Annals of Botany **90**: 549–557, 2002
doi:10.1093/aob/mcf228, available online at www.aob.oupjournals.org

BOTANICAL BRIEFING

Glutamate Receptors in Plants

ROMOLA DAVENPORT*

Department of Plant Science, Downing Street, Cambridge CB2 3EA, UK

Received: 5 April 2002 Returned for revision: 22 May 2002 Accepted: 2 July 2002 Published electronically: 2 October 2002

Ionotropic glutamate receptors function in animals as glutamate-gated non-selective cation channels. Numerous glutamate receptor-like (*GLR*) genes have been identified in plant genomes, and plant GLRs are predicted, on the basis of sequence homology, to retain ligand-binding and ion channel activity. Non-selective cation channels are ubiquitous in plant membranes and may function in nutrient uptake, signalling and intra-plant transport. However, there is little evidence for amino acid gating of plant ion channels. Recent evidence suggests that plant *GLR*s do encode non-selective cation channels, but that these channels are not gated by amino acids. The functional properties of these proteins and their roles in plant physiology remain a mystery. The problems surrounding characterization and assignation of function to plant *GLR*s are discussed in this Botanical Briefing, and potential roles for GLR proteins as non-selective cation channels involved in metabolic signalling are described.

Key words: Review, gutamate receptor, plant glutamate receptor, GLR, GluR, iGluR, *Arabidopsis thaliana*, calcium, signalling, non-selective cation channel, nitrogen metabolism.

INTRODUCTION

Plant membranes contain a variety of ion channels involved in transport of nutrients, ions used for signalling, osmotica and metabolites. While early work focused on highly selective ion channels, it has recently become clear that a diverse array of cations is transported via non-selective cation channels (NSCCs). NSCCs are ion channels that are selectively permeable for cations over anions, but that do not discriminate strongly between monovalent cations. Some NSCCs do not permit permeation of divalent cations, while others are relatively selective for divalent over monovalent cations (although still transporting cations of both valencies from mixed solutions). Non-selective cation movements are ubiquitous in the plasma membrane of plant cells and dominate tonoplast ion transport. In animals, NSCCs coexist with highly selective Na^+, Ca^{2+} and K^+ channels. In plants, K^+-selective channels have been characterized but it is not yet clear whether highly selective Ca^{2+} channels exist, or whether Ca^{2+} fluxes are in fact mediated by NSCCs. There is no evidence for plant Na^+-selective channels, and Na^+ appears to enter plant cells via NSCCs. NSCCs form a heterogeneous category varying in ion selectivity and determinants of channel gating. Many plant NSCCs are voltage-independent, and appear to have a high probability of opening in patch-clamp conditions. Little is known about the mechanisms that control gating in this type of channel. Plant non-selective cation currents are in the initial stages of characterization and the physiological roles of these currents are not well understood (Demidchik *et al*., 2002).

* For correspondence. Fax +44 (0)1223 333953, e-mail rjd23@cus.cam.ac.uk

The proteins responsible for non-selective cation currents have not been identified. However, sequencing of the arabidopsis genome revealed, for the first time, a complete complement of gene candidates for plant cation channels. The result was surprising. The arabidopsis genome contains 15 putative K^+ channel sequences, a single sequence with distant similarity to animal voltage-gated Ca^{2+} channels, and 40 putative NSCCs (Demidchik *et al*., 2002). The relative abundance of NSCC-type genes underscores their ubiquity in electrophysiological studies. More surprising was the fact that these putative NSCC genes were related to ion channel types found in animals and for which no physiological evidence existed in plants: glutamate receptors and cyclic nucleotide-gated ion channels. Members of the latter family have been shown to form inwardly rectifying, cyclic nucleotide-activated K^+-permeable ion channels (Leng *et al*., 1999, 2002) but, *in vivo*, the scant evidence that exists for plants points to an inhibitory effect of cyclic nucleotides on plasma membrane NSCC currents (Maathuis and Sanders, 2001). Plant glutamate receptor-like (*GLR*) genes were initially identified by similarity searches of EST databases, and 20 *GLR*s have been identified in the arabidopsis genome. Evidence is emerging that plant GLRs function as NSCCs, but there is no evidence of glutamate gating of these channels. There is also very little evidence, *in vivo*, for the existence of glutamate-activated NSCCs.

This Botanical Briefing focuses on the arabidopsis GLRs (AtGLRs) because the entire gene family is known and some characterization has been performed. However *GLR*-like genes have been identified in other dicotyledonous and monocotyledonous species as well as in gymnosperms, indicating that these genes are of general importance in plant physiology.

AMINO ACID SIGNALLING IN PLANTS

In animals, glutamate functions as a neurotransmitter and activates glutamate receptor cation channels (iGluRs), which trigger electrical or Ca^{2+} signal cascades. In plants, amino acids are involved in signalling of both plant nitrogen status and plant nitrogen : carbon ratios. Endogenous glutamine has been implicated in feedback inhibition of root N uptake, via the suppression of transcription of genes encoding inorganic nitrogen transporters (Rawat *et al*., 1999; Zhuo *et al*., 1999). Transcription of key enzymes involved in assimilation of inorganic nitrogen to amino acids is controlled in a reciprocal fashion by N (amino acids) and C (light and/or sugars) (reviewed in Lam *et al*., 1996; Oliviera *et al*., 2001). The key amino acids in plant N assimilation, storage and long-distance transport are glutamate and glutamine (the first products of N assimilation) and aspartate and asparagine (synthesized from glutamate and glutamine) (see 'Prediction of physiological function', below).

The evident ability of plants to sense internal N levels led Coruzzi and co-workers to search for N sensors, and resulted in the identification of plant *GLR* genes (Lam *et al*., 1998) as well as a plant homologue of the bacterial N sensor PII (Hsieh *et al*., 1998).

Physiological evidence for glutamate receptor function in arabidopsis

GLR genes were identified by sequence homology with animal iGluRs, and evidence for their involvement in carbon/nitrogen signalling is indirect. GLRs have been implicated in light signalling on the basis of plant responses to agonists and inhibitors of animal iGluRs. The iGluR inhibitor DNQX [6,7 dinotropuinoxaline 2,3(1H,4H) dione] caused an etiolated phenotype (hypocotyl elongation and reduced chlorophyll levels) in light-grown arabidopsis seedlings. Dark-grown seedlings were unaffected by DNQX, indicating that light signalling was specifically impaired (Lam *et al*., 1998). The cycad toxin BMAA [S(+)-β-methyl-α, β-diaminopropionic acid] caused similar symptoms in arabidopsis seedlings, and these could be prevented by supply of exogenous glutamate or glutamine, but not aspartate (Brenner *et al*., 2000). These results were surprising because BMAA, like glutamate, is an agonist of animal iGluRs, whereas DNQX is an inhibitor. It is quite possible that these compounds all targeted an enzyme with an amino acid-binding domain, although there was no evidence for involvement of ion channels.

Following the discovery of the arabidopsis *AtGLR* family, Dennison and Spalding (2000) demonstrated that addition of glutamate (1 mM) to the medium around arabidopsis seedlings stimulated a rapid increase in cytosolic Ca^{2+} and caused membrane depolarization of root tip cells. These effects were attributed to activation of plasma membrane Ca^{2+}-permeable channels, although the possibility of secondary activation of Ca^{2+} influx by glutamate uptake was not excluded. Addition of arginine, aspartate, D-glutamate and the animal iGluR agonists NMDA and AMPA (see below) had no comparable effect on cytosolic Ca^{2+} levels. Demidchik *et al*. (2001) reported an increase in voltage-independent non-selective cation currents (Na^+ and Ca^{2+}) in 20 % of arabidopsis root protoplasts upon exposure to low millimolar levels of glutamate. These reports constitute the only evidence of glutamate gating of plant ion channels, despite the frequent use of glutamate as a balancing anion in patch-clamping solutions. Extracellular glutamate had no effect on plasma membrane NSCC currents in wheat root protoplasts (Tyerman *et al*., 1997) or on a wheat root plasma membrane NSCC characterized in planar lipid bilayers (Davenport and Tester, 2000).

IONOTROPIC GLUTAMATE RECEPTORS IN ANIMALS

Plant *GLR* genes are predicted to encode ion channels with close sequence and structural similarities to animal ionotropic glutamate receptors (iGluRs). iGluRs are non-selective cation channels that function predominantly as glutamate-gated Na^+ and Ca^{2+} influx pathways at neuronal (vertebrate) or neuromuscular (invertebrate) junctions (Dingledine *et al*., 1999).

Mammalian iGluRs are divided into four groups (AMPA, NMDA, kainate and delta receptors), partly on the basis of sensitivity to agonists [AMPA α(-amino-3-hydroxy-5-methyl-4-isoazolepropionate); NMDA (*N*-methyl-D-aspartate); kainic acid]. AMPA and kainate receptors generally show low Ca^{2+} permeability (depending upon post-transcriptional RNA editing of a single residue in the pore region, from glutamine to arginine) and rapid desensitization. NMDA receptors are Ca^{2+}-permeable and desensitize more slowly. The delta iGluRs do not show ion channel activity and do not bind glutamate. The gating mechanism of iGluRs is voltage-insensitive, but NMDA receptors show voltage-dependent block by Mg^{2+}, and some kainate and AMPA receptors exhibit current rectification due to polyamines. NMDA receptors are activated by aspartate as well as glutamate, and require glycine binding for activity.

Homologues of the mammalian iGluR subtypes are also found in invertebrates (Sprengel *et al*., 2001). In addition to iGluRs, fish, amphibians and birds also express low molecular weight kainate-binding proteins (KBPs). KBPs lack the long N-terminus (Fig. 1) but otherwise resemble iGluRs in sequence. However, although they bind glutamate with high affinity, and the pore regions are capable of ion conduction (Villmann *et al*., 1997), KBPs do not appear to function as ion channels. Only one bacterial iGluR has been discovered, GluR0, from the cyanobacterium *Synechocystis*. GluR0 is K^+-selective and is activated by glutamate and glutamine (Chen *et al*., 1999). No iGluR-like genes have been discovered in any of the fungal genomes yet sequenced.

Animals also possess metabotropic glutamate receptors (mGluRs) which contain glutamate-binding domains homologous with the N-terminal region of iGluRs (Fig. 1) but that do not form ion channels. Glutamate-gated anion channels have been identified in invertebrates, but these channels are considered to be related to the acetylcholine receptor-like

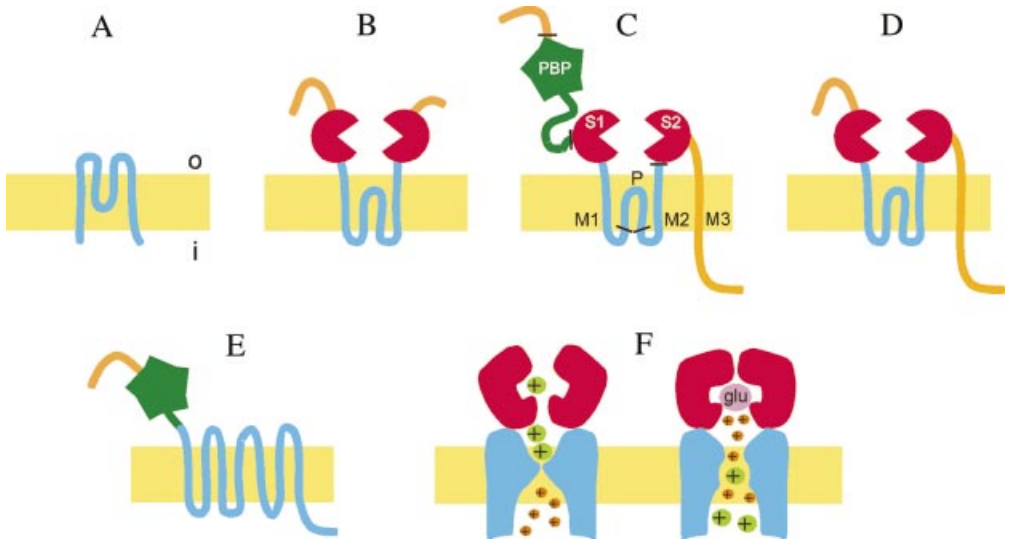

FIG. 1. Predicted structure and evolutionary relationships of ionotropic glutamate receptors, kainate binding proteins and a prokaryotic K^+ channel. A, KcsA, a prokaryotic K^+ channel subunit with M1PM2 structure. B, GluR0, a prokaryotic glutamate-gated K^+ channel subunit. C, Mammalian iGluR and plant GLR subunits. Consensus AtGLR splice sites are marked (the third splice is found only in group III genes). D, Kainate-binding protein subunits, found in fish, amphibians and birds. E, Mammalian mGluR subunits. F, The putative 'Venus flytrap' mechanism of animal iGluR channel gating. On the left the channel subunit is in the closed state. When glutamate binds to the active site of the S1–S2 complex (right) then the conformation of the transmembrane domains is converted to an ion-permeant state. This diagram represents a single subunit but it is predicted that four or possibly five subunits assemble to form a functional ion channel, with each subunit contributing residues to the membrane-spanning pore. Pore conductance level appears to depend on the number of S1–S2 complexes binding glutamate. The putative membrane orientation is as indicated in A. I, Indicates cytosol; o, extracellular or other compartment; PBP, periplasmic amino acid binding protein; S1, S2, ligand binding domains; M, transmembrane domain; P, pore. Colours indicate putative homology between protein domains.

cation channels rather than the cation-transporting glutamate receptors (Xue, 1998).

PREDICTED STRUCTURE OF ANIMAL AND PLANT GLUTAMATE RECEPTORS

Plant *GLR* genes are predicted to encode proteins with high sequence and structural homology to animal iGluRs. Animal iGluR proteins are thought to function as tetramers (Rosenmund *et al*., 1998) or possibly pentamers (Premkumar and Auerbach, 1997), with each subunit containing three membrane-spanning domains and a hydrophobic loop lining the pore region and conferring ion selectivity on the channel (Fig. 1C). The ligand-binding domain is formed by two regions (S1, S2), separated by the first two transmembrane domains (M1, M2) and pore region (P). S1 and S2 are thought to interact to bind glutamate by a 'Venus flytrap' mechanism similar to that of bacterial periplasmic amino acid binding proteins (Fig. 1F). It is conjectured that this structure arose from the insertion of an inverted K^+ channel domain between the two lobes of a bacterial amino acid-binding protein (Wo and Oswald, 1995). This is supported by the recent discovery of the cyanobacterial homologue GluR0 (Chen *et al*., 1999). GluR0 lacks the long N-terminus and third transmembrane domain of plant and animal glutamate receptors, but contains S1/S2 domains separated by an M1PM2 pattern (Fig. 1). The GluR0 pore region contains the GYGD motif, which confers K^+ selectivity in K^+ channels, and GluR0 functions as a glutamate-activated, K^+-selective ion channel.

Plant GLRs preserve the predicted channel structure of animal iGluRs, and show high amino acid sequence identity in the ligand binding domains and in the M1 and M2 transmembrane domains (Chiu *et al*., 1999). The M2 domain is most highly conserved, although interestingly plant sequences lack the P,K/R residues at the beginning of M2 which are highly conserved in all animal iGluRs except the delta receptors (Fig. 2). The most striking difference between plant GLRs and animal iGluRs is in the sequence of the putative pore region (Fig. 2). The plant pore regions contain cationic residues in the 'GYGD' region, which in other cation channels is predicted to interact closely with the permeant cation and effect selectivity. Most cation channels have anionic or polar residues in these positions, although post-transcriptional editing of a nearby pore residue [from a polar glutamine (Q) to a cationic arginine (R)] occurs in certain AMPA receptors and results in reduction of both Ca^{2+} permeability and single channel conductance (Dingledine *et al*., 1999). The unusual pore sequence of plant GLRs suggests either novel selectivity, or a novel mechanism of selectivity. The selectivity of the pore loop cannot be predicted by comparison with, for example, anion channels, because the pore loop structure is only found in the voltage-gated cation channels and some of the ligand-gated cation channel families.

Like the animal iGluR proteins, plant GLRs are predicted to have a long N-terminal domain of unknown function, with homology to the ligand-binding domain of mGluRs,

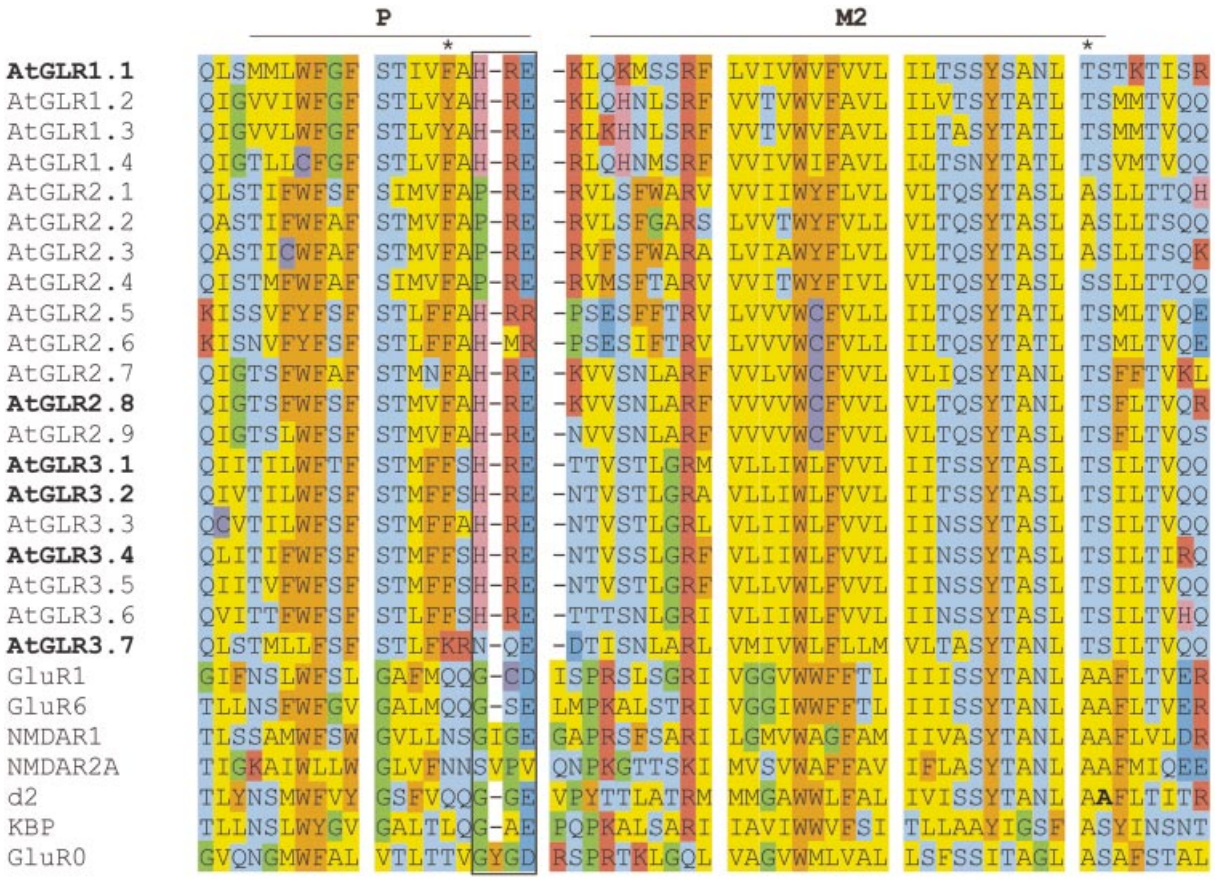

FIG. 2. Alignment of the P1 and M2 regions of the arabidopsis AtGLRs (cloned full-length cDNAs indicated in bold) with Genbank sequences of rat GluR1 (X17184), human GluR6 (CAC67487·1), human NR1 (Q05586), rat NR2A (D13211), frog KBP (X17314), mouse δ2 (D13266) and *Synechocystis* GluR0 (slr1257). The 'Q/R/N' site of RNA editing in the pore of some AMPA and kainate receptors is indicated by an asterisk, and the pore region corresponding to the 'GYGD' selectivity filter of K^+ channels is boxed. The position of the δ2 Lurcher mutation in M2 is indicated by an asterisk: the wildtype alanine ('A') is mutated to threonine ('T') in Lurcher mutants. P, Pore region; M2, second transmembrane domain. Chemical properties of amino acid side-groups are represented by colours: red = basic; pink = histidine (pKa = 6·5); dark blue = acidic; light blue = hydrophilic; yellow = aliphatic; orange = aromatic; green = proline and glycine; and purple = cysteine.

$GABA_B$ receptors and extracellular Ca sensors. This domain can be deleted from vertebrate iGluRs without loss of function, although it has been implicated in desensitization, membrane targeting, and allosteric modulation by Zn^{2+} (Zheng *et al*., 2001). Indeed the function of this type of large extracellular domain is still little explored in the metabotropic sensors (Hammerland *et al*., 1999). It has been suggested that the plant GLRs resemble an ancestral glutamate receptor from which both iGluRs and various metabotropic receptors derived (Turano *et al*., 2001). The homologies of the N-terminal domain suggest interesting possibilities for allosteric modulation of GLR function by Ca^{2+} or metabolites.

Mammalian iGluRs are thought to function as heteromers, and some subunits do not form functional ion channels when expressed as homomers in heterologous systems. The delta receptors, NR3a and the KBPs do not form functional ion channels even in heteromeric assemblies, and it is thought that these may play some modulatory role in association with other channel-forming subunits (Villmann *et al*., 1997, 1999). Transplantation of the pore regions of KBPs into functional iGluR subunits demonstrated that the pore regions themselves were capable of ion conduction, although similar experiments with the pore regions of NR3 and the delta receptors did not produce ionic currents (Villmann *et al*., 1997, 1999). Curiously, however, the delta receptor δ2 acquires ion channel function when mutated in the second transmembrane domain (M2) (from LAA to LAT). This gain of function mutation results in the mouse neurodegenerative 'Lurcher' phenotype, and caused constitutive, glutamate-insensitive cation currents when the mutant δ2 subunit was expressed in *Xenopus oocytes* (Zuo *et al*., 1997). Plant GLRs are predicted to possess a Lurcher-like sequence in the M2 region (Fig. 2), and it has been suggested that plant GLRs may produce constitutive ion channel activity (Chiu *et al*., 1999). This is consistent with the functional characterization of AtGLRs performed so far (see below). However, GluR0 also has a Lurcher-like M2 sequence (LAS) but retained glutamate sensitivity (Chen *et al*., 1999), and Lurcher-type mutations (LAA to LAT) of

TABLE 1. *The arabidopsis GLR family (Lacombe* et al.*, 2001b)*

Name	MIPS locus	Old cDNA name	Chromosome	Number of exons	Number of ESTs	Transcription/expression data	Predicted membrane location
AtGLR1·1	At3g04110	*GLR1*	III	5	4	Mixed tissue[a] above-ground 2–6 w[b]	S (0·97)
AtGLR1·2	*At5g48400*		V	5	0		S (0·99)
AtGLR1·3	*At5g48410*		V	5	0		S (0·98)
AtGLR1·4	At3g07520		III	5	0		S (0·99)
AtGLR2·1	At5g27100		V	5	1	Rosette 4–7 w[c]	S (0·99)
AtGLR2·2[1]	*At2g24720*		II	4	0		S (0·98)
AtGLR2·3	*At2g24710*		II	5	0		S (0·98)
AtGLR2·4[2]	At4g31710		IV	5	0		S (0·97)
AtGLR2·5[3]	*At5g11210*		V	5	0		S (0·95)
AtGLR2·6[3]	*At5g11180*		V	5	0		S (0·93)
AtGLR2·7	*At2g29120*		II	5	1	Above-ground 2–6 w[d]	S (0·94)
AtGLR2·8	*At2g29110*	*GluR9*	II	5	0	cDNA[e]	S (0·99)
AtGLR2·9	*At2g29100*		II	5	0		S (0·91)
AtGLR3·1a	At2g17260	*GLR2*[4]	II	6	(2)	Roots[f] mixed[g]	S (0·93)
AtGLR3·1b	(At2g17260)	*ACL1*[4]	II	5	(2)		S (0·83)
AtGLR3·2	At4g35290	*AtGluR2*	IV	6	3	Mixed[h] Stelar cells of root and shoot[i]	S (0·87)
AtGLR3·3	At1g42540		I	6	0		M (0·47)
AtGLR3·4	At1g05200	*GluR3*	I	6	4	Above-ground 2–6 w[j] green siliques[k] Root, shoot 2 w[l]	C (0·65)
AtGLR3·5[5]	*At2g32390*	*GLR6*	II	6	0	Root, shoot 2 w[l]	S (0·85)
AtGLR3·6	At3g51480		III	6	1	Root 4–7 w[m]	S (0·71)
AtGLR3·7	*At2g32400*	*GLR5*	II	6	1	Root[n] root, shoot 2w[l]	S (0·94)

Full-length cDNAs are indicated in bold: other sequences are predicted from genomic DNA (for prediction of exons, see Appendix in supplementary data at www.aob.oupjournals.org). Genes that are next to or very close to each other on a chromosome are indicated in italics in column 2. Transcription data indicate the source of the RNA ('w', weeks from planting; 'above-ground', all aerial parts of the plant). Membrane localization was predicted using TargetP (v1·01; Emanuelsson *et al*., 2000): numbers in parentheses indicate the program's prediction of probability.

S, Secretory pathway; M, mitochondrial; C, chloroplastic. MIPS, Munich Information Center for Protein Sequences (http://mips.gsf.de/proj/thal/db/).

[1]Appears in MIPS as At2g24730, and is misdescribed as a pseudogene. Lacks last intron, giving a large fourth exon. [2]Second splice differs from consensus. [3]These genes are separated by two ORFs and are clearly the products of a duplication event. Both are immediately upstream of (transcribed) RNA helicase genes. [4]These full-length cDNAs are splice variants differing at the 5′ end. *AtGLR3·1a* has an extra 5′ exon. Both cDNAs lack the 'typical' second intron. The ESTs are 3′ sequences so not distinguishable. [5]Cloned as a misspliced cDNA, *GLR6*, but only correctly spliced transcripts were detected in RNA populations.

[a]T22862, AA585839, CD4-7 library (Coruzzi *et al*., 1999). [b]AV526778, AV520459. [c]AI998138. [d]AV523736. [e]Full-length clone AJ311495, no data on RNA source. [f]AV546686. [g]AW004425. [h]AI993537, R29880, AA650976. [i]Kim *et al*., 2001. [j]AV524083, AV529672. [k]AV555202, AV567289. [l]Davenport, unpubl. res. [m]AI996656. [n]AV547071.

the animal NMDA receptors NR1 and NR2B did not cause constitutive channel opening (Kashiwagi *et al*., 2002).

ORGANIZATION OF THE ARABIDOPSIS GLUTAMATE RECEPTOR FAMILY

In arabidopsis, the entire complement of predicted *GLR*s is known, and the 20 genes have been assigned to three subfamilies on the basis of parsimony analysis (Lacombe *et al*., 2001*b*) (Table 1). Only six of the 20 genes have been cloned as full-length cDNAs, although 11 have been identified as transcribed. The hypothetical cDNA sequences presented in Table 1 are predicted on the basis of conservation of common splice sites and amino acid sequence in common with cloned cDNAs, and differ in some cases from those available in public databases (see Appendix in supplementary data in www.aob.oupjournals.org). Splice sites are conserved between groups (Fig. 1) with some exceptions (documented in Table 1). The high proportion of group III genes that is known to be transcribed suggests that this group may play more ubiquitous roles than the other groups. *AtGLR3·1* is subject to alternative splicing, giving rise to two cDNAs which differ at the 5′ end, with possible consequences for membrane targeting.

The arabidopsis *GLR* family appears to have developed by multiple local gene duplication events, as indicated by the high frequency of occurrence of adjacent pairs and triplets of very similar *GLR* genes (Table 1). It is possible that some of these genes have no function. There is at least one *GLR* pseudogene in the arabidopsis genome. At2g24750 is predicted to lie two open reading frames 5′ of *AtGLR2·2* and appears to be a genuine pseudogene: it lacks the conserved splice sites and is transcribed in reverse orientation in the EST AV539801.

FUNCTIONAL CHARACTERIZATION OF PLANT GLUTAMATE RECEPTORS

Transport functions

Evidence is emerging for non-selective cation channel function of AtGLRs. Arabidopsis plants over-expressing genomic *AtGLR3·2* under the control of the cauliflower mosaic virus 35S promoter showed symptoms of Ca deficiency and hypersensitivity to K^+ and Na^+ (Kim *et al*.,

2001). Total Ca content of the plants did not differ from that of the wildtype, but the plants required three times more Ca^{2+} for optimal growth, suggesting that distribution of Ca within the plant, rather than uptake, was affected. Supplemental Ca^{2+} abolished sensitivity of transgenic plants to monovalent cations. Unfortunately, uptake of monovalent cations was not measured. Fusion of the putative promoter/enhancer region of *AtGLR3·2* to the GUS reporter gene resulted in GUS expression in stelar cells surrounding the vasculature of root and shoot tissue, suggesting a role for AtGLR3·2 in unloading Ca^{2+} from the xylem. It was proposed that over-expression of AtGLR3·2 subunits may have interfered with the stoichiometry of native GLR heteromers and reduced their Ca^{2+} transport capacity.

AtGLR3·7 has been expressed successfully in *Xenopus* oocytes and appears to function as a constitutively active, Ca^{2+}-permeable non-selective cation channel, with no evidence of activation by glutamate or other iGluR agonists (Cheffings, 2001).

AtGLR3·4 has been expressed in oocytes and human embryonic kidney (HEK) cells, and was reported to mediate Ca^{2+} influx (Lacombe *et al*., 2001*a*). Currents attributed to AtGLR3·4 were insensitive to agonists of animal glutamate receptors (unnamed amino acids, NMDA, kainate, AMPA, BMAA and concanavalin A) applied to the external side of the oocyte membrane. ABA and auxin also had no effect. AtGLR2·8 was also tested in oocytes but no currents were detected.

These studies suggest that plant GLRs function in Ca^{2+} and monovalent cation transport, and may form constitutively active ion channels. The lack of gating effects of animal GluR agonists on plant GLRs is, at first, surprising. It has been suggested that the 'Lurcher'-type M2 sequence of plant GLRs could cause constitutive activation (Chiu *et al*., 1999). However, it is still necessary to account for the conservation of the ligand-binding domain sequences. Several animal iGluRs show either low sensitivity to ligands (GluR7) or no affinity for ligands (e.g. the delta receptors), while retaining ligand-binding domain-like sequences (Lomeli *et al*., 1993; Strutz *et al*., 2001). It is possible that in plants only some GLR subunits, or some heteromeric combinations, confer ligand sensitivity, or that the ligand sensitivity of GLR proteins differs *in vivo* from that of homomeric subunits in heterologous systems. Alternatively, while GLR currents appeared to be constitutive, they may in fact be only partially activated, and require a novel ligand for full activation.

Tissue localization

Analysis of *AtGLR3·2* promoter expression patterns (Kim *et al*., 2001) coincided with an earlier anecdotal report of GLR localization to vascular tissues in tomato (Knight, 1999). Other information about localization comes from ESTs and unpublished data and is summarized in Table 1. *AtGLR3·4* and *AtGLR3·5* transcripts were detected by RT-PCR in RNA from both root and shoot tissue of 2-week-old arabidopsis plants (Davenport, unpubl. res.). *AtGLR3·4* ESTs exist from above-ground tissue and green siliques, indicating spatially and temporally widespread transcription of the gene. *AtGLR3·7* transcripts were detected by RT-PCR in RNA from both leaf and root (Davenport, unpubl. res.), and an *AtGLR3·7* EST was extracted from root tissue (AV547071). Group III gene transcripts appear to be most abundant, and this is the only group for which there is evidence of transcription in roots. However, as yet there is insufficient information to suggest any specialization of function within the three subgroups. Some glutamate receptor transcripts may elude detection due to very low abundance (e.g. *AtGLR3·2* was not detectable by Northern blotting; Kim *et al*., 2001), or to unusual expression patterns. Some of these genes could be untranscribed pseudogenes. This possibility is suggested by the high frequency of closely spaced or tandem repeat GLRs (11 of 20), some of very similar sequence and promoter patterns. This suggests recent duplication, and may indicate linked expression or, alternatively, loss of function of one of the duplicates. [Note added in proof: a recent publication (Chiu *et al*., 2000) reports tissue specificity of transcription of all 20 *AtGLRs* and describes patterns of expression of the GUS reporter gene under control of promoter regions of *AtGLR1.1*, *2.1* and *3.1*.]

Membrane localization

The intracellular localization of plant GLRs is unknown, although it is generally assumed to be in the plasma membrane (PM). Most of the arabidopsis GLRs are predicted by computer-based programs to be targeted to the secretory pathway on the basis of hydrophobic N-terminal sequences (Table 1), although the N termini are not always predicted to be extracellular. The secretory pathway includes all proteins processed by the endoplasmic reticulum (ER) and not retained within the ER, so embraces plasma membrane, tonoplast and some peroxisomal membrane proteins (Johnson and Olsen, 2001; Mullen *et al*., 2001). Attempts to visualize the membrane localization of several GLRs by fusion of green fluorescent protein (GFP) to the C-terminus under control of the constitutively active 35S promoter have been unsuccessful, suggesting that C-terminal fusions may prevent proper processing of the chimaera, or prevent proper folding of the GFP (Davenport, unpubl. res.). Fusions of GFP to the C-terminus of N-terminal putative signal sequences suggested that only some of the sequences tested were targeted to the ER (Davenport, unpubl. res.). It is possible that membrane targeting of plant GLRs involves internal or C-terminal signal sequences. Animal iGluRs contain N-terminal signal sequences and several C-terminal GFP fusion constructs were correctly targeted to the plasma membrane (Marshall *et al*., 1995; Marsh *et al*., 2001). The cyanobacterial GluR0 required replacement of the native N-terminal hydrophobic sequence with the rat GluR6 signal sequence for plasma membrane targeting in oocytes (Chen *et al*., 1999).

Endomembrane targeting of plant GLRs *in planta* could confound heterologous characterization. For instance, the apparent lack of sensitivity of GLRs to amino acids could reflect the orientation of the proteins within the heterologous host membrane, rather than insensitivity to ligands. If the proteins were incorporated into target membranes with

opposite orientation to that of animal iGluRs (i.e. with a cytosolic ligand-binding domain), this would produce an apparent insensitivity to exogenous amino acids (and possibly cause constant activation by endogenous cytosolic amino acids). Incorporation of plant GLRs into host endomembranes could result in failure to detect activity of the foreign protein at all (as in the case of AtGLR2·8, but see below for an alternative explanation).

PREDICTION OF PHYSIOLOGICAL FUNCTION

What little published evidence there is indicates that plant GLRs function as constitutively active non-selective cation channels. The lack of gating of these channels is difficult to interpret. Given the ubiquity of apparently constitutively active non-selective cation currents observed in the plasma membrane (Demidchik *et al*., 2002), and the paucity of gene families predicted to encode them (at least in arabidopsis), it is quite likely that at least some GLRs contribute to these currents. However, the apparent conservation of the ligand-binding S1–S2 domains makes it likely that at least some subunits or subunit combinations show sensitivity to ligands. Attributions of function in the literature are mostly vague, but generally propose that GLRs function in Ca^{2+} signalling of extracellular amino acid levels at the plasma membrane.

In fact, the limited variety of NSCC gene candidates suggests that GLRs may have a number of different physiological roles, and could vary in ligand sensitivity and membrane location. Thus some GLR subunits could function as constitutively active NSCCs, and play roles in Ca^{2+} signalling and nutrient uptake at the plasma membrane, whereas others could function in amino acid-regulated intracellular signalling or metabolism. Plant chloroplast inner and thylakoid membranes, ER membranes and mitochondria possess a variety of non-porin ion channels, the regulation of which is relatively poorly known, and NSCC channels are abundant in the tonoplast membrane (Demidchik *et al*., 2002). In the case of glutamate, the intracellular distribution of glutamate is critical to its diverse roles, and endomembrane-localized GLRs could signal local N status. Glutamate is synthesized in the cytosol, plastids and possibly in mitochondria, de-aminated in mitochondria, and functions as an important intermediate in the photorespiratory cycle in peroxisomes (Fig. 3). The contributions of the different organelles to N metabolism vary with tissue type, light exposure and nutrient status of the plant. Endomembrane GLRs could release Ca^{2+} from intracellular stores to alter gene transcription of N assimilation enzymes in response to intracellular amino acid levels.

Alternatively, or in addition to Ca^{2+} signalling, GLRs could function as amino acid-gated NH_4^+ channels. NH_4^+ uptake has been proposed as a major physiological function of PM NSCCs in leguminous nodules and cereal roots (Tyerman *et al*., 1995; White, 1996; Davenport and Tester, 2000). NH_4^+ is taken up by plant roots and it is also the major intermediate of N assimilation (from reduction of NO_3^-) and N reassimilation (during photorespiration, protein recycling and seed germination). NH_4^+ is usually assimilated to amino acids for long-distance transport, but within cells it is shuttled between the chloroplast, mitochondrion and cytosol during release and assimilation, and is stored in the vacuole (Fig. 3). GLRs in the PM and endomembranes could function to transport NH_4^+ into cells and between intracellular compartments in response to changes in intracellular amino acid levels indicating a requirement for increased N assimilation or storage.

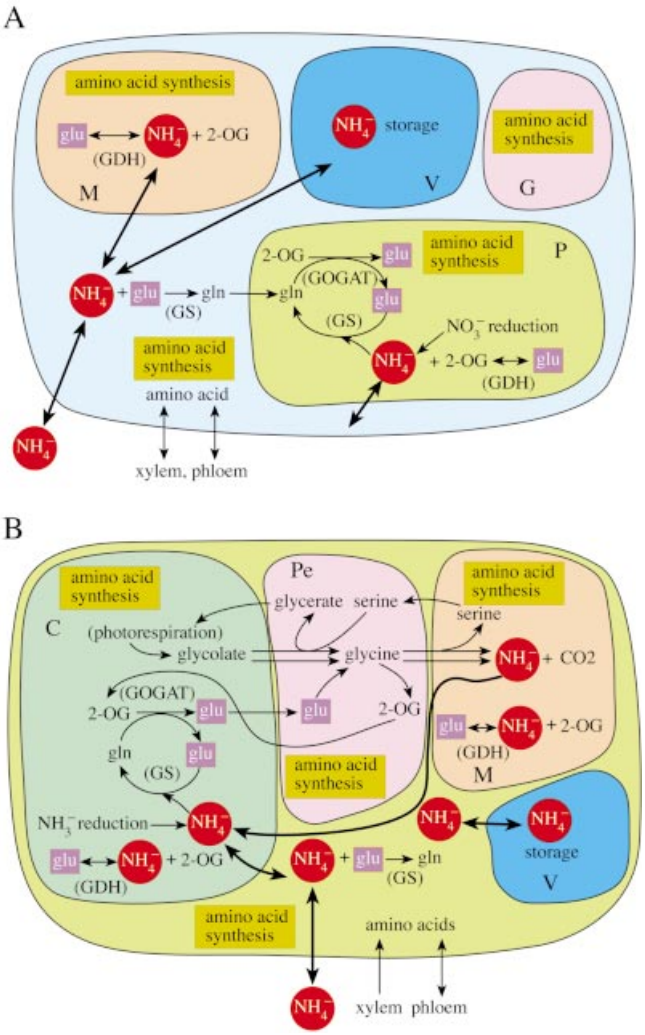

FIG. 3. Intracellular compartmentation of glutamate and ammonium metabolism in a generalized non-photosynthetic cell (A) and a generalized photosynthetic cell (B). The enzymes involved in glutamate (glu) and glutamine (gln) synthesis and de-amination are shown: GS, glutamine synthetase; GOGAT, glutamate synthase; GDH, glutamate dehydrogenase; and 2-OG, 2-oxoglutarate. The enzymic pathways shown will not necessarily all be present in a particular cell. The synthetic capacity of GDH *in planta* is disputed (Miflin and Habash, 2002). Enzymes for synthesis of other amino acids are present in the cytosol, mitochondria (M), plastids (P) including chloroplasts (C), glyoxysomes (G) and peroxisomes (Pe). In non-photosynthetic cells, NH_4^+ derives from nitrate reduction, amino acid deamination and from the apoplast including the soil solution. In photosynthetic cells, NH_4^+ derives from these pathways and also from photorespiration. Putative transmembrane transport of NH_4^+ is indicated in bold lines.

The advantages of NSCCs to species with highly selective ion channels is largely unknown in animals or plants. NSCCs may be used for Ca^{2+} transport because the bidirectional movement of cations in the channel (including K^+ efflux) would reduce the depolarizing effects of Ca^{2+} influx on membrane voltage. NH_4^+-conducting channels may be non-selective by biophysical necessity: no NH_4^+-selective channels have been discovered to date.

DIFFICULTIES IN CHARACTERIZATION OF PLANT GLUTAMATE RECEPTORS

Characterization of plant GLR genes has been very slow. There appear to be a number of problems affecting both cloning of cDNAs, and reconstitution of expressed cDNAs as functional ion channels.

Toxicity of AtGLR proteins in Escherichia coli *and eukaryotic hosts*

There is evidence for toxicity of some plant *GLR*s in *E. coli*. This is indicated by the prevalence of misspliced, incompletely spliced, or nonsense clones of these genes in arabidopsis cDNA libraries and populations cloned by RT-PCR (Davenport, unpubl. res.). These dysfunctional transcripts were selected by the biological cloning process, since sequencing of populations of cDNAs amplified from RNA without cloning indicated that the populations contained mainly correctly spliced RNAs. This selection for dysfunctional clones encoding truncated or frame-shifted proteins suggests that plant *GLR* cDNAs are transcribed and translated in *E. coli*, and encode toxic proteins. Correct *GLR* sequences possibly encode constitutively active or non-desensitizing ion channels, which cause toxic ion imbalances in *E. coli*. A number of plant transporters have been demonstrated to be functionally expressed in *E. coli* (Uozumi, 2001). Selection for dysfunctional clones occurs even in promotorless cloning vectors, presumably due to adventitious promoter activity of cryptic promoter sites present in common cloning vectors or within the N-terminal region of the gene itself. For some types of characterization, the problem of toxic cDNA expression can be avoided by use of genomic clones, as in the case of homologous overexpression of *AtGLR3·2*. In cases where the cDNA is required, it may be desirable to clone plant *GLR* cDNAs with inserted introns recognized by the intended eukaryotic host but which produce dysfunctional proteins in *E. coli*.

Problems with expression of cDNAs may also arise due to toxicity in eukaryotic hosts. Several groups have reported difficulties in obtaining functional expression of plant GLRs in oocytes. Interestingly, similar problems with toxicity have been encountered with plant cyclic nucleotide-gated channels, the other main group of non-selective cation channels identified in the arabidopsis genome.

Heteromerization

One problem in obtaining functional expression of GLRs may be a requirement for heteromerization to form functional ion channels. The absence of novel currents in oocytes injected with *AtGLR2·8* mRNA could be due to a requirement for additional subunits. Among the NMDA receptor subunits, NR1 produces a functional homomer, and is thought to act as the basic subunit required for functional heteromer formation with other NMDA subunits (Conley, 1995). One explanation for the higher frequency of type III transcripts may be that these genes encode the basic subunits of plant GLRs. Thus some GLRs may require coexpression with other subunits to demonstrate ion channel activity. Moreover, the characteristics of homomeric channels observed in heterologous systems may not match those of native channels, and extensive coexpression and colocalization studies may be required to determine the subunits contributing to ion channel activity *in vivo*. Since the *in vivo* activity of native GLRs is still unknown, the close matching of genes and function will be difficult.

CONCLUSIONS

At present we know too little about plant glutamate receptors and plant NSCCs to predict the relationship between them. Matching of plant GLRs to physiological function will require extensive characterization of tissue and membrane localization, as well as determination of ion selectivity and ligand sensitivities of homomeric and heteromeric GLRs. Transgenic plants and mutants should help to identify both gene function and the physiological roles of NSCCs, although prediction of phenotype is difficult, given the lack of information about NSCC function *in planta*. Along with cyclic nucleotide-gated channels, GLRs are the major candidates for NSCCs in plants. While GLRs have apparently maintained the major structural features of their animal homologues, they can be expected to show radical differences in functional character.

ACKNOWLEDGEMENTS

I thank the Royal Society for a Dorothy Hodgkin Research Fellowship, Newnham College, Cambridge for research support, Chris Cheffings for useful discussions and Mark Tester for critical comments.

LITERATURE CITED

Brenner ED, Martinez-Barboza N, Clark AP, Liang QS, Stevenson DW, Coruzzi GM. 2000. Arabidopsis mutants resistant to S(+)-β-methyl-α,β-diaminopropionic acid, a cycad-derived glutamate receptor agonist. *Plant Physiology* **124**: 1615–1624.

Cheffings CM. 2001. Calcium channel activity of a plant glutamate receptor homologue. *12th International Workshop on Plant Membrane Biology*. Madison, WI.

Chen G-Q, Cui C, Mayer ML, Gouax E. 1999. Functional characterisation of a potassium-selective prokaryotic glutamate receptor. *Nature* **402**: 817–821.

Chiu J, DeSalle R, Lam H-M, Meisel L, Coruzzi G. 1999. Molecular evolution of glutamate receptors: a primitive signaling mechanism that existed before plants and animals diverged. *Molecular Biology and Evolution* **16**: 826–838.

Conley EC. 1995. *The ion channels factsbook Vol. I. Extracellular ligand-gated channels.* San Diego: Academic Press.

Coruzzi G, Oliviera I, Lam H-M, Hsieh M-H. 1999. Plant glutamate receptors. *U.S. patent 5,981,703.*

Davenport RJ, Tester M. 2000. A weakly voltage-dependent, nonselective cation channel mediates toxic sodium influx in wheat. *Plant Physiology* **122**: 823–834.

Demidchik V, Davenport RJ, Tester M. 2002. Nonselective cation channels in plants. *Annual Reviews in Plant Physiology and Plant Molecular Biology* **53**: 67–107.

Demidchik V, Essah PA, Tester M. 2001. Effects of glutamate on nonselective cation currents in Arabidopsis root protoplasts. *12th International Workshop on Plant Membrane Biology*. Madison, WI.

Dennison KL, Spalding EP. 2000. Glutamate-gated calcium fluxes in Arabidopsis. *Plant Physiology* **124**: 1511–1514.

Dingledine R, Borges K, Bowie D, Traynelis SF. 1999. The glutamate receptor ion channels. *Pharmacological Reviews* **51**: 7–61.

Emanuelsson O, Nielsen H, Brunak S, von Heijne G. 2000. Predicting subcellular localization of proteins based on their N-terminal amino acid sequence. *Journal of Molecular Biology* **300**: 1005–1016.

Hammerland LG, Krapcho KJ, Garrett JE, Alasti N, Hung BCP, Simin RT, Levinthal C, Nemeth EF, Fuller FH. 1999. Domains determining ligand specificity for Ca^{2+} receptors. *Molecular Pharmacology* **77**: 642–648.

Hsieh M-H, Lam H-M, van de Loo FJ, Coruzzi G. 1998. A PII-like protein in *Arabidopsis*: putative role in nitrogen sensing. *Proceedings of the National Academy of Sciences of the USA* **95**: 13965–13970.

Johnson TL, Olsen LJ. 2001. Building new models for peroxisome biogenesis. *Plant Physiology* **127**: 731–739.

Kashiwagi K, Masuko T, Nguyen CD, Kuno T, Tanaka I, Igarishi K, Williams K. 2002. Channel blockers acting at *N*-methyl-D-aspartate receptors: differential effects of mutations in the vestibule and ion channel pore. *Molecular Pharmacology* **61**: 533–545.

Kim SA, Kwak JM, Jae S-K, Wang M-H, Nam HG. 2001. Overexpression of the *AtGluR2* gene encoding an Arabidopsis homolog of mammalian glutamate receptors impairs calcium utilisation and sensitivity to ionic stress in transgenic plants. *Plant and Cell Physiology* **42**: 74–84.

Knight J. 1999. Acting on impulse. *New Scientist* **163**: 7.

Lacombe B, Meyerhoff O, Steinmeyer R, Becker D, Hedrich R. 2001*a*. Role of Arabidopsis ionotropic glutamate receptors. Abstract. Association 'Canaux Ioniques' 12eme Colloque, La Londe les Maures.

Lacombe B *et al.* 2001*b*. The identity of plant glutamate receptors. *Science* **292**: 1486–1487.

Lam H-M, Coschigano KT, Oliviera IC, Melo-Oliviera R, Coruzzi GM. 1996. The molecular-genetics of nitrogen assimilation into amino acids in higher plants. *Annual Reviews in Plant Physiology and Plant Molecular Biology*: **47**: 569–593.

Lam H-M, Chiu J, Hsieh M-H, Meisel L, Oliviera IC, Shin M, Coruzzi G. 1998. Glutamate-receptor genes in plants. *Nature* **396**: 125–126.

Leng Q, Mercier RW, Yao W, Berkowitz GA. 1999. Cloning and first functional characterisation of a plant cyclic nucleotide-gated cation channel. *Plant Physiology* **121**: 753–761.

Leng Q, Mercier RW, Hua B-G, Fromm H, Berkowitz GA. 2002. Electrophysiological analysis of cloned cyclic nucleotide-gated ion channels. *Plant Physiology* **128**: 400–410.

Lomeli H, Sprengel R, Laurie DJ, Kohr G, Herb A, Seeburg PH, Wisden W. 1993. The rat delta1 and delta2 subunits extend the excitatory amino acid receptor family. *FEBS Letters* **315**: 139–143.

Maathuis FJM, Sanders D. 2001. Sodium uptake in Arabidopsis roots is regulated by cyclic nucleotides. *Plant Physiology* **127**: 1617–25.

Marsh DR, Holmes KD, Dekaban GA, Weaver LC. 2001. Distribution of an NMDA receptor:GFP fusion protein in sensory neurons is altered by a C-terminal construct. *Journal of Neurochemistry* **77**: 23–33.

Marshall J, Molloy R, Moss GW, Howe JR, Hughes TE. 1995. The jellyfish green fluorescent protein: a new tool for studying ion channel expression and function. *Neuron* **14**: 211–215.

Miflen BJ, Habash DZ. 2002. The role of glutamine synthetase and glutamate dehydrogenase in nitrogen assimiliation and possibilities for improvement in the nitrogen utilisation of crops. *Journal of Experimental Botany* **53** (Special Issue): 979–987.

Mullen RT, Flynn CR, Trelease RN. 2001. How are peroxisomes formed? *Trends in Plant Science* **6**: 256–261.

Oliviera IC, Brenner E, Chiu J, Hsieh M-H, Kouranov A, Lam H-M, Shin MJ, Coruzzi G. 2001. Metabolite and light regulation of metabolism in plants: lessons from the study of a single metabolic pathway. *Brazilian Journal of Medical and Biological Research* **34**: 567–575.

Premkumar LS, Auerbach A. 1997. Stoichiometry of recombinant *N*-methyl-D-aspartate receptor channels inferred from single-channel current patterns. *Journal of General Physiology* **110**: 485–502.

Rawat SR, Silim SN, Kronzucker HJ, Siddiqi MY, Glass ADM. 1999. AtAMT1·1 gene expression and NH_4^+ uptake in roots of *Arabidopsis thaliana*: evidence for regulation by root glutamine levels. *Plant Journal* **19**: 143–152.

Rosenmund C, Stern-Bach Y, Stevens CF. 1998. The tetrameric structure if a glutamate receptor channel. *Science* **280**: 1596–1599.

Sprengel R, Aronoff R, Volkner M, Schmitt B, Mosbach R, Kuner T. 2001. Glutamate receptor channel signatures. *Trends in Pharmacological Science* **22**: 7–10.

Strutz N, Villmann C, Thalhammer A, Kizelsztein P, Eisenstein M, Teichberg VI, Hollmann M. 2001. Identification of domains and amino acids involved in GluR7 ion channel function. *Journal of Neuroscience* **21**: 401–411.

Turano FJ, Panta GR, Allard MW, van Berkum P. 2001. The putative glutamate receptors from plants are related to two superfamilies of animal neurotransmitter receptors via distinct evolutionary mechanisms. *Molecular Biology & Evolution* **18**: 1417–1420.

Tyerman SD, Whitehead LF, Day DA. 1995. A channel-like transporter for NH_4^+ on the symbiotic interface of N_2 fixing plants. *Nature* **378**: 629–632.

Tyerman SD, Skerrett M, Garrill A, Findlay GP, Leigh RA. 1997. Pathways for the permeation of Na^+ and Cl^- into protoplasts derived from the cortex of wheat roots. *Journal of Experimental Botany* **48** (Special Issue): 459–480.

Uozumi N. 2001. *Escherichia coli* as an expression system for K^+ transport systems from plants. *American Journal of Cell Physiology* **281**: C733–C739.

Villmann C, Bull L, Hollmann M. 1997. Kainate binding proteins possess functional ion channel domains. *Journal of Neuroscience* **17**: 7634–7643.

Villmann C, Strutz N, Morth T, Hollmann M. 1999. Investigation by ion channel domain substitution of rat glutamate receptor subunits, orphan receptors and a putative NMDA receptor subunit. *European Journal of Neuroscience* **11**: 1765–1778.

White PJ. 1996. The permeation of ammonium through a voltage-independent K^+ channel in the plasma membrane of rye roots. *Journal of Membrane Biology* **152**: 89–99.

Wo Z, Oswald R. 1995. Unravelling the modular design of glutamate-gated ion channels. *Trends in Neuroscience* **18**: 161–168.

Xue H, 1998. Identification of major phylogenetic branches of inhibitory ligand-gated channel receptors. *Journal of Molecular Evolution* **47**: 323–333.

Zheng F, Erreger K, Low C-M, Banke T, Lee CJ, Conn PJ, Traynelis SF. 2001. Allosteric interaction between the amino terminal domain and the ligand binding domain of NR2A. *Nature Neuroscience* **4**: 894–901.

Zhuo D, Okamoto M, Vidmar JJ, Glass ADM. 1999. Regulation of a putative high-affinity nitrate transporter (*Nrt2;1At*) in roots of *Arabidopsis thaliana*. *Plant Journal* **17**: 563–568.

Zuo J, De Jager PL, Takahashi KA, Jiang W, Linden DL, Heintz N. 1997. Neurodegeneration in Lurcher mice caused by mutation in δ2 glutamate receptor gene. *Nature* **388**: 769–773.