Glutamate receptor-like channels in plants: a role as amino acid sensors in plant defence?
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Abstract
Plant glutamate receptor-like genes (GLRs) are homologous to the genes for mammalian ionotropic glutamate receptors (iGluRs), after which they were named, but in the 16 years since their existence was first revealed, progress in elucidating their biological role has been disappointingly slow. Recently, however, studies from a number of laboratories focusing on the model plant species Arabidopsis thaliana (L.) have thrown new light on the functional properties of some members of the GLR gene family. One important finding has been that plant GLR receptors have a much broader ligand specificity than their mammalian iGluR counterparts, with evidence that some individual GLR receptors can be gated by as many as seven amino acids. These results, together with the ubiquity of their expression throughout the plant, open up the possibility that GLR receptors could have a pervasive role in plants as non-specific amino acid sensors in diverse biological processes. Addressing what one of these roles could be, recent studies examining the wound response and disease susceptibility in GLR knockout mutants have provided evidence that some members of clade 3 of the GLR gene family encode important components of the plant’s defence response. Ways in which this family of amino acid receptors might contribute to the plant’s ability to respond to an attack from pests and pathogens are discussed.

Introduction
iGluRs are ligand-gated ion channels best known for their role in fast excitatory neurotransmission in the mammalian central nervous system. Therefore, the discovery in 1998 that plants have a large family of iGluR-like genes (the GLR genes) presented something of an enigma [1]. What could this gene family be doing in an organism with no nervous system? Despite the excitement that their discovery provoked, GLR genes have been slow to relinquish their secrets, and it is only very recently that efforts to understand their biological functions have really begun to bear fruit. The structure and evolutionary origins of the plant GLRs have been the subject of two detailed reviews [2,3], and here we focus only on the most recent advances. Amongst the most important recent findings are that plant GLRs appear to have a much broader ligand specificity than their mammalian homologues and that some members of the GLR family in Arabidopsis have a role in the innate immune response.

Ligand promiscuity of the plant glutamate-like receptors
Once the Arabidopsis thaliana genome had been fully sequenced, it was found that this model plant species has 20 GLR genes (Arabidopsis thaliana glutamate-like receptor [AtGLRs]) that can be grouped into three clades [4]. The plant GLRs are predicted to have the same modular structure as their mammalian homologues, with an amino-terminal domain, a ligand-binding domain, a transmembrane domain that includes the pore region, and a carboxyl-terminal domain [4]. Functionally, mammalian iGluRs are glutamate-gated cation channels, selective for Na⁺, K⁺, and Ca²⁺ ions.
Aspartate is also an iGluR agonist, but a weak one, and glycine and D-serine act as co-agonists with glutamate for some iGluRs [5].

In contrast to the ligand specificity of the iGluRs, in planta studies using GLR knockout mutants [6-8] recently supported by heterologous expression experiments [9,10] indicate that, collectively, the GLR receptors in Arabidopsis are gated by a broad spectrum of amino acids. The data for the three AtGLR genes that have been studied in most detail (summarised in Table 1) suggest that at least 12 of the 20 proteinogenic amino acids, as well as the tripeptide glutathione (GSH), can serve as GLR agonists. Although it is possible that the phenotype of GLR knockout mutants could be influenced by pleiotropic effects on other channels, the heterologous expression experiments found that AtGLR1.4 was gated by seven amino acids and AtGLR3.4 by three (different) amino acids [9,10]. Significantly, glutamate was not amongst the group of agonists identified for either AtGLR1.4 or AtGLR3.4 in these experiments. The heterologous expression studies also found that AtGLR1.4, like mammalian iGluRs, acted as a non-selective, Ca\(^{2+}\)-permeable cation channel [9] but that AtGLR3.4 was highly selective for Ca\(^{2+}\) over Na\(^{+}\) [10]. These findings are consistent with in planta evidence that glutamate and other amino acids trigger membrane depolarisation and Ca\(^{2+}\) influx in a GLR-dependent manner (reviewed in [3]).

On the basis of the homology modelling of their ligand-binding domains, it has been concluded that all members of the AtGLR family are likely to be gated by amino acid residues and that the broad ligand specificity observed experimentally is consistent with the high degree of sequence diversity found within the region of the ligand-binding domain that is predicted to bind the agonist side chain [9]. Therefore, even though only a few of the 20 AtGLRs have so far been characterised, it seems safe to conclude that collectively the plant GLR family is likely to be gated by a significant proportion of the 20 proteinogenic amino acids as well as by an unknown number of related molecules, such as small peptides like GSH. In the N-methyl D-aspartate (NMDA) subgroup of mammalian iGluRs (the subgroup most closely related to plant GLRs), the amino-terminal domain contains sequences related to the bacterial periplasmic-binding domain that binds a variety of small molecules and ions and that plays an important role in both negative and positive allosteric regulation of the receptor [11]. Plant GLRs possess a similar conserved sequence in their amino-terminal domain [12,13], suggesting the potential for additional sensory complexity arising from allosteric interactions between this domain and as-yet-unknown regulatory molecules.

**Role of plant glutamate-like receptors in the immune response**

The first indication that GLRs might have a role in the plant defence response came from the finding that transgenic Arabidopsis plants overexpressing a radish GLR complementary DNA (RsGluR) showed an increased expression of a number of defence-related genes and enhanced the resistance to a necrotic fungal pathogen (*Botrytis cinerea*) [14]. Two later pharmacological studies reported that antagonists of mammalian iGluRs were able to interfere with aspects of the immune response in tobacco suspension culture cells [15] and Arabidopsis seedlings [16]. These early findings have now been backed up by genetic evidence from the study of a number of AtGLR knockout mutants [17,18]. Analysis of the immune response in *atglr3.3* mutants found increased susceptibility to the bacterial pathogen *Pseudomonas syringae*, which was
correlated with a defect in the activation of defence gene expression in response to infection [17]. The same study demonstrated that the abilities of GSH to activate defence gene expression and to enhance the immunity to \textit{P. syringae} were also dependent on \textit{AtGLR3.3}. This aspect of the disease resistance phenotype was specific to a small subset of \textit{AtGLR3.3} agonists, since of the ligands able to elicit \textit{AtGLR3.3}-dependent membrane depolarisation in roots [6], only GSH and cysteine were able to suppress bacterial growth in an \textit{AtGLR3.3}-dependent manner [17]. In an independent study, \textit{AtGLR3.3} was also found to be required for basal resistance to downy mildew disease, caused by the oomycete pathogen, \textit{Hyaloperonospora arabidopsidis} [18]. Again, \textit{atglr3.3} mutants failed to activate defence gene expression in response to infection. Both \textit{P. syringae} and \textit{H. arabidopsidis} are biotrophic pathogens (they do not directly kill plant tissues), but when \textit{atglr3.3} mutants were infected with the necrotrophic fungal pathogen \textit{Botrytis cinerea}, no difference in resistance was detected [18]. This may suggest that \textit{AtGLR3.3} is required for the full activation of salicylic acid-dependent plant defences, which are typically associated with resistance against biotrophic, but not necrotrophic, pathogens. So far, all GLR genes implicated in the defence response (including \textit{RsGlur}) belong to clade 3 of the GLR family. The finding that mutations in four other clade 3 GLRs (\textit{AtGLR3.1}, \textit{AtGLR3.4}, \textit{AtGLR3.5}, and \textit{AtGLR3.7}) had no impact on the resistance to either \textit{H. arabidopsidis} or \textit{B. cinerea} [18] suggests either that roles in pathogen resistance are restricted to a subset of clade 3 GLRs or that there is functional redundancy amongst some of these \textit{AtGLR} genes.

Basal resistance to virulent pathogens, such as \textit{P. syringae} and \textit{H. arabidopsidis}, is conferred principally by so-called PAMP (pathogen-associated molecular patterns)-triggered immunity (PTI), which is activated by the recognition of PAMPs [19]. iGluR-related receptors were previously suggested to have a role in PAMP-mediated signaling in Arabidopsis, based on the ability of the antagonists of mammalian iGluRs to block cytoplasmic Ca\textsuperscript{2+} transients triggered by two common PAMPs perceived by plants: the peptides flg22 and eff18 [16]. Although the specificity and precise targets of iGluR antagonists in plants are still unclear, these findings suggest the possibility that plant GLRs mediate PTI by acting as Ca\textsuperscript{2+} channels downstream of PAMP perception by pattern-recognition receptors (PRRs).

\textit{AtGLR3.3} has also been implicated in the defence response to mechanical wounding [20], which has many features in common with the defence response to feeding by insects and related pests [21]. Wounding and herbivore feeding elicit responses both in the injured leaf and, systemically, in undamaged parts of the plant. Several long-distance signals, including chemical, hydraulic, and electrical signals, have previously been suggested to carry information to systemic leaves [21]. The concept of electrical signalling in plants often generates much interest but with the exception of a few specific responses, such as rapid movements in plants such as the Venus flytrap [22], it is relatively poorly defined. However, Mousavi and colleagues identified several GLRs from amongst a panel of membrane transporters as proteins required for systemic transmission of a wound-induced electrical signal in Arabidopsis [20]. Importantly, systemic wound-induced gene expression was tightly correlated with electrical signal transmission, and both responses were eliminated in \textit{atglr3.3 atglr3.6} double mutants. Although the wound-induced electrical signal moves as a wave of plasma membrane depolarisation [20], which would be consistent with GLR activity as an inward cation channel, it has yet to be established whether GLRs are directly responsible for the propagation of the electrical signal, or whether they act indirectly, in the upstream signalling process that generates the initial signal. The data supporting the role of GLRs in plant defence responses are summarised in Table 2.

**Concluding remarks**

Had the plant GLR gene family been characterised before their mammalian iGluR homologues, it now seems likely they would have been referred to as general or non-specific amino acid receptors rather than glutamate receptors. In the breadth of their ligand specificity, they are analogous to other general amino acid sensors like the yeast Ssy1p transceptor [23] and some members of the mammalian Ca\textsuperscript{2+}-sensing receptor superfamily [24]. The ubiquity of their expression in the plant [2,25] and their potential localisation on both the plasma membrane and the chloroplast inner membrane [9,26,27] place the GLRs in an ideal location to serve multiple roles in sensing amino acids (and related molecules) endogenously, intercellularly, and in the soil environment. In their potential role as sensors of both internal and environmental chemical cues, they resemble the recently uncovered family of ionotropic receptors (IRs) in Drosophila, which is a variant subfamily of the iGluRs that function as odorant and taste receptors for a diverse range of molecules [28]. Root growth and branching are known to be sensitive to signals from external glutamate [29] and many biological processes in plants are potentially regulated by amino acid signalling [30].

At least in the case of \textit{AtGLR3.3} and perhaps other members of GLR clade 3, there is now strong evidence for a role in the innate immune response but what might
this role be? Like animals, plants use conserved molecules of their enemies as PAMPs (or HAMPs in the case of herbivore-associated molecular patterns) to stimulate defence responses [19,31], but it is becoming increasingly clear that they also use certain molecules released by their own damaged cells (called damage-associated molecular patterns, or DAMPs) for the recognition of "damaged-self" [32]. It has been hypothesised that these DAMPs can act in concert with PAMPs to help the host differentiate between beneficial or harmless microbes and those that are causing pathological damage [32]. For example, extracellular ATP released from damaged cells is sensed by neighbouring intact cells to stimulate defence responses in both animals and plants [33,34]. It is possible that the ability of AtGLR3.3 and other clade 3 GLRs to act as sensors of changes in the extracellular amino acid concentration provides an important additional signal to confirm that tissue damage has taken place (e.g. after wounding or herbivore activity). In the case of pathogen attack, increased concentrations of extracellular amino acids have been found in infected tissues [35], and PAMP-induced exocytosis of glutamate has been reported in tobacco cells [14].

In Figure 1, we present a speculative model that attempts to integrate the evidence that GLRs have a role in plant defence, with what is known of other pathways by which plants activate their defence responses. The model incorporates the idea that GLR ligands might in some cases function as DAMPs and suggests that the sensing of different combinations of host- and non-host-derived cues by GLRs and other receptors may be integrated to differentially regulate alternative plant defence pathways. In the model, jasmonic acid-dependent defences are activated by increased amino acid concentrations (perceived by the GLR receptors [20]), by increased extracellular ATP (eATP) concentrations (perceived by the newly identified DORN1 [does not respond to nucleotides 1] eATP receptor [36]), and by specific herbivore-derived elicitors (HAMPs) perceived by PRRs [31]. Salicylic acid-mediated defence activated following perception of PAMPs by their cognate PRRs, may also be partly dependent on GLR signalling activated by pathogen-induced increases in apoplastic amino acid concentrations [15,35], and on DORN1 signalling in response to reduced levels of eATP [37].

Establishing whether amino acids (and other potential GLR ligands) do indeed function as DAMPs will require a much better understanding of the positioning of GLRs in defence signalling pathways and, more importantly, a clearer definition of which of the potential ligands are biologically relevant for defence responses. Given the ligand promiscuity of plant GLRs,
the number of candidates for the biologically relevant ligands may be very large.

Finally, it is worth noting that in the intervening years since the GLRs were discovered in plants, the presence of iGluRs in a variety of cell types beyond the synapse has been recognised (even if their functions in those cells are not necessarily very clear) [38], so that even in mammals, iGluRs are no longer thought of as strictly being associated with the nervous system. Perhaps significantly, in the context of the new research on plant GLRs discussed here, iGluRs are abundant on the surface of T cells and other cells of the mammalian immune system and glutamate has been identified as an important immunomodulator [39,40]. Given the ancient evolutionary origins of the innate immune
system and the homologies that exist between some of its key components in plants and animals [41]. Perhaps parallels will yet be found between the roles of iGluRs and GLRs in their respective defence response systems.

**Abbreviations**

AtGLR, *Arabidopsis thaliana* glutamate-like receptor; DAMP, damage-associated molecular pattern; DORN1, does not respond to nucleotides 1; eATP, extracellular ATP; GLR, glutamate-like receptor; GSH, glutathione (reduced); HAMP, herbivore-associated molecular pattern; iGluR, ionotropic glutamate receptor; PAMP, pathogen-associated molecular pattern; PRR, pattern-recognition receptor; Pti, PAMP-triggered immunity.

**Disclosures**

The authors declare that they have no disclosures.

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