Anjard and Loomis, 2006).

Homologues of iGluRs/GLRs even exist in cyanobacteria (GluR0) where they function as glutamate-gated K+ channels (Chen et al., 1999). Although these receptors lack the extended C-terminal and N-terminal domains that characterize the eukaryotic members of the family (Fig. 2), it is likely that iGluRs suitable for intercellular communication evolved from an ancestral GluR0-type channel (Oswald, 2004).

In summary, it appears that both iGluR/GLR-type and mGluR/GABAR-type receptors were already present in the progenitors of the Viridiplantae and the Metazoa. Since multicellularity is thought to have arisen independently in the evolution of the major eukaryotic kingdoms (Viridiplantae, Fungi, and Metazoa) (Schopf, 1993), it seems that glutamate/GABA signalling mechanisms may have been a feature of the most primitive unicellular life forms. It is possible that the earliest function of glutamate/GABA signalling in single-celled organisms was in triggering chemotactic responses. Chemotaxis is recognized as an important adaptive mechanism which enhances an organism’s ability to exploit nutrient patches (Fenchel, 2002), and glutamate has been identified as a chemotactic signal in a number of modern day unicellular organisms, both prokaryotic (Brown and Berg, 1974; Barbour et al., 1991) and eukaryotic (Lee et al., 1999; Van Houten et al., 2000). Glutamate also acts as a cue that initiates a range of feeding-related responses in a variety of marine metazoans (Bellis et al., 1991; Trott et al., 1997; Daniel et al., 2001; Kidawa, 2005). In some instances there is preliminary evidence suggesting that chemodetection of environmental glutamate may involve iGluR-type receptors (Bellis et al., 1991; Murphy and Hadfield, 1997; Van Houten et al., 2000). In this context, it is intriguing that glutamate, acting through iGluR-type receptors, has a role as a guidance cue for neuronal migration during embryogenesis (Behar et al., 1999; Manent et al., 2005; Matsugami et al., 2006; McGowan, 2006), perhaps echoing an evolutionarily ancient role as a chemotactrant.

If it is the case that glutamate signalling evolved in a primitive progenitor of plants and animals, clues to the function of glutamate signalling pathways in plants may come from a greater understanding of non-neuronal glutamate signalling in animals, and vice versa.

**Glutamate signalling and root apical meristem activity**

An unusual and striking effect of external glutamate on *Arabidopsis* root growth and branching has recently been reported (Walch-Liu et al., 2006b). When *Arabidopsis* roots were exposed to low concentrations of L-glutamate there was a marked inhibition of primary root growth and an increase in root branching near the root apex. This effect on root architecture resulted from inhibition of meristematic activity at the primary root tip and an initial stimulation of lateral root outgrowth in the apical region of the primary root. Lateral roots were also glutamate sensitive, but only after they had reached 5–10 mm in length, indicating a developmentally delayed response. A similar response to glutamate was found in roots of a number of other species, including *Thlaspi caerulescens*, *Thellungiella halophila*, wild poppy, and tomato (Walch-Liu et al., 2006a, b). Intriguingly, different *Arabidopsis*
ecotypes displayed widely differing sensitivities to glutamate, the most sensitive being C24 and the least sensitive RLD1.

Both positive and negative effects of amino acids on plant growth have been reported by other authors (Skinner and Street, 1953; Rognes et al., 1986; Katonoguchi et al., 1994; Barazani and Friedman, 2000), and a rapid inhibition of primary root growth by glutamate was reported by Sivaguru et al. (2003). However, the effects observed by Walch-Liu et al. (2006b) are distinctive in a number of respects. Most notably, the effects were detected at much lower concentrations than the millimolar concentrations commonly used in previous studies and were strongly genotype dependent. Furthermore, the effects were highly specific to L-glutamate, not being mimicked by related amino acids such as aspartate, glutamine, and GABA, or even by the D-stereoisomer of glutamate. The authors were able to rule out the possibility that the L-glutamate treatment was causing any kind of general nutritional or metabolic disturbance to the plant. Indeed, glutamate was only inhibitory to primary root growth if it was applied directly to the primary root tip.

The specificity of the glutamate effect, its localized nature, and its occurrence at low concentrations (>20 μM) led to the suggestion that the root tip is probably responding to changes in the apoplastic L-glutamate concentration, and that some kind of signalling mechanism is involved (Walch-Liu et al., 2006b). Attempts to use agonists and antagonists of mammalian iGluRs to investigate the possible involvement of AtGLR-encoded glutamate receptors proved unfruitful: the antagonists tested [DNQX, MK-801, and 2-amino-5-phosphonopentanoate (AP-5)] neither suppressed the L-glutamate effect nor had any direct effect on root growth (Walch-Liu and Forde, 2007). Furthermore, although the agonist BMAA was inhibitory to root growth, the root phenotype it produced was very different from that seen with L-glutamate (Walch-Liu and Forde, 2007).

Glutamate signalling in plants: autocrine/paracrine, endocrine, or environmental?

What might be the functional relevance of an exogenous glutamate-sensing mechanism? It has been noted that the range of concentrations reported for apoplastic glutamate in a variety of tissues and species is 0.3–1.3 mM, which matches fairly closely the range of glutamate concentrations in which glutamate-elicited currents are half activated (0.2–0.5 mM) (Demidchik et al., 2004). Although very little is known about glutamate fluxes and the dynamics of apoplastic glutamate in plant tissues, it seems possible that glutamate could participate in intercellular signalling, and even in long-distance signalling within the plant.

There is a striking precedent for an amino acid-like signalling molecule in plants, in the form of auxin. The major form of auxin in plant tissues, indole-3-acetic acid (IAA), is an analogue of tryptophan and its role as a plant hormone is implicated in almost every aspect of plant biology. Like glutamate, auxin added exogenously to plant cells elicits rapid membrane depolarization and a transient increase in \([\text{Ca}^{2+}]_{\text{cyt}}\) (Zimmermann et al., 1999). It is clear that exogenous auxin is able to modify root growth at very much lower concentrations than glutamate (10^{-11} M versus 10^{-5} M) (Evans et al., 1994), an observation that might be taken to indicate that their signalling properties are very different. However, this disparity may simply reflect the fact that glutamate, unlike auxin, has to fulfil the dual role of being both a signal molecule and a key primary metabolite whose endogenous concentration must be maintained at levels appropriate for catalytic activity (see above).

For glutamate to act as an autocrine or paracrine signal in plants, it would be necessary to have efficient mechanisms for its efflux and re-absorption, as there are in the synapse (Moriyama and Yamamoto, 2004). Auxin efflux in plant tissues appears to be mediated by a vesicular cycling mechanism (Blakeslee et al., 2005), and parallels have been drawn between this process and the mechanism by which glutamate is secreted during synaptic transmission in mammals (Baluška et al., 2005). It is therefore possible that glutamate effluxes into the apoplast in a similar way. Alternatively, glutamate could be released from plant cells directly via anion channels such as those that facilitate organic acid efflux in roots (Roberts, 2006).

Effective mechanisms for glutamate re-absorption also exist. Six members of the Arabidopsis AAP family (AAP1–AAP6) have been heterologously expressed and shown to catalyse the low affinity influx of a broad range of amino acids, including glutamate (\(K_a=0.36–5.0\) mM) (Fischer et al., 2002), while the related LHT1 gene appears to encode a high-affinity glutamate influx system (\(K_a=14\) mM) (Hirner et al., 2006).

There is preliminary evidence that an autocrine/paracrine signalling pathway involving glutamate might be operating in Al^{3+}-treated Arabidopsis root tips (Sivaguru et al., 2003). Rapid responses to Al^{3+}, including membrane depolarization and microtubule depolymerization, could be mimicked by glutamate treatment, but were evoked more rapidly by glutamate. The authors proposed that Al^{3+} signalling in the root tip may be initiated by efflux of glutamate (or a glutamate-like ligand) via an Al^{3+}-gated anion channel, with subsequent binding of this ligand to a glutamate receptor (Fig. 3). This may be an example of the stress response mechanism originally postulated by Dennison and Spalding (2000), in which endogenous or environmental factors trigger glutamate efflux into the apoplast to activate glutamate-gated channels, causing a transient change in \([\text{Ca}^{2+}]_{\text{cyt}}\) and a cascade of downstream responses (Dennison and Spalding, 2000).
As an amino acid commonly found in the phloem sap, glutamate could potentially also perform a long-distance signalling role. It was the observation that amino acids cycle between the shoot and root that first prompted the hypothesis that these amino acids could enable the shoot to communicate its changing N/C status to the root and thereby regulate the nitrate uptake system appropriately (Cooper and Clarkson, 1989). Although evidence supporting this hypothesis is still largely lacking (Forde, 2002), it is nevertheless an attractive concept that deserves further investigation. One possible way in which a long-distance glutamate signal might operate in the context of root growth is depicted in Fig. 3. In this model, shoot-derived glutamate arriving at the root tip [via a partially apoplastic route similar to the one taken by shoot-derived auxin (Marchant et al., 2002)] is sensed by plasma membrane glutamate receptors, potentially enabling meristematic activity in the root tip to respond to changes in the N/C status of the shoot.

A further possibility, and one that does not exclude the potential for glutamate to act as an endogenous signal, is that roots have evolved the capacity to respond to variations in glutamate concentration in the external environment (Walch-Liu et al., 2006a, b) (Fig. 3). Glutamate is one of the most abundant amino acids in the soil, being a component of the pool of dissolved organic nitrogen (DON), as well as a constituent of root exudates (Paynel et al., 2001). In the bulk soil solution, glutamate concentrations may be <10 μM (Paynel et al., 2005), but concentrations are expected to be much higher locally, particularly in the vicinity of decomposing animal or vegetable matter (Walch-Liu et al., 2006a). As the root system develops and the growing root tips continuously explore the soil volume, they can therefore expect to encounter significant variations in the external glutamate concentration. In agar plate experiments, locally high glutamate concentrations of ≈50 μM were shown to slow primary root growth and encourage root branching behind the primary root tip (Walch-Liu et al., 2006b). It was pointed out that this response, if replicated in the field, would lead to an increase in the density of the root system within glutamate-rich patches of soil. Although differing from the commonly observed root foraging response to localized supplies of inorganic nutrients (NO₃, Pi, etc.), which is based on the direct stimulation of lateral root growth and branching (Robinson, 1994), this response to glutamate can similarly be interpreted as a means to enhance the precision of root placement within the soil. The outcome in both cases is to focus root growth in the region of the soil where resources are most abundant and therefore to enhance the plant’s ability to compete (with its neighbours and microorganisms) for those resources.

There is an increasing appreciation of the potential importance of organic N as a source of N for plants (Jones et al., 2005; Weigelt et al., 2005), and it has been argued that plants will be most effective at competing with microorganisms in organic N-rich patches where amino acid concentrations are highest (Jones et al., 2005). It will be interesting to establish whether the variation in glutamate sensitivity observed between different Arabidopsis ecotypes has an adaptive significance, related, for example, to the importance of organic N as an N source in their native soils.

**Concluding remarks**

Glutamate plays a central role in plant nitrogen metabolism. It may be synthesized and metabolized by a number of different pathways (Fig. 1). There is evidence that under most circumstances the plant is able to maintain the soluble concentration within fairly narrow limits. The mechanism by which this occurs involves a number of enzymes present within different cellular compartments, in particular GS, glutamate synthase, and GDH. It is likely that, ultimately, the supply of 2-oxoglutarate catalysed by isocitrate dehydrogenase will play a key role in regulating the accumulation of glutamate.

The mounting evidence in favour of a signalling role for glutamate has been reviewed here. However, until glutamate can conclusively be shown to be a ligand of a specific GLR-type receptor, or until a receptor for glutamate of a different type is identified, the case must be considered unproven. As has been discussed elsewhere (Lam et al., 2006), glutamate signalling may be part of a much broader network of N signalling pathways that enable the plant to monitor and adapt to changes in its N status and the N supply.

In terms of the effect of external L-glutamate on root architecture, several key questions need to be addressed. First, what is the molecular basis for L-glutamate sensing and signal transduction at the root tip? Secondly, what accounts for the remarkable natural variation in L-glutamate sensitivity between Arabidopsis ecotypes? Thirdly,
does the L-glutamate-elicited change in root architecture constitute an adaptive response, perhaps by enhancing the plant’s ability to compete for localized patches of organic N? The first two questions are currently being addressed using forward and reverse genetics and quantitative trait loci analysis (P. Walch-Liu, T. Remans, and BG Forde unpublished data). Furthermore, mutants and near-isogenic lines differing in their glutamate sensitivity that are emerging from these studies will make it possible to test experimentally the adaptive significance of the root architectural response to external L-glutamate.

Acknowledgements

Work in BGF’s laboratory has been supported by grants from the Biotechnology and Biological Sciences Research Council (BBSRC) and by European Commission Research Training Network grant no. HPRN-CT-2002-00247 (PLUSN).

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