

How do plants sense their nitrogen status?

Lucas Gent and Brian G. Forde*

Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK

*Corresponding author: b.g.forde@lancaster.ac.uk

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1 **Abstract**

2 The primary processes that contribute to the efficient capture of soil nitrate are the development of a
3 root system that effectively explores the soil and the expression of high-affinity nitrate uptake systems
4 in those roots. Both these processes are highly regulated to take into account the availability and
5 distribution of external nitrate pools and the endogenous N status of the plant. Whilst significant
6 progress has been made in elucidating the early steps in sensing and responding to external nitrate,
7 there is much less clarity about how the plant monitors its N status. This review specifically addresses
8 the questions of what N compounds are sensed and in which part of the plant, as well as the identity of
9 the signalling pathways responsible for their detection. Candidates that are considered for the role of N
10 sensory systems include the Target of Rapamycin (TOR) signalling pathway, the General Control Non-
11 derepressible 2 (GCN2) pathway, the plastidic PII-dependent pathway and the family of Glutamate-Like
12 Receptors (GLRs). However, despite significant recent progress in elucidating the function and mode
13 of action of these signalling systems, there is still much uncertainty about the extent to which they
14 contribute to the process by which plants monitor their N status. The possibility is discussed that the
15 large GLR family of Ca²⁺ channels, which are gated by a wide range of different amino acids and
16 expressed throughout the plant, could act as amino acid sensors upstream of a Ca²⁺-regulated
17 signalling pathway such as the TOR pathway to regulate the plant's response to changes in N status.

18 **Key words:** amino acids; GCN2; glutamate receptors; nitrate uptake; PII; root development; signal
19 transduction; target of rifampicin.

20 **Abbreviations:** 2-OG, 2-oxoglutarate; AspRS, aspartyl-tRNA synthetase; BABA, β-aminobutyric acid;
21 eIF2α eukaryotic translation initiation factor alpha; GCN2, general amino acid control non-derepressible
22 2; GLR, glutamate-like receptor; GS, glutamine synthetase; hVps34, human vacuolar protein sorting
23 34; iGluR, ionotropic glutamate receptor; NAGK, *N*-acetyl-L-glutamate kinase; NR, nitrate reductase;
24 PP2A, protein phosphatase 2A; TAP46, 2A phosphatase-associated protein of 46 kDa; TORC, target
25 of rapamycin complex.

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29 Introduction

30 It has long been appreciated that plants are able to regulate their nutrient uptake to match their demand
31 for those nutrients. As far back as 1906, J. F. Breazeale demonstrated that wheat plants that were
32 nitrogen starved for the first 15 d after germination subsequently showed much higher capacities for
33 absorbing nitrate than plants that had received sufficient N (Breazeale, 1906). Developmental
34 processes in both roots and shoots are also known to be modified by signals related to the N status of
35 the plant (Forde and Lorenzo, 2001). As there is no evidence that plants have evolved separate N
36 sensing mechanisms for controlling N uptake and plant development, this review will consider the most
37 recent evidence relating to the control of both physiological and developmental processes, focusing
38 specifically on how plants sense their internal N status. To learn more about the downstream
39 components of N signalling pathways in plants the reader is referred to a number of excellent reviews
40 that have taken a broader view of the topic (Krapp, 2015; Li *et al.*, 2014; Liu *et al.*, 2015; Medici and
41 Krouk, 2014; O'Brien *et al.*, 2016; Ruffel *et al.*, 2014; Sirohi *et al.*, 2016; Vidal *et al.*, 2015).

42 What is sensed and where is it sensed?

43 An ability to respond to changes in the internal N status implies the existence of mechanisms that can
44 monitor the abundance of one or more key effector molecules that provide reliable information on the
45 availability of N in specific plant tissues. In molecular terms we would expect to find molecules (usually
46 proteins), that act as sensors by binding to specific N-containing compounds and transmitting that
47 information to downstream components of a signal transduction pathway. We will begin by considering
48 what the key effector molecules might be and in which parts of the plant their abundance is likely to be
49 monitored.

50 Glutamine, as the product of the first step in the pathway of N assimilation in bacteria and fungi
51 as well as in plants (Lea *et al.*, 1990), is the organic form of N that has been most commonly considered
52 to be a candidate for the key effector in the sensing of the intracellular N status in many organisms. In
53 *Aspergillus nidulans* and other filamentous fungi, when glutamine levels are high, pathways responsible
54 for assimilating energetically unfavourable N sources (like nitrate) are down-regulated through a
55 process called *nitrogen metabolite repression* (Crawford and Arst, 1993). However, although much is
56 known about the complex genetic control of nitrogen metabolite repression, the precise mechanism by
57 which glutamine is sensed has not been determined (Tudzynski, 2014), which leaves open the
58 possibility that it is not glutamine itself that is the effector. In mammals and the budding yeast

59 *Saccharomyces cerevisiae*, the evolutionarily conserved Target Of Rapamycin Complexes (TORCs)
60 are involved in sensing amino acid-derived signals to stimulate a variety of metabolic processes.
61 However, glutamine sensing appears to be only part of the story (Fumarola *et al.*, 2005; Nakajo *et al.*,
62 2005), with other amino acids such as leucine and arginine also being implicated in some of the multiple
63 pathways of TORC activation (Kingsbury *et al.*, 2015; Shimobayashi and Hall, 2016). For example,
64 SLC38A9, a solute carrier family protein with a proposed role in transporting glutamine and arginine
65 into the mammalian lysosome, has recently been identified as an arginine sensor upstream of
66 mammalian TORC1 (mTORC1) (Shimobayashi and Hall, 2016) and two distinct leucine sensors
67 operating in this pathway leucyl-tRNA synthetase (Han *et al.*, 2012) and Sestrin2 (Wolfson *et al.*, 2016)
68 have also been uncovered. Another important mechanism for monitoring the internal N status in yeast
69 and mammals is the General amino acid Control Non-derepressible 2 (GCN2) pathway, which does not
70 sense amino acids *per se* but rather the uncharged tRNAs that accumulate during amino acid
71 deprivation (Chantranupong *et al.*, 2015).

72 In bacteria, it is the glutamine:2-oxoglutarate (2-OG) ratio that is the key indicator of N status,
73 regulating as it does glutamine synthetase activity through a PII-mediated mechanism (discussed
74 below). In *E coli*, the PII proteins are encoded by the related GlnB and GlnK genes and their main
75 function is to regulate glutamine synthetase (GS) activity (Arcondeguy *et al.*, 2001). The PII-modifying
76 enzyme GlnD, which uridylylates and deuridylylates PII proteins, is inhibited by binding to glutamine
77 and has the role of glutamine sensor, while GlnB itself binds to 2-OG and acts as a 2-OG sensor.

78 In plants, the question of the signalling role of glutamine in feedback regulation of nitrate uptake
79 has often been asked, but without producing a definitive answer. It has long been thought likely that
80 phloem-mediated shoot-to-root signalling is responsible for regulating NO₃⁻ uptake activity to match the
81 plant's demand for N (Forde and Clarkson, 1999; Imsande and Touraine, 1994). Amino acids, as
82 significant components of phloem sap, have generally been considered strong candidates for the
83 relevant phloem transmissible compounds (Cooper and Clarkson, 1989). However, attempts to identify
84 glutamine or other individual amino acids as key effectors in this pathway have proved inconclusive.
85 For example, when individual amino acids were indirectly loaded into the phloem through the cut surface
86 of soybean cotyledons, 8 of the 14 amino acids tested were effective in down-regulating nitrate uptake
87 in the roots, but glutamine was significantly less potent than arginine or alanine (Muller and Touraine,
88 1992). Despite these and other reports indicating a negative correlation between the amino acid content
89 of the phloem and the rate of nitrate uptake, there is also evidence from experiments with split-root

90 systems that feedback regulation of nitrate uptake can occur independently of any change in the amino
91 acid content of the phloem (Tillard *et al.*, 1998). Furthermore, it has been found that the local amino
92 acid content of the root itself is not correlated with nitrate uptake rates (Lainé *et al.*, 1995) and that
93 feedback regulation of the expression of the *NRT2.1* nitrate transporter in roots is dependent on the
94 global N status of the plant and not the local supply of N to the root (Gansel *et al.*, 2001). These pieces
95 of evidence support the idea of long distance shoot-to-root signals that communicate the plant's N status
96 to the root nitrate uptake system and but argue against those signals being amino acids. A detailed
97 discussion of the identity of alternative long-distance signals is beyond the scope this review, but
98 includes a variety of phloem-mobile molecules that have been implicated in N signalling, including auxin
99 (Forde, 2002), microRNAs (Zeng *et al.*, 2014) and small peptides (Araya *et al.*, 2014). Very relevantly
100 in this context, a recent report identified Arabidopsis ELONGATED HYPOCOTYL5 (HY5), a bZIP
101 transcription factor, as a shoot-to-root phloem-mobile signal that stimulates both root growth and nitrate
102 uptake in response to illumination of the shoot (Chen *et al.*, 2016). This is particularly significant for the
103 integration of N and C metabolism because, as well as acting as a hub for multiple hormonal and abiotic
104 signalling networks, HY5 is also known to regulate C fixation in the shoot (Lau and Deng, 2010).

105 In terms of the identity of the key metabolite(s) directly relevant to N sensing, candidates other
106 than glutamine have been identified. A detailed transcriptomics and metabolomics analysis in
107 Arabidopsis found evidence that leucine abundance was strongly correlated to the expression of a set
108 of several hundred genes (Hannah *et al.*, 2010), leading to the suggestion that in plants, as in yeast
109 and mammals, leucine could be a key regulator of gene expression. There is also evidence that tissue
110 nitrate concentrations can also contribute to the plant's perception of its N status. Using tobacco lines
111 with varying levels of nitrate reductase (NR) activity it was found that accumulation of high
112 concentrations of nitrate in the shoot led to stimulation of organic acid metabolism, repression of starch
113 synthesis and inhibition of root growth (Scheible *et al.*, 1997a; Scheible *et al.*, 1997b; Stitt and Feil,
114 1999). These are responses associated with high N status, yet were seen even though the NR-defective
115 plants were severely deficient in organic N. Similarly, early lateral root development in an NR-deficient
116 mutant was found to be more sensitive than the wild-type to the inhibitory effects of high nitrate
117 concentrations (Zhang *et al.*, 1999), rather than less sensitive as would have been expected if
118 assimilation of nitrate into organic forms of N was required for feedback repression. Thus we must
119 consider the possibility that nitrate sensors present in the shoot have an important role to play in global
120 N-status sensing.

121 It is of course over-simplistic to consider N status sensing in isolation since the plant must
122 integrate signals from a wide range of other metabolites, most notably those related to carbon
123 metabolism (Nunes-Nesi *et al.*, 2010; Zheng, 2009). The PII regulatory pathway in bacteria, which
124 senses the glutamine:2-oxoglutarate ratio (Arcondeguy *et al.*, 2001), is a good example of how sensing
125 of the C/N balance can be achieved. However, the mechanisms by which nitrogen and carbon signalling
126 are integrated in plants are poorly understood and likely to be much more complex than in bacteria, so
127 will not be dealt with in detail here. We have already noted above the important identification of HY5 as
128 a novel regulatory molecule that has the ability to integrate C and N metabolism both within and between
129 distant plant organs (Chen *et al.*, 2016). In the following sections, we review the most recent advances
130 in our understanding of the multiple mechanisms by which N-containing compounds are sensed in
131 plants, as candidates for the role of sensing global N status.

132

133 **Glutamine sensing in plastids by PII proteins**

134 PII proteins belong to one of the most evolutionarily conserved families of signalling proteins, being
135 widely distributed in bacteria and plants as well as in many species of the archaeal kingdom
136 (Forchhammer and Luddecke, 2016). However, despite their conservation at the amino acid sequence
137 level, the signalling roles of PII proteins are diverse. In plants, the plastid-localized PII protein (encoded
138 by the nuclear-localized *GlnB* gene) has been shown to interact with *N*-acetyl-L-glutamate kinase
139 (NAGK) (Burillo *et al.*, 2004; Sugiyama *et al.*, 2004), which catalyses an important rate-limiting step in
140 arginine biosynthesis, and with acetyl-CoA carboxylase (Bourrellier *et al.*, 2010), a key enzyme in fatty
141 acid biosynthesis. More recently it has been discovered that glutamine binds to a plant-specific C-
142 terminal extension of the PII protein and it is only after binding that the PII protein is able to form a
143 complex with and activate NAGK (Chellamuthu *et al.*, 2014). By this means plants have evolved a
144 simplified glutamine-sensing mechanism for the PII regulatory pathway, circumventing the need for
145 covalent modification of PII by a glutamine-sensitive uridyl transferase as occurs in *E. coli*. It has been
146 proposed that the low-affinity binding site for glutamine on PII allows for activation of NAGK only at high
147 plastidic glutamine concentrations, enabling increased biosynthesis of arginine (which can be used for
148 N storage) under conditions of N over-supply (Chellamuthu *et al.*, 2014). Although the glutamine-binding
149 motif in PII is highly conserved in plants, it is surprisingly missing in Arabidopsis and other members of
150 the *Brassicaceae* family (Chellamuthu *et al.*, 2014), yet two PII knock-out mutants of Arabidopsis
151 showed strongly reduced accumulation of arginine when supplied with ammonium after N starvation

152 (Chellamuthu *et al.*, 2014; Ferrario-Mery *et al.*, 2006). Therefore, it appears that Arabidopsis must use
153 an alternative mechanism for PII-dependent regulation of arginine metabolism.

154 Despite the significance of PII signalling in plastids, and evidence that over-expressing PII in a
155 leguminous plant leads to a decline in rates of N-fixation in nodules (D'Apuzzo *et al.*, 2015), its primary
156 function would appear to be limited to the regulation of the arginine biosynthesis and fatty acid
157 metabolism and therefore a more global role in sensing the shoot's N status does not currently appear
158 likely.

159

160 **TOR Signalling**

161 Arabidopsis has a single TOR kinase gene (*AtTOR*) (Menand *et al.*, 2002) and also carries homologues
162 of many, but not all, of the genes encoding components of the TORC1 complex in mammals (Xiong and
163 Sheen, 2014). TOR kinase is known to have diverse roles in regulating plant growth and development
164 and to act as an integrator of multiple signalling networks to coordinate growth and developmental
165 transitions (Dobrenel *et al.*, 2016; Xiong and Sheen, 2014). The TOR complex is therefore ideally placed
166 to play a key role in N status sensing in plants, as it does in yeast and mammals (discussed above).
167 There are several lines of evidence indicating that the TOR complex in Arabidopsis is involved in
168 regulating N metabolism as well as in coordinating N and C metabolism. A number of studies that have
169 investigated the metabolic effects of down-regulating TOR itself, or of down-regulating genes encoding
170 TOR-associated proteins, have observed major shifts in metabolism that include an accumulation of
171 amino acids and additional effects on the abundance of starch and sugars (Caldana *et al.*, 2013;
172 Deprost *et al.*, 2007; Moreau *et al.*, 2012). Because TOR is a positive regulator of protein synthesis and
173 a negative regulator of protein turnover, this accumulation of amino acids in TOR-deficient plants could
174 be accounted for by a combination of a decline in the rate of protein synthesis and an up-regulation of
175 the rate of protein degradation (Caldana *et al.*, 2013). However, other factors may additionally be at
176 play because, in Arabidopsis lines that are defective in expression of either the *TOR* or *TAP46* (2A
177 *PHOSPHATASE ASSOCIATED PROTEIN OF 46 kDa*) genes, effects are seen at the mRNA level that
178 could contribute to the changes in N metabolism, namely down-regulation of nitrate assimilatory genes
179 and an induction of genes involved in amino acid recycling (Ahn *et al.*, 2011). TAP46 is a protein
180 phosphatase 2A (PP2A)-associated protein that regulates PP2A activity in Arabidopsis and that has

181 been identified as a downstream effector of the TOR complex (Ahn *et al.*, 2011). This implies that TOR,
182 acting at the transcriptional level through TAP46 and PP2A, may be important for regulating the balance
183 between primary N assimilation and N recycling to accommodate changes in the plant's N status.
184 However, while there is evidence that TOR activity in plants is regulated by intracellular sugar availability
185 (Dobrenel *et al.*, 2016), there is currently no corresponding information in plants on the mechanism of
186 N sensing upstream of TOR, or on which N metabolites are sensed.

187

188 **The GCN2 protein kinase**

189 The GCN2 protein kinase is a key component of the general amino acid control mechanism in yeast,
190 which is responsible for suppressing global protein synthesis under conditions of N deficiency to help
191 maintain amino acid homeostasis (Schneper *et al.*, 2004). During N starvation, the hyperaccumulation
192 of uncharged tRNAs directly stimulates the kinase activity of GCN2 which then phosphorylates its target,
193 the eukaryotic translation initiation factor eIF2 α . Phosphorylation of eIF2 α stimulates the translation of
194 a specific set of mRNAs (e.g. the mRNA for the GCN4 transcription factor in yeast) whilst reducing the
195 efficiency of translation initiation for most mRNAs, leading to a general decline in protein synthesis.
196 Plants possess genes for GCN2 and two types of eIF2 α kinases, although no plant GCN4 homologue
197 has been identified. In yeast, activation of the kinase activity of GCN2 involves binding of the uncharged
198 tRNAs to a histidyl-tRNA synthetase-related domain located at its C-terminus. Despite the plant
199 homologue of GCN2 having a truncated version of the C-terminal domain, it has recently been
200 demonstrated using *in vitro* assays that it too is activated by binding to uncharged tRNAs and is then
201 able to phosphorylate both of the eIF2 α homologues present in Arabidopsis (Li *et al.*, 2013b).

202 A recent insight into the role and mechanism of amino acid sensing in plant defence has come
203 from studies into the mechanism of β -aminobutyric acid (BABA)-induced priming of the plant immune
204 response. A screen for Arabidopsis mutants defective in BABA-induced priming revealed that plant
205 perception of BABA is mediated by an aspartyl-tRNA synthetase (AspRS) encoded by the *Impaired in*
206 *BABA-induced Immunity 1 (IBI1)* gene (Luna *et al.*, 2014). As well as priming the defence response in
207 leaves, BABA also inhibits plant growth and the same study found evidence that this response to BABA
208 (but not the priming response) operates through a GCN2-dependent pathway. This has led to a model
209 in which amino acid uptake by a parasitizing plant pathogen leads to a decline in cellular aspartate

210 levels, reducing the canonical AspRS activity of IBI1 and thereby activating a secondary IBI1 activity to
211 prime the defence response. In this model, R-BABA (the active enantiomer of BABA, which has
212 stereochemical similarity to L-Asp) acts by blocking the binding of L-Asp to IBI1 and thereby 'tricks' the
213 protein into sensing low L-Asp levels. Thus, it appears that an enzyme whose primary function is in
214 protein synthesis (AspRS) has been co-opted to serve a role in assisting the plant to mobilise its
215 defences to pathogen attack. It has been proposed that activation of GCN2 by BABA arises from its
216 inhibition of AspRS activity, leading to accumulation of the uncharged tRNAs (Luna *et al.*, 2014).
217 Subsequent GCN2-catalysed phosphorylation of eIF2 α would then trigger downstream responses such
218 as inhibition of plant growth and presumably other eIF2 α -regulated responses to amino acid deficiency.

219

220 **GSI-like genes**

221 In addition to the well-studied cytoplasmic and plastidic glutamine synthetases (GSs) that are so
222 important for N metabolism, plants also carry another form of GS that is more closely related to
223 prokaryotic GS (GSI-type) than to these eukaryotic forms (GSII-type). Most plant GSI-type genes
224 encode a protein with an N-terminal aminohydrolase domain related to the nodulin 6 protein
225 (Dorskocilova *et al.*, 2011) and hence are referred to as NodGS. The first fusion protein of this form to
226 be identified was the fungal FluG protein, and in *Aspergillus* sp. FluG has a regulatory role as a
227 morphogenetic factor that stimulates asexual sporulation under conditions of N starvation (but not C
228 starvation), a role that was shown to reside in the GSI-like domain of the protein (D'Souza *et al.*, 2001).
229 In Arabidopsis, downregulation of NodGS by RNAi led to multiple developmental effects, including a
230 shortened primary root and disruption of the root cap, suggesting a possible regulatory role in root
231 morphogenesis (Dorskocilova *et al.*, 2011). The model legume *Medicago truncatula* has two NodGS
232 genes, *MtGS1a* and *MtGS1b*, which are preferentially expressed in roots and root nodules and whose
233 expression is down-regulated by externally applied amino acids (Silva *et al.*, 2015). Neither FluG nor
234 the plant NodGSs so far analysed possess significant GS enzymatic activity (D'Souza *et al.*, 2001;
235 Dorskocilova *et al.*, 2011; Silva *et al.*, 2015), leading to speculation that their GSI-like domains might
236 perform a different function, perhaps in the production of some form of signalling molecule. The lack of
237 GS activity in FluG/NodGS is associated with the lack of conservation of two key amino acid residues
238 in the active site (Dorskocilova *et al.*, 2011), but the overall sequence conservation of the protein
239 indicates that it could still provide binding sites for NH₄⁺, glutamate and/or glutamine and therefore

240 potentially perform a role as some form of N sensor. The general idea of a sensory role for a GS enzyme
241 is supported by a study in the rice fungal pathogen *Fusarium fujikuroi* (Wagner *et al.*, 2013), which found
242 evidence that the GSII-type enzyme in that species has a regulatory function involving NH₄⁺ sensing
243 that could be separated from its catalytic activity by site-directed amino acid substitutions.

244

245 **The GLR family of glutamate receptor-like proteins**

246 Plants possess a large family of membrane proteins with homology to the ionotropic glutamate receptor
247 (iGluR) family that was first identified and characterised in mammals (Price *et al.*, 2012). Mammalian
248 iGluRs are primarily known for their role as fast excitatory neurotransmitters in the central nervous
249 system where they act as glutamate-gated cation channels, selective for Na⁺, K⁺ and Ca²⁺ ions. In
250 *Arabidopsis* there are 20 *GLR (Glutamate-Like Receptor)* genes encoding proteins with the same
251 modular structure as their mammalian homologues: an N-terminal domain, a ligand-binding domain, a
252 transmembrane domain that includes a pore region, and a C-terminal domain (Davenport, 2002). Since
253 their discovery in 1998 (Lam *et al.*, 1998) there has been considerable interest in what role the GLR
254 proteins might play in plants, where a nervous system is obviously lacking. At the molecular level the
255 accumulated evidence indicates that GLRs act as amino acid-gated Ca²⁺ channels and that members
256 of the family are located in different cell membranes, including the plasma membrane, the inner and
257 outer chloroplast membranes and the mitochondrion (reviewed by Weiland *et al.* 2016). However, while
258 the iGluRs are largely glutamate-specific (with only aspartate acting as an alternative, weak agonist
259 (Flores-Soto *et al.*, 2012), plant GLRs appear to be gated by a broad range of different amino acids.
260 Evidence for this has come from studies using both *GLR* knockout mutants (Michard *et al.*, 2011; Qi *et*
261 *al.*, 2006; Stephens *et al.*, 2008; Tapken *et al.*, 2013) and heterologous expression (Tapken *et al.*, 2013;
262 Vincill *et al.*, 2012). For example, when expressed in *Xenopus* oocytes the *Arabidopsis* AtGLR1.4
263 protein was found to be gated to varying degrees by methionine, tryptophan, phenylalanine, leucine,
264 tyrosine, asparagine and threonine, but not by L-glutamate or other proteinogenic amino acids (Tapken
265 *et al.*, 2013), while the AtGLR3.4 protein expressed in Human Embryonic Kidney 293 cells was sensitive
266 to asparagine, L-serine and glycine, but not to L-glutamate, alanine, cysteine or phenylalanine (Vincill
267 *et al.*, 2012). These findings are consistent with the evidence from various *Arabidopsis* knock-out
268 mutants (Michard *et al.*, 2011; Qi *et al.*, 2006; Stephens *et al.*, 2008; Tapken *et al.*, 2013) that, in contrast
269 to their mammalian homologues, the plant GLRs are relatively promiscuous in their ligand specificity.
270 These experimental data are further supported by homology modelling studies which not only indicate

271 that all members of the AtGLR family could potentially bind amino acids but also that the sequence
272 diversity that exists in their ligand-binding domains would be consistent with them being gated by a
273 diverse set of agonists (Tapken *et al.*, 2013). In addition, there is the possibility that amino acids or
274 other metabolites could allosterically regulate the plant GLRs by binding to sequences in their extended
275 N-terminal domains (Weiland *et al.*, 2016). Some mammalian iGluRs have a similarly long N-terminal
276 domain that allows both positive and negative allosteric regulation of the receptor by a variety of small
277 molecules and ions (Kumar and Mayer, 2013). Should the N-terminal domain in plant GLRs serve a
278 similar function it would greatly amplify the potential of this family of receptors to integrate a multitude
279 of metabolic signals.

280 Of the candidates for a role in sensing N status the GLRs are amongst the most compelling. As
281 a family they are ubiquitously expressed throughout the plant, their products are located on both the
282 plasma membrane and organellar membranes, collectively their activity is gated by a diverse set of
283 amino acids, and their ability to control the movement of Ca²⁺ ions across these membranes potentially
284 provides a direct link to multiple Ca²⁺ signalling pathways (Dodd *et al.*, 2010). Experimental evidence
285 for their physiological role comes primarily from two early papers that described the pleiotropic effects
286 of disrupting the expression of the *AtGLR1.1* gene on aspects of carbon, nitrogen and hormone
287 metabolism (Kang *et al.*, 2004; Kang and Turano, 2003). It was reported that germination of *AtGLR1.1*
288 antisense lines on N-free full-strength Murashige and Skoog medium was inhibited by the presence of
289 3% sucrose (but not by other C sources) and that the inhibitory effect of sucrose could be overcome by
290 the inclusion of 5 mM NO₃⁻ (but not 5 mM NH₄⁺) (Kang and Turano, 2003). Other aspects of the
291 pleiotropic phenotype of the *antiAtGLR1.1* line included effects on the expression of some enzymes of
292 C and N metabolism (Kang and Turano, 2003) and an increased sensitivity of germination and root
293 growth to external abscisic acid (ABA) treatment (Kang *et al.*, 2004). Taken together with additional
294 effects on the expression of some genes related to ABA signalling and biosynthesis (Kang *et al.*, 2004)
295 these observations were interpreted as indicating a role for AtGLR1.1 in linking changes in C/N status
296 to ABA signalling and other metabolic and developmental responses.

297 There are three clades of *GLR* genes in plants and most studies into the physiological role of
298 plant GLRs have focussed on members of clade 3 (principally AtGLR3.3, AtGLR3.4 and AtGLR3.5)
299 (Weiland *et al.*, 2016). Diverse functions that have been assigned to these GLRs on the basis of the
300 phenotype of knock-out mutants or over-expressing lines have so far included roles in stomatal closure,
301 root branching, maintenance of the primary root meristem, gravitropism, pollen tube signalling and the

302 defence response (reviewed Weiland *et al.* 2016). However, there is surprisingly little experimental
303 evidence directly linking any of these phenotypes to activation of a GLR by its known agonist(s). One
304 notable exception is a study of the role of the *AtGLR3.3* gene in the immune response (Li *et al.*, 2013a),
305 where it was found that cysteine and the tripeptide glutathione (both of which were identified as
306 *AtGLR3.3* agonists) were each able to suppress growth of the bacterial pathogen *Pseudomonas*
307 *syringae* pv tomato DC3000 in Arabidopsis leaves and that this response was defective in an *atglr3.3*
308 knock-out mutant. A more recent paper reported that L-glutamate was able to trigger stomatal closure
309 in Arabidopsis leaves and that this was dependent on a functional *AtGLR3.5* gene (Yoshida *et al.*,
310 2016). The difficulty in achieving this degree of definition in our understanding of the physiological role
311 of most of the GLRs is likely to be due to a combination of genetic redundancy in the large gene family
312 and the multiplicity of agonists that can activate them. Nevertheless, based on what we know so far, it
313 seems plausible that many or all the diverse and pleiotropic phenotypes caused by disruption or
314 overexpression of members of the *GLR* family are due, directly or indirectly, to perturbations in amino
315 acid (or small peptide) sensing. If this is the case then it points to the GLR family having important roles
316 throughout the plant in monitoring changes in amino acid distribution (between different cellular and
317 extracellular compartments), amino acid composition and overall amino acid abundance. It remains to
318 be established how important the Ca²⁺ signals generated as a result of this GLR activity are in initiating
319 the effects on gene expression and root development that are symptomatic of changes in the plant's N
320 status.

321

322 **Conclusions**

323 In this review, we have attempted to assess the most recent advances in our understanding of how
324 plants monitor their N status. The main candidates for this N sensing role belong to signalling pathways
325 that have mostly been chosen for investigation on the basis of their homology to nutrient sensing
326 systems previously identified in other organisms (bacteria, yeast or mammals). The exception to this
327 rule is the GLR family of glutamate receptor-like proteins, whose homologues in mammals are primarily
328 (but not exclusively) associated with the nervous system and the process of neurotransmission. Despite
329 the progress that has been made in recent years, particularly in elucidating the TOR-, PII- and GCN2-
330 mediated pathways in higher plants, there is still little clarity about which N-compounds are being
331 monitored to maintain amino acid homeostasis or the identity of the molecular sensors for those

332 compounds. In the absence of hard evidence, some speculation may be permissible. Is it possible that
333 members of the GLR family, whose credentials for the role of ubiquitous amino acid sensors have been
334 outlined above, are the upstream components required by a TOR-mediated amino acid signalling
335 pathway? Although yeast has no GLR/iGluR homologues, there is a precedent for this in mammals
336 where there is evidence that iGluRs belonging to the NMDA group are able to regulate mTOR signalling
337 activity in neurons (Burket *et al.*, 2015; Gong *et al.*, 2006; Huang *et al.*, 2007). Some experimental
338 support for this hypothesis exists in the form of the phenotype of the *AtGLR1.1* antisense line described
339 above, involving changes in the expression of enzymes involved in C and N metabolism and ABA
340 signalling (Kang *et al.*, 2004; Kang and Turano, 2003), which is not dissimilar to phenotypes described
341 for lines defective in components of the TOR signalling pathway (Dobrenel *et al.*, 2016).

342 It is also worth noting that there are examples in both mammals and yeast of interactions
343 between Ca²⁺ signalling and TOR signalling (Deutsch *et al.*, 2014; Mulet *et al.*, 2006) and a study using
344 human cell lines found evidence that amino acids activate the mTOR complex *via* a pathway involving
345 Ca²⁺-dependent activation of the human vacuolar protein sorting 34 (hVps34), a type III
346 phosphatidylinositol 3-kinase (Gulati *et al.*, 2008). To date there are no reports of a role for Ca²⁺
347 signalling in the activation of the TOR complex in plants, however Arabidopsis does have a homologue
348 of hVp34 (AtVPS34) in which the Ca²⁺-dependent lipid-binding domain is conserved between plants
349 and humans (Welters *et al.*, 1994). Thus we consider that the possibility of a link between Ca²⁺ influx
350 through amino acid-gated members of the GLR family and an AtVPS34-dependent TOR signalling
351 pathway in plants is one that is worthy of investigation.

352 Fig. 1 illustrates how amino acid signalling through a panel of GLR complexes with multiple and
353 differing ligand specificities could, *via* a Ca²⁺-dependent signalling pathway, provide a mechanism for
354 regulating the TOR complex in response to changes in the overall amino acid pool. The multiplicity of
355 the amino acids that are able to act as ligands for plant GLRs could help to explain why, as discussed
356 above, previous research has failed to pinpoint any particular amino acid(s) for the role of effector in N
357 status sensing. Overcoming genetic redundancy amongst GLR family members to test the postulated
358 link between GLRs and TOR in amino acid signalling will be a challenge, but as previously discussed
359 in more detail (Forde, 2014), opportunities may arise through the application of chemical genetic
360 approaches if small molecule agonists or antagonists can be identified that target specific clades or
361 sub-groups of the GLRs. The mechanism by which plants monitor their N status is so fundamental to

362 how they regulate the processes of N acquisition, N metabolism and N storage that a better
363 understanding of these is expected to be important for future efforts to improve the efficiency with which
364 crop plants capture and utilize soil nitrate.

365

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References

- Ahn CS, Han JA, Lee HS, Lee S, Pai HS.** 2011. The PP2A regulatory subunit Tap46, a component of the TOR signaling pathway, modulates growth and metabolism in plants. *Plant Cell* **23**, 185-209.
- Araya T, Miyamoto M, Wibowo J, Suzuki A, Kojima S, Tsuchiya YN, Sawa S, Fukuda H, von Wirén N, Takahashi H.** 2014. CLE-CLAVATA1 peptide-receptor signaling module regulates the expansion of plant root systems in a nitrogen-dependent manner. *Proceedings of the National Academy of Sciences USA* **111**, 2029-2034.
- Arcondeguy T, Jack R, Merrick M.** 2001. P-II signal transduction proteins, pivotal players in microbial nitrogen control. *Microbiology and Molecular Biology Reviews* **65**, 80-105.
- Bourrellier ABF, Valot B, Guillot A, Ambard-Bretteville F, Vidal J, Hodges M.** 2010. Chloroplast acetyl-CoA carboxylase activity is 2-oxoglutarate-regulated by interaction of PII with the biotin carboxyl carrier subunit. *Proceedings of the National Academy of Sciences USA* **107**, 502-507.
- Breazeale JF.** 1906. The relation of sodium to potassium in soil and solution cultures. *Journal of the American Chemical Society* **28**, 1013-1025.
- Burillo S, Luque I, Fuentes I, Contreras A.** 2004. Interactions between the nitrogen signal transduction protein PII and N-acetyl glutamate kinase in organisms that perform oxygenic photosynthesis. *Journal of Bacteriology* **186**, 3346-3354.
- Burket JA, Benson AD, Tang AH, Deutsch SI.** 2015. NMDA receptor activation regulates sociability by its effect on mTOR signaling activity. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* **60**, 60-65.
- Caldana C, Li Y, Leisse A, Zhang Y, Bartholomaeus L, Fernie AR, Willmitzer L, Giavalisco P.** 2013. Systemic analysis of inducible target of rapamycin mutants reveal a general metabolic switch controlling growth in *Arabidopsis thaliana*. *Plant Journal* **73**, 897-909.
- Chantranupong L, Wolfson RL, Sabatini DM.** 2015. Nutrient-sensing mechanisms across evolution. *Cell* **161**, 67-83.
- Chellamuthu VR, Ermilova E, Lapina T, Luddecke J, Minaeva E, Herrmann C, Hartmann MD, Forchhammer K.** 2014. A widespread glutamine-sensing mechanism in the plant kingdom. *Cell* **159**, 1188-1199.
- Chen XB, Yao QF, Gao XH, Jiang CF, Harberd NP, Fu XD.** 2016. Shoot-to-root mobile transcription factor HY5 coordinates plant carbon and nitrogen acquisition. *Current Biology* **26**, 640-646.
- Cooper HD, Clarkson DT.** 1989. Cycling of amino nitrogen and other nutrients between shoots and roots in cereals: a possible mechanism integrating shoot and root in the regulation of nutrient uptake. *Journal of Experimental Botany* **40**, 753-762.
- Crawford NM, Arst HN.** 1993. The molecular genetics of nitrate assimilation in fungi and plants. *Annual Review of Genetics* **27**, 115-146.
- D'Apuzzo E, Valkov VT, Parlati A, Omrane S, Barbulova A, Sainz MM, Lentini M, Esposito S, Rogato A, Chiurazzi M.** 2015. PII overexpression in *Lotus japonicus* affects nodule activity in permissive low-nitrogen conditions and increases nodule numbers in high nitrogen treated plants. *Molecular Plant-Microbe Interactions* **28**, 432-442.

- D'Souza CA, Lee BN, Adams TH.** 2001. Characterization of the role of the FluG protein in asexual development of *Aspergillus nidulans*. *Genetics* **158**, 1027-1036.
- Davenport R.** 2002. Glutamate receptors in plants. *Annals of Botany* **90**, 549-557.
- Deprost D, Yao L, Sormani R, Moreau M, Leterreux G, Nicolai M, Bedu M, Robaglia C, Meyer C.** 2007. The Arabidopsis TOR kinase links plant growth, yield, stress resistance and mRNA translation. *Embo Reports* **8**, 864-870.
- Deutsch SI, Tang AH, Burket JA, Benson AD.** 2014. NMDA receptors on the surface of cancer cells: Target for chemotherapy? *Biomedicine & Pharmacotherapy* **68**, 493-496.
- Dietrich P, Anschutz U, Kugler A, Becker D.** 2010. Physiology and biophysics of plant ligand-gated ion channels. *Plant Biology* **12**, 80-93.
- Dobrenel T, Caldana C, Hanson J, Robaglia C, Vincenz M, Veit B, Meyer C.** 2016. TOR signaling and nutrient sensing. *Annual Review of Plant Biology* **67**, 261-285.
- Dodd AN, Kudla J, Sanders D.** 2010. The language of calcium signaling. *Annual Review of Plant Biology* **61**, 593-620.
- Doskocilova A, Plihal O, Volc J, Chumova J, Kourova H, Halada P, Petrovska B, Binarova P.** 2011. A nodulin/glutamine synthetase-like fusion protein is implicated in the regulation of root morphogenesis and in signalling triggered by flagellin. *Planta* **234**, 459-476.
- Ferrario-Mery S, Besin E, Pichon O, Meyer C, Hodges M.** 2006. The regulatory PII protein controls arginine biosynthesis in Arabidopsis. *FEBS Letters* **580**, 2015-2020.
- Flores-Soto ME, Chaparro-Huerta V, Escoto-Delgadillo M, Vazquez-Valls E, Gonzalez-Castaneda RE, Beas-Zarate C.** 2012. Structure and function of NMDA-type glutamate receptor subunits. *Neurologia* **27**, 301-310.
- Forchhammer K, Luddecke J.** 2016. Sensory properties of the P-II signalling protein family. *Febs Journal* **283**, 425-437.
- Forde BG.** 2002. Local and long-range signaling pathways regulating plant responses to nitrate. *Annual Review of Plant Biology* **53**, 203-224.
- Forde BG.** 2014. Glutamate signalling in roots. *Journal of Experimental Botany* **65**, 779-787.
- Forde BG, Clarkson DT.** 1999. Nitrate and ammonium nutrition of plants: physiological and molecular perspectives. *Advances in Botanical Research* **30**, 1-90.
- Forde BG, Lorenzo H.** 2001. The nutritional control of root development. *Plant and Soil* **232**, 51-68.
- Fumarola C, La Monica S, Guidotti GG.** 2005. Amino acid signaling through the mammalian target of rapamycin (mTOR) pathway: Role of glutamine and of cell shrinkage. *Journal of Cellular Physiology* **204**, 155-165.
- Gansel X, Munos S, Tillard P, Gojon A.** 2001. Differential regulation of the NO₃⁻ and NH₄⁺ transporter genes *AtNRT2.1* and *AtAMT1.1* in Arabidopsis: relation with long-distance and local controls by N status of the plant. *Plant Journal* **26**, 143-155.
- Gong R, Park CS, Abbassi NR, Tang SJ.** 2006. Roles of glutamate receptors and the mammalian target of rapamycin (mTOR) signaling pathway in activity-dependent dendritic protein synthesis in hippocampal neurons. *Journal of Biological Chemistry* **281**, 18802-18815.

- Gulati P, Gaspers LD, Dann SG, Joaquin M, Nobukuni T, Natt F, Kozma SC, Thomas AP, Thomas G.** 2008. Amino acids activate mTOR Complex 1 via Ca²⁺/CaM signaling to hVps34. *Cell Metabolism* **7**, 456-465.
- Han JM, Jeong SJ, Park MC, Kim G, Kwon NH, Kim HK, Ha SH, Ryu SH, Kim S.** 2012. Leucyl-tRNA synthetase is an intracellular leucine sensor for the mTORC1-signaling pathway. *Cell* **149**, 410-424.
- Hannah MA, Caldana C, Steinhäuser D, Balbo I, Fernie AR, Willmitzer L.** 2010. Combined transcript and metabolite profiling of arabidopsis grown under widely variant growth conditions facilitates the identification of novel metabolite-mediated regulation of gene expression. *Plant Physiology* **152**, 2120-2129.
- Huang YF, Kang BN, Tian J, Liu Y, Luo HR, Hester L, Snyder SH.** 2007. The cationic amino acid transporters CAT1 and CAT3 mediate NMDA receptor activation-dependent changes in elaboration of neuronal processes via the mammalian target of rapamycin mTOR pathway. *Journal of Neuroscience* **27**, 449-458.
- Imsande J, Touraine B.** 1994. N demand and the regulation of nitrate uptake. *Plant Physiology* **105**, 3-7.
- Kang JM, Mehta S, Turano FJ.** 2004. The putative glutamate receptor 1.1 (AtGLR1.1) in *Arabidopsis thaliana* regulates abscisic acid biosynthesis and signaling to control development and water loss. *Plant and Cell Physiology* **45**, 1380-1389.
- Kang JM, Turano FJ.** 2003. The putative glutamate receptor 1.1 (AtGLR1.1) functions as a regulator of carbon and nitrogen metabolism in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences USA* **100**, 6872-6877.
- Kingsbury JM, Sen ND, Cardenas ME.** 2015.) Branched-chain aminotransferases control TORC1 signaling in *Saccharomyces cerevisiae*. *Plos Genetics* 10.1371/journal.pgen.1005714.
- Krapp A.** 2015. Plant nitrogen assimilation and its regulation: a complex puzzle with missing pieces. *Current Opinion in Plant Biology* **25**, 115-122.
- Kumar J, Mayer ML.** 2013. Functional insights from glutamate receptor ion channel structures. *Annual Review of Physiology* **75**, 313-337.
- Lainé P, Ourry A, Boucaud J.** 1995. Shoot control of nitrate uptake rates by roots of *Brassica napus* L. Effects of localized nitrate supply. *Planta* **196**, 77-83.
- Lam H-M, Chiu J, Hsieh M-H, Meisel L, Oliveira IC, Shin M, Coruzzi G.** 1998. Glutamate receptor genes in plants. *Nature* **396**, 125-126.
- Lau OS, Deng XW.** 2010. Plant hormone signaling lightens up: integrators of light and hormones. *Current Opinion in Plant Biology* **13**, 571-577.
- Lea PJ, Robinson SA, Stewart GA.** 1990. The enzymology and metabolism of glutamine, glutamate and asparagine. In: Mifflin BJ, Lea PJ, eds. *The Biochemistry of Plants Vol 16: Intermediary Metabolism*. San Diego: Academic Press, 121-157.
- Li F, Wang J, Ma CL, Zhao YX, Wang YC, Hasi A, Qi Z.** 2013a. Gutamate Receptor-Like channel3.3 is involved in mediating glutathione-triggered cytosolic calcium transients, transcriptional changes, and innate immunity responses in *Arabidopsis*. *Plant Physiology* **162**, 1497-1509.

- Li MW, AuYeung WK, Lam HM.** 2013b. The GCN2 homologue in *Arabidopsis thaliana* interacts with uncharged tRNA and uses *Arabidopsis* eIF2 alpha molecules as direct substrates. *Plant Biology* **15**, 13-18.
- Li Y, Krouk G, Coruzzi GM, Ruffel S.** 2014. Finding a nitrogen niche: a systems integration of local and systemic nitrogen signalling in plants. *Journal of Experimental Botany* **65**, 5601-5610.
- Liu Q, Chen XB, Wu K, Fu XD.** 2015. Nitrogen signaling and use efficiency in plants: what's new? *Current Opinion in Plant Biology* **27**, 192-198.
- Luna E, van Hulten M, Zhang YH, Berkowitz O, Lopez A, Petriacq P, Sellwood MA, Chen BN, Burrell M, van de Meene A, Pieterse CMJ, Flors V, Ton J.** 2014. Plant perception of beta-aminobutyric acid is mediated by an aspartyl-tRNA synthetase. *Nature Chemical Biology* **10**, 450-456.
- Medici A, Krouk G.** 2014. The Primary Nitrate Response: a multifaceted signalling pathway. *Journal of Experimental Botany* **65**, 5567-5576.
- Menand B, Desnos T, Nussaume L, Berger F, Bouchez D, Meyer C, Robaglia C.** 2002. Expression and disruption of the *Arabidopsis* TOR (target of rapamycin) gene. *Proceedings of the National Academy of Sciences USA* **99**, 6422-6427.
- Michard E, Lima PT, Borges F, Silva AC, Portes MT, Carvalho JE, Gilliam M, Liu LH, Obermeyer G, Feijo JA.** 2011. Glutamate receptor-like genes form Ca²⁺ channels in pollen tubes and are regulated by pistil D-serine. *Science* **332**, 434-437.
- Moreau M, Azzopardi M, Clement G, Dobrenel T, Marchive C, Renne C, Martin-Magniette ML, Taconnat L, Renou JP, Robaglia C, Meyer C.** 2012. Mutations in the *Arabidopsis* Homolog of LST8/G beta L, a partner of the Target of Rapamycin kinase, impair plant growth, flowering, and metabolic adaptation to long days. *Plant Cell* **24**, 463-481.
- Mulet JM, Martin DE, Loewith R, Hall MN.** 2006. Mutual antagonism of target of rapamycin and calcineurin signaling. *Journal of Biological Chemistry* **281**, 33000-33007.
- Muller B, Touraine B.** 1992. Inhibition of NO₃⁻ uptake by various phloem translocated amino acids in soybean seedlings. *Journal of Experimental Botany* **43**, 617-623.
- Nakajo T, Yamatsuji T, Ban H, Shigemitsu K, Haisa M, Motoki T, Noma K, Nobuhisa T, Matsuoka J, Gunduz M, Yonezawa K, Tanaka N, Naomoto Y.** 2005. Glutamine is a key regulator for amino acid-controlled cell growth through the mTOR signaling pathway in rat intestinal epithelial cells. *Biochemical and Biophysical Research Communications* **326**, 174-180.
- Nunes-Nesi A, Fernie AR, Stitt M.** 2010. Metabolic and signaling aspects underpinning the regulation of plant carbon nitrogen interactions. *Molecular Plant* **3**, 973-996.
- O'Brien JA, Vega A, Bouguyon E, Krouk G, Gojon A, Coruzzi G, Gutierrez RA.** 2016. Nitrate transport, sensing, and responses in plants. *Molecular Plant* **9**, 837-856.
- Price MB, Jelesko J, Okumoto S.** 2012.) Glutamate receptor homologs in plants: functions and evolutionary origins. *Frontiers in Plant Science* 10.3389/fpls.2012.00235.
- Qi Z, Stephens NR, Spalding EP.** 2006. Calcium entry mediated by GLR3.3, an *Arabidopsis* glutamate receptor with a broad agonist profile. *Plant Physiology* **142**, 963-971.
- Robaglia C, Thomas M, Meyer C.** 2012. Sensing nutrient and energy status by SnRK1 and TOR kinases. *Current Opinion in Plant Biology* **15**, 301-307.

Ruffel S, Gojon A, Lejay L. 2014. Signal interactions in the regulation of root nitrate uptake. *Journal of Experimental Botany* **65**, 5509-5517.

Scheible WR, Gonzalez-Fontes A, Lauerer M, Muller-Rober B, Caboche M, Stitt M. 1997a. Nitrate acts as a signal to induce organic acid metabolism and repress starch metabolism in tobacco. *Plant Cell* **9**, 783-798.

Scheible WR, Gonzalez-Fontes A, Morcuende R, Lauerer M, Geiger M, Glaab J, Gojon A, Schulze ED, Stitt M. 1997b. Tobacco mutants with a decreased number of functional *nia* genes compensate by modifying the diurnal regulation of transcription, post-translational modification and turnover of nitrate reductase. *Planta* **203**, 304-319.

Schneper L, Duvel K, Broach JR. 2004. Sense and sensibility: nutritional response and signal integration in yeast. *Current Opinion in Microbiology* **7**, 624-630.

Shimobayashi M, Hall MN. 2016. Multiple amino acid sensing inputs to mTORC1. *Cell Research* **26**, 7-20.

Silva LS, Seabra AR, Leitao JN, Carvalho HG. 2015. Possible role of glutamine synthetase of the prokaryotic type (GSI-like) in nitrogen signaling in *Medicago truncatula*. *Plant Science* **240**, 98-108.

Sirohi G, Pandey BK, Deveshwar P, Giri J. 2016. Emerging trends in epigenetic regulation of nutrient deficiency response in plants. *Molecular Biotechnology* **58**, 159-171.

Stephens NR, Qi Z, Spalding EP. 2008. Glutamate receptor subtypes evidenced by differences in desensitization and dependence on the *GLR3.3* and *GLR3.4* genes. *Plant Physiology* **146**, 529-538.

Stitt M, Feil R. 1999. Lateral root frequency decreases when nitrate accumulates in tobacco transformants with low nitrate reductase activity: consequences for the regulation of biomass partitioning between shoots and root. *Plant and Soil* **215**, 143-153.

Sugiyama K, Hayakawa T, Kudo T, Ito T, Yamaya T. 2004. Interaction of N-Acetylglutamate kinase with a PII-Like protein in rice. *Plant and Cell Physiology* **45**, 1768-1778.

Tapken D, Anschutz U, Liu LH, Huelsken T, Seeböhm G, Becker D, Hollmann M. 2013.) A plant homolog of animal glutamate receptors is an ion channel gated by multiple hydrophobic amino acids. *Science Signaling* 10.1126/scisignal.2003762.

Teardo E, Formentin E, Segalla A, Giacometti GM, Marin O, Zanetti M, Lo Schiavo F, Zoratti M, Szabo I. 2011. Dual localization of plant glutamate receptor AtGLR3.4 to plastids and plasmamembrane. *Biochimica et Biophysica Acta: Bioenergetics* **1807**, 359-367.

Tillard P, Passama L, Gojon A. 1998. Are phloem amino acids involved in the shoot to root control of NO₃⁻ uptake in *Ricinus communis* plants? *Journal of Experimental Botany* **49**, 1371-1379.

Tudzynski B. 2014. Nitrogen regulation of fungal secondary metabolism in fungi. *Frontiers in Microbiology* **5**.

Vidal E, Álvarez J, Moyano T, Gutiérrez R. 2015. Transcriptional networks in the nitrate response of *Arabidopsis thaliana*. *Current Opinion in Plant Biology* **27**, 125-132.

Vincill ED, Bieck AM, Spalding EP. 2012. Ca²⁺ conduction by an amino acid-gated ion channel related to glutamate receptors. *Plant Physiology* **159**, 40-46.

Wagner D, Wiemann P, Huss K, Brandt U, Fleissner A, Tudzynski B. 2013. A sensing role of the glutamine synthetase in the nitrogen regulation network in *Fusarium fujikuroi*. *Plos One* **8**.

- Weiland M, Mancuso S, Baluska F.** 2016. Signalling via glutamate and GLRs in *Arabidopsis thaliana*. *Functional Plant Biology* **43**, 1-25.
- Welters P, Takegawa K, Emr SD, Chrispeels MJ.** 1994. AtVPS34, a phosphatidylinositol 3-kinase of *Arabidopsis thaliana*, is an essential protein with homology to a calcium-dependent lipid-binding domain. *Proceedings of the National Academy of Sciences USA* **91**, 11398-11402.
- Wolfson RL, Chantranupong L, Saxton RA, Shen K, Scaria SM, Cantor JR, Sabatini DM.** 2016. Sestrin2 is a leucine sensor for the mTORC1 pathway. *Science* **351**, 43-48.
- Xiong Y, Sheen J.** 2014. The role of target of rapamycin signaling networks in plant growth and metabolism. *Plant Physiology* **164**, 499-512.
- Yoshida R, Mori IC, Kamizono N, Shichiri Y, Shimatani T, Miyata F, Honda K, Iwai S.** 2016. Glutamate functions in stomatal closure in *Arabidopsis* and fava bean. *Journal of Plant Research* **129**, 39-49.
- Zeng HQ, Wang GP, Hu XY, Wang HZ, Du LQ, Zhu YY.** 2014. Role of microRNAs in plant responses to nutrient stress. *Plant and Soil* **374**, 1005-1021.
- Zhang HM, Jennings A, Barlow PW, Forde BG.** 1999. Dual pathways for regulation of root branching by nitrate. *Proceedings of the National Academy of Sciences USA* **96**, 6529-6534.
- Zheng Z-L.** 2009. Carbon and nitrogen nutrient balance signaling in plants. *Plant Signaling & Behavior* **4**, 584-591.

Legends

Fig. 1. Hypothetical model for activation of the TOR complex by amino acid sensing through the family of GLR glutamate receptor-like Ca^{2+} channels present in plants. The TOR complex plays a central role in regulating plant growth and metabolism in response to changes in nutrient availability, working in conjunction with the Snf1-related kinase (SnRK1) which largely acts antagonistically to TOR (Dobrenel *et al.*, 2016; Robaglia *et al.*, 2012). The diagram depicts a cell in which a diverse set of GLRs are expressed, each activated by a variety of different amino acid ligands (only a proportion of which are indicated). Collectively this panel of amino acid sensors would be able to respond to changes in the overall amino acid pool, therefore potentially providing an accurate reflection of the N status of the tissue. Although the GLRs are shown as located in the plasma membrane, there is evidence that they are also to be found on internal (plastidic) membranes (Teardo *et al.*, 2011), so that their sensory role would not be restricted to amino acids in the apoplast. Ligand binding by plant GLRs is known to trigger Ca^{2+} influx (Dietrich *et al.*, 2010) and it is proposed that this could be linked to activation of the TOR complex, perhaps through a pathway involving the Ca^{2+} -dependent regulation of VPS34 and the downstream production of phosphatidic acid which is an activator of TOR in animals (Gulati *et al.*, 2008). See text for further details.

Fig. 1

