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Review

*Tansley insight*

# Structural insights into long-distance signal transduction pathways mediated by plant glutamate receptor-like channels

Author for correspondence:
*Alex Costa*
*Email: alex.costa@unimi.it*

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**Matteo Grenzi[1], Maria Cristina Bonza[1], Andrea Alfieri[2] and Alex Costa[1,3]**

[1]Department of Biosciences, University of Milan, Via G. Celoria 26, Milano 20133, Italy; [2]Centro Grandi Strumenti, University of Pavia, via Ferrata 9, Pavia 27100, Italy; [3]Institute of Biophysics, National Research Council of Italy (CNR), Via G. Celoria 26, Milano 20133, Italy

## Contents



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## Summary

In recent years, studies have shed light on the physiological role of plant glutamate receptor-like channels (GLRs). However, the mechanism by which these channels are activated, and in particular, what is the physiological role of their binding to amino acids, remains elusive. The first direct biochemical demonstration that the *Arabidopsis thaliana* GLR3.3 isoform binds glutamate and other amino acids in a low micromolar range of concentrations was reported only recently. The first crystal structures of the ligand-binding domains of *At*GLR3.3 and *At*GLR3.2 isoforms also have been released. We foresee that these new experimental pieces of evidence provide the basis for a better understanding of how GLRs are activated and modulated in different physiological responses.

## I. Introduction: plant glutamate receptor-like channels

Calcium ($Ca^{2+}$) is a key second messenger in plant cells. It is universally involved in different developmental programs as well as in plant local and systemic responses to changing environments. The generation of free cytosolic $Ca^{2+}$ transients requires the opening of $Ca^{2+}$-permeable channels which regulate cytosolic $Ca^{2+}$ influx (Kudla *et al.*, 2018).

Ionotropic glutamate receptors (iGluRs) are ligand-gated nonselective cation channels that mediate neurotransmission in the animal central nervous system (Traynelis *et al.*, 2010). Homologous proteins were identified in plants, namely glutamate receptor-like channels (GLRs). In *Arabidopsis thaliana*, 20 GLR members were grouped into three clades (I, II and III; Lam *et al.*, 1998), and have been found to be involved in root development, seed germination, ion transport, metabolic pathways and $Ca^{2+}$ signaling (reviewed in Wudick *et al.*, 2018a). Several members of the Arabidopsis GLRs are expressed in pollen and are crucial for the generation of the $Ca^{2+}$-tip gradient as well as for proper pollen tube growth, attraction and fertility (Michard *et al.*, 2011; Wudick *et al.*, 2018b; Mou *et al.*, 2020). In addition, the two *Physcomitrella patens* GLRs are essential for both chemotaxis and reproduction (Ortiz-Ramírez *et al.*, 2017). Intriguingly, both Arabidopsis and *Solanum lycopersicum* GLRs, particularly those belonging to Clade III, are elemental in long-distance signaling (Mousavi *et al.*, 2013; Farmer *et al.*, 2014; Nguyen *et al.*, 2018; Toyota *et al.*, 2018; Wang *et al.*, 2019; Goto *et al.*, 2020; Shao *et al.*, 2020).

Original studies and valuable reviews have plausibly adopted the model of regulation/activation of the animal iGluRs for plant GLRs; however, several sets of data have described the functionality of some GLRs without the need for the ligand. Here, in the light of recent biochemical and structural characterization of GLRs, we provide insights into how to use this information to clarify the ways in which these proteins exert their roles.

## II. Structural features and conservation of GLRs

The architecture and stoichiometry of plant GLRs are believed to be similar to those of iGluRs, with the assembly of four subunits (homomeric or heteromeric) arranged to form a functional channel (Wudick *et al.*, 2018a). Each subunit hosts an extracellular amino-terminal domain, a ligand-binding domain (LBD), three full transmembrane helices plus a partial one, and a cytoplasmic tail. As a general feature, the LBD has a conserved clamshell architecture (Fig. 1). In animals the binding of a ligand/agonist induces a variable degree of closure of the LBD that pulls on the transmembrane segments, opening the channel pore (Traynelis *et al.*, 2010). The first crystal structures of the LBDs of the two plant isoforms *At*GLR3.2 and *At*GLR3.3 were recently released. They displayed the predicted bilobed structure resembling prokaryotic and eukaryotic LBDs (Gangwar *et al.*, 2020; Alfieri *et al.*, 2020; Mayer, 2020). Binding experiments performed on *At*GLR3.3-LBD revealed its preference not only for L-Glu, but also for sulfur-containing amino acids (AA) (i.e. L-Cys and L-Met). Four solved *At*GLR3.3-LBD crystal structures in complex with L-Glu, Gly, L-Cys and L-Met provided a rationale for how the plant LBD binding site evolved to accommodate diverse AA and identified key residues involved in their binding (Alfieri *et al.*, 2020). A key feature coming from both structural and biochemical data is that, in contrast to the selectivity profiles of prokaryotic and other eukaryotic GLRs, where a restricted preference for 1 or 2 L-AA is usually observed, *At*GLR3.3 is able to bind and accommodate different AA. Remarkably, despite their different affinities and their different abilities to evoke cytosolic $Ca^{2+}$ increases in root tip cells, the extent of the *At*GLR3.3-LBD clamshell closure is the same for all ligands (Alfieri *et al.*, 2020). Structural data of *At*GLR3.2 and modeling analyses predict that other Arabidopsis GLRs also show 'ligand promiscuity' even if with important differences. As examples, GLR1.2, GLR1.4 and GLR3.1/GLR3.5, are predicted to accommodate D-Ser or bulkier hydrophobic AAs (Michard *et al.*, 2011; Tapken *et al.*, 2013; Kong *et al.*, 2016). Besides, the nonproteinogenic amino acid 1-Aminocyclopropane-1-carboxylic acid (ACC) is another possible *At*GLR3.3 ligand (Mou *et al.*, 2020), a suggestion well-supported by a modeling approach (Fig. 1).

Overall, the *At*GLR3.2-LBD and *At*GLR3.3-LBD crystal structures represent a rational tool to generate homology models for other Arabidopsis GLRs and to derive clues about their binding specificities, thus helping to define, by *in vivo* approaches, the functional role of GLR ligands which is still unclear.

## III. GLRs in long-distance electrical and $Ca^{2+}$ signaling

Although plants do not have a nervous system, distant organs can 'communicate' the perception of environmental stimuli by means of various mechanisms (Choi *et al.*, 2016), including the fast propagation of electrical signals (Hedrich *et al.*, 2016) which can be coupled with changes in free cytosolic $Ca^{2+}$, as in the local and systemic wounding responses (Kiep *et al.*, 2015; Vincent *et al.*, 2017; Nguyen *et al.*, 2018; Shao *et al.*, 2020). Besides electrical and $Ca^{2+}$ signals, reactive oxygen species (ROS) also play a role in long-distance signaling with clear connections to $Ca^{2+}$ signaling. The ROS–$Ca^{2+}$ crosstalk has been the subject of recent reviews; therefore, we direct the readers to them (e.g. Gilroy *et al.*, 2016).

The first demonstration that plants generate electrical signals moving within the body in response to different stimuli dates to the end of the 19th Century (reviewed in Hedrich *et al.*, 2016, and Farmer *et al.*, 2020), with an important contribution from Bowles' group in tomato (Wildon *et al.*, 1992). A leap forward to the understanding of their physiological roles came from Farmer's group who demonstrated that leaf wounding, by triggering long-distance electrical signals, elicits an increase in the jasmonic acid (JA) concentrations and the expression of genes involved in JA signaling in systemic leaves (Mousavi *et al.*, 2013). The genetic demonstration that electrical signals were dependent on the activity of several isoforms of the Clade III *At*GLRs (3.2, 3.3 and 3.6) provided the first evidence that these channels mediate long-distance signaling. It was notable that the double *glr3.3/glr3.6* mutant showed no electrical signals in the systemic leaves upon wounding (Mousavi *et al.*, 2013). The following demonstration that *At*GLR3.3 and *At*GLR3.6 are expressed in different vascular tissues (phloem and xylem parenchyma cells, respectively) proved that both tissues are synergistically involved in the electrical signal transmission (Nguyen *et al.*, 2018). However, it appeared that, in this specific case, these two GLRs do not assemble in a heteromeric complex, as instead predicted in root epidermal cells (Mou *et al.*, 2020) where they are co-expressed (Vincill *et al.*, 2013; Singh *et al.*, 2016).

In Arabidopsis, by simultaneously performing surface membrane potential measurements and cytosolic $Ca^{2+}$ imaging, the coupling between wounding-induced GLR-mediated electrical signals and propagating $Ca^{2+}$ waves was demonstrated, albeit the maximum $Ca^{2+}$ increase temporally followed the depolarization (Nguyen *et al.*, 2018). Evidence exists that different GLRs show $Ca^{2+}$ permeability (e.g. *At*GLR1.4, *At*GLR3.2, *At*GLR3.3, *At*GLR3.4, *At*GLR3.6, *Os*GLR2.1; *Pp*GLR1) (Vincill *et al.*, 2012; Tapken *et al.*, 2013; Kong *et al.*, 2016; Ni *et al.*, 2016; Ortiz-Ramirez *et al.*, 2017; Wudick *et al.*, 2018b; Shao *et al.*, 2020), and thus, based on the results reported in Nguyen *et al.* (2018), it is plausible that at least *At*GLR3.3 and *At*GLR3.6 can directly couple these two long-distance signals induced by wounding. However, more complex scenarios cannot be excluded with GLRs responsible for the surface depolarization that in turn activates voltage-dependent $Ca^{2+}$-permeable channels (Zimmermann & Felle, 2009). Admittedly, many unsolved questions regarding the link between GLRs activation, membrane depolarization and $Ca^{2+}$ increase still need to be clarified, and we direct interested readers to a recent review (Farmer *et al.*, 2020). Nevertheless, although there may be a direct or indirect role of GLRs in triggering the free cytosolic $Ca^{2+}$ increase, this rise stimulates JA biosynthesis that helps plants to cope overall with injuries caused by pathogens (e.g.

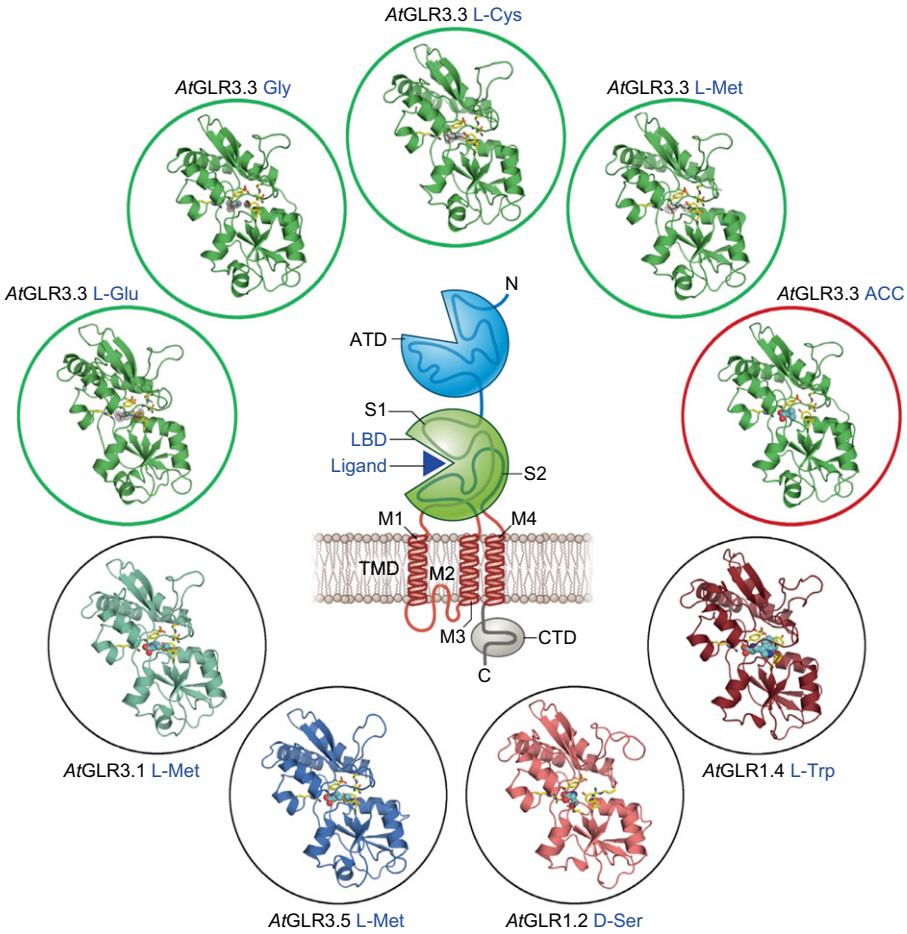


**Fig. 1** Predicted architecture of plant glutamate receptor-like channels (GLRs) and structural determinants of the ligand-binding domain. The schematic drawing shows the predicted membrane structure of a single GLR subunit which hosts an extracellular amino-terminal domain (ATD), a ligand-binding domain (LBD) composed of segments S1 and S2, four membrane helices (M1 to M4, one of which – M2 – is not fully transmembrane), and a cytoplasmic tail (CTD), arranged in the order ATD-S1-M1-M2-M3-S2-M4-CTD. The four upper green-lined circles show the structures in ribbon representation of the *At*GLR3.3-LBD bound to different ligands (from the left to the right: L-Glu, Gly, L-Cys and L-Met). The atomic coordinates and structure factors are deposited in the Protein Data Bank, http://www.wwpdb.org (PDB ID codes 6R85, 6R88, 6R89 and 6R8A for the complexes of the *At*GLR3.3-LBD with L-Glu, Gly, L-Cys and L-Met, respectively). The red-lined circle shows that the same *At*GLR3.3-LBD structure is in principle able to accommodate the Aminocyclopropane-1-carboxylic acid (ACC) ligand without any obvious steric hindrance. The remaining four bottom black circles show homology modeling, based on the above-mentioned *At*GLR3.3-LBD structure, of the LBDs of other GLR isoforms. The modeling approach appears to justify the binding of their respective predicted ligands (L-Met for *At*GLR3.1 and *At*GLR3.5, D-Ser for *At*GLR1.2 and L-Trp for *At*GLR1.4) and, more generally, the preference of these isoforms for bulkier hydrophobic amino acid ligands.

by chewing insects) (Nguyen *et al.*, 2018; Yan *et al.*, 2018). As an example, larvae of the African cotton leafworm, *Spodoptera littoralis*, gained more weight feeding on the *glr3.3* and *glr3.3/glr3.6* (as well as *glr3.1/glr3.3*) mutants than on the wild-type (Nguyen *et al.*, 2018). The strict relationship between pathogen attack, long-distance GLR-mediated electrical signaling and JA biosynthesis also has been found in tomato. Grafting experiments demonstrated that *Sl*GLR3.5 (homologous to *At*GLR3.3) is required for the root-to-shoot systemic transmission of electrical signals generated in response to the root-knot nematode *Meloidogyne incognita,* the root attacks of which led to an increase of jasmonates in leaves (Wang *et al.*, 2019).

## IV. How do plant GLRs work?

A wealth of genetic information on the role played by the Clade III GLR isoforms in long-distance signaling is currently available, but the mechanisms of their *in planta* activation/regulation need to be refined. In the glutamatergic synapse of the animal nervous system, the glutamate is released from the presynaptic neuron with the consequent activation of the iGluR receptors in the postsynaptic neuron, allowing the passage of sodium ($Na^+$) and $Ca^{2+}$ (Fig. 2a) (Traynelis *et al.*, 2010). As proposed previously by Nguyen *et al.* (2018), making a simple analogy with iGluRs activation, we can foresee that in plants mechanical wounding or insect chewing might induce the release of glutamate or other AAs in the apoplast, thus activating – through direct binding – the GLRs with a consequent $Ca^{2+}$ influx into the cytosol (Fig. 2c) (Vincent *et al.*, 2017; Nguyen *et al.*, 2018; Toyota *et al.*, 2018; Shao *et al.*, 2020). In Arabidopsis this scenario is supported by two pieces of evidence: (1) the use of the genetically encoded glutamate fluorescent sensor (iGluSnFR) reported a local increase in the apoplastic Glu concentration of the wounded leaf (Toyota *et al.*, 2018); and (2) treatment of Arabidopsis leaves or roots with a high concentration

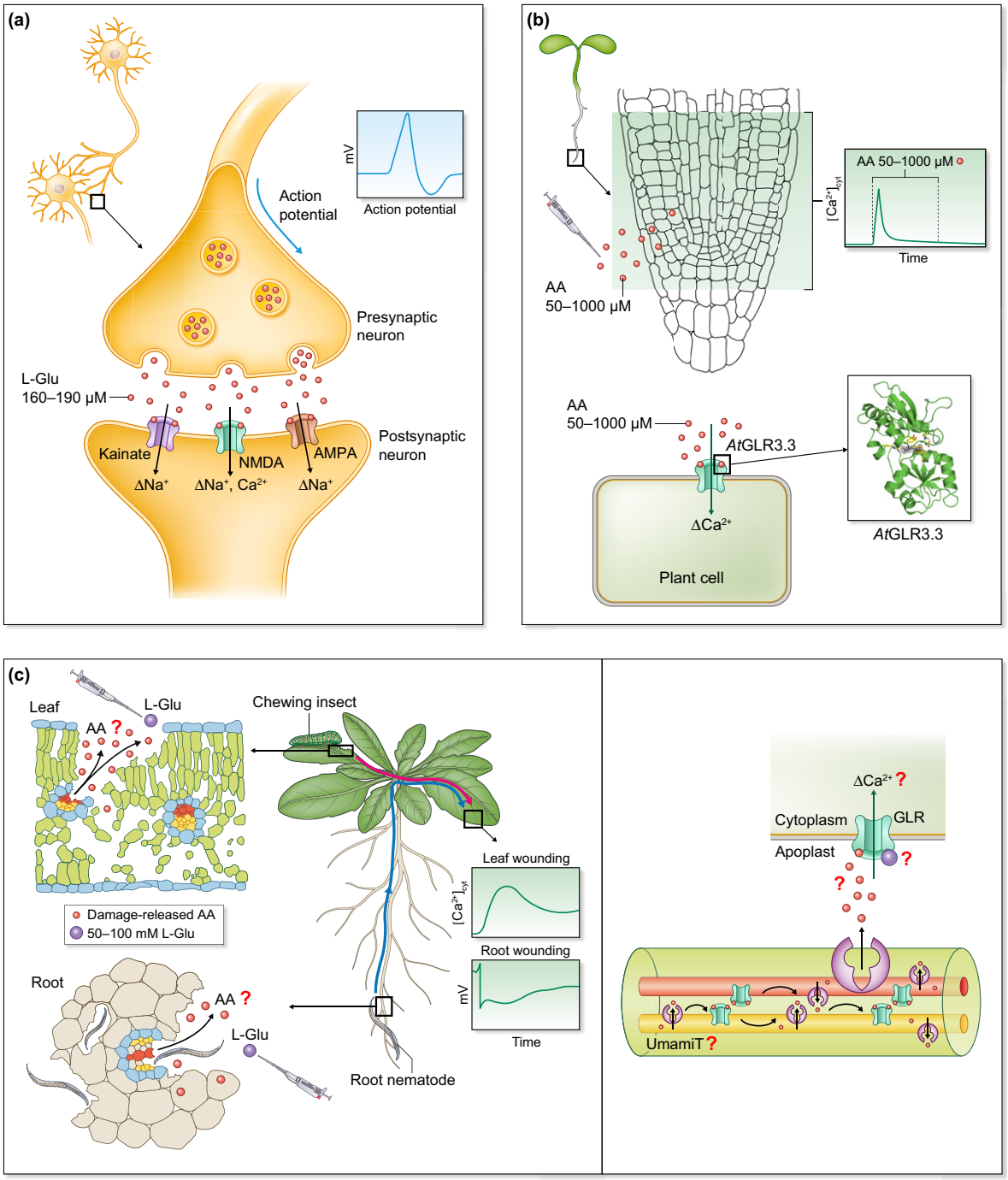


**Fig. 2** Functional comparison of iGluRs and glutamate receptor-like channels (GLRs) activation in animal and plant cells. (a) The accepted model of iGluR activation mechanism in an animal synapse. An action potential in the presynaptic neuron triggers the release of Glu (μM range of concentrations) (Dzubay & Jahr, 1999) with the following activation of the plasma membrane-localized Kainate, NMDA and AMPA receptors in the postsynaptic neuron, allowing the passage of sodium ($Na^+$) and calcium ($Ca^{2+}$). (b) In the plant root tip cells, exogenous administration of L-Glu and other amino acids (AA) in a μM concentration range (50–1000 μM with a maximum response to 500 μM) triggers a transient increase in the free cytosolic $Ca^{2+}$ concentration that in Arabidopsis is dependent on the presence of the *At*GLR3.3 isoform (for which the *in vitro* $K_d$ for L-Glu is 2.2 μM). (c) Leaf and root wounding triggers long-distance surface potential depolarizations and free cytosolic $Ca^{2+}$ increases that are largely dependent on clade III GLRs. Likewise, chewing insects trigger depolarization and $Ca^{2+}$ waves from leaf to leaf, and root nematode attack elicits a depolarization wave from root to shoot. The presented model would predict that local wounding (mechanical or resulting from the interaction with pathogens) induces a local AA release that activates the GLR receptors. In support of this, the local administration of 50–100 mM L-Glu (a concentration 1000-fold higher than the one required in root tip cells) to wounded tissues (leaf or root) triggers long-distance $Ca^{2+}$ waves. Whereas the local release of L-Glu or other amino acids in response to wounding can indeed activate the GLRs, the mechanism of their activation in distant vascular tissues is not yet defined. It might be hypothesized that in the vascular tissues the GLR activation also requires AA binding; however, in this case, owing to the absence of any damaged cells, a controlled release of AA should occur. If the AA transporters UmamiT are expressed in the vascular tissues, their activity could be upstream of the GLR activation. The question marks represent possible speculative models that need to be proven experimentally.

of Glu triggered long-distance $Ca^{2+}$ signaling (Toyota *et al.*, 2018; Shao *et al.*, 2020). Moreover, in Arabidopsis, several studies reported that Glu (and other AAs) induced free cytosolic $Ca^{2+}$ increase and plasma membrane (PM) depolarization in seedlings and root cells, two events dependent on *At*GLR3.3 (Qi *et al.*, 2006; Alfieri *et al.*, 2020) (Fig. 2b). The fact that *At*GLR3.3-LBD binds Glu and other AAs in the low micromolar range of concentrations matches with the demonstration that concentrations as low as 50 μM of L-Glu, L-Cys and Gly can induce $Ca^{2+}$ increase in Arabidopsis root cells (Stephens *et al.*, 2008; Alfieri *et al.*, 2020), and that different AAs, including ACC, when administrated at a maximum of 500 μM, stimulate ion transport in mammalian COS-7 cells expressing *Pp*GLR1 (Mou *et al.*, 2020). However, this high binding affinity is at odds with the high glutamate concentration required to trigger the long-distance plant defense signaling in Arabidopsis (50–100 mM) (Toyota *et al.*, 2018; Shao *et al.*, 2020), and the systemic potentials transmission (10 mM) (Zimmermann & Felle, 2009) and action potentials (1–100 mM) in *Hordeum vulgare* (Felle & Zimmermann, 2007). Whereas the requirement for high AA concentrations *in planta* can be explained by the presence of physical barriers, the need for very high AA concentrations to trigger $Ca^{2+}$ increases in mammalian HEK293T cells expressing the *At*GLR3.3 and *At*GLR3.6, as reported in Shao *et al.* (2020), might be carefully considered because this does not match up to both *in vitro* and *in vivo* measurements (Alfieri *et al.*, 2020; Mou *et al.*, 2020), and certainly needs clarification.

We might hypothesize that in response to wounding – not only locally, but also in vascular tissues – a release of AA into the apoplast may trigger the activation of PM-localized GLRs (e.g. *At*GLR3.3 and 3.6), thus driving $Ca^{2+}$ influx (Fig. 2b). If this is so, we can foresee that the AA release must be fine-tuned through the activity of AA transporters. In this scenario, members of the UmamiT (Usually multiple acids move in and out Transporter) family which are expressed in vascular tissues (Tegeder & Hammes, 2018) may be potential candidates (Fig. 2c). However, there are no data in support of this hypothesis as yet. Further research is required to understand whether a systemic apoplastic release of Glu or other AAs (possibly from phloem) occurs, and whether the speed of the release lies in the same time range as that of the long-distance electrical and $Ca^{2+}$ waves, possibly anticipating them (Mousavi *et al.*, 2013; Nguyen *et al.*, 2018; Toyota *et al.*, 2018). To achieve this goal, the use of plants expressing the apoplastic localized glutamate iGluSnFR (Toyota *et al.*, 2018) or the recently developed FRET-based FLIPE (fluorescent indicator proteins for glutamate) sensors (Castro-Rodrígue *et al.*, 2020), together with a $Ca^{2+}$ sensor (e.g. R-Geco1) (Keinath *et al.*, 2015) could be pursued. However, proper controls need to be used, because, for example, iGluSnFR has been shown to exhibit pH-sensitivity (Marvin *et al.*, 2013).

Nevertheless, to complete the picture we should point out that some evidence might not fully support the model of apoplastic release of AAs in long-distance signaling with the consequent activation of GLRs. First, it has been shown that different GLRs (*Pp*GLRs, *At*GLR3.2 and *At*GLR3.3) can mediate ion fluxes ($Na^+$ and $Ca^{2+}$) without the need for an externally added ligand when expressed heterologously (Ortiz-Ramírez *et al.*, 2017; Wudick *et al.*, 2018b). Secondly, Clade III GLRs are apparently not detected in the PM of vascular tissue cells. *At*GLR3.1- and *At*GLR3.3-VENUS chimeric proteins localize mainly at the endoplasmic reticulum in the xylem contact cells and in the phloem cells, respectively, whereas *At*GLR3.6-VENUS localizes at the tonoplast (Nguyen *et al.*, 2018). To shed light on this critical point, immunogold labeling with specific GLR antibodies should be pursued, because only a few proteins can reach PM, and then only to a concentration insufficient for fluorescent tag detection.

Based on the collected evidence, is it therefore possible to reconcile the 'animal model' of GLR activation with the data currently available for plants? The fact that GLRs might be differentially activated/regulated in different tissues could be an option: for instance, in root cells (where the *At*GLR3.3 is expressed) or stomatal guard cells (where the *At*GLR3.1/*At*GLR3.5 heterocomplex forms) GLRs could be gated by the AA binding (including ACC) (Qi *et al.*, 2006; Stephens *et al.*, 2008; Kong *et al.*, 2016; Alfieri *et al.*, 2020; Mou *et al.*, 2020), whereas in pollen the interaction with the CORNICHON chaperones is essential for their sorting and activation (Wudick *et al.*, 2018b). In the vascular cells, other mechanisms may be required instead and might be different between local and systemic. One plausible alternative mechanism for working at long distance has been called the 'squeeze cell hypothesis', in which rapid axial changes in xylem hydrostatic pressure, occurring for example in response to wounding, lead to radially dispersed pressure changes that activate GLRs (Farmer *et al.*, 2014). The possible apoplastic pH regulation of GLR activity also could be taken into consideration (Shao *et al.*, 2020) because changes in apoplastic pH are associated with long-distance electrical signals (Felle & Zimmermann, 2007).

## V. Concluding remarks and perspectives

Plant GLRs have various physiological functions and their role in long-distance signaling places them as key players of plant acclimation to biotic and abiotic stress. However, unsolved question remain, such as GLRs' activation and regulation in different tissues (e.g. the vasculature). The recent release of GLRs–LBD structures represents a new tool helpful in driving rational mutagenesis approaches to alter or eliminate the AA binding. The expression of mutated forms of different GLRs in heterologous and homologous systems will help to define the functional role of LBDs and demonstrate the physiological importance of the ligand binding, which is still pending. This aspect also needs to be re-evaluated in the light of the ACC activation of GLRs (Mou *et al.*, 2020). The work by Tapken *et al.* (2013) pioneered the mutagenesis approach in *Xenopus* oocytes for the *At*GLR1.4, but nowadays the identification of accessory proteins (e.g. CORNICHON) making Clade III GLRs functional also in animal cells (e.g. *At*GLR3.3 in COS-7) (Wudick *et al.*, 2018b), can allow us to extend this approach to other isoforms (e.g. Ortiz-Ramirez *et al.*, 2017; Mou *et al.*, 2020). Both electrophysiological and imaging techniques will be instrumental to reveal the role of LBD AA binding. The availability of Arabidopsis mutants with easy-to-analyze phenotypes (e.g. *glr3.3/glr3.6*) (Mousavi *et al.*, 2013), will enable researchers to design straightforward complementation or

genome-editing strategies to test – *in planta* – the effects of LBD mutations, giving key information regarding the functional role of the GLRs amino acid-binding both in the local sensing of AA as well as in long-distance signaling.

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## VII. Author contributions

MG prepared Figs 1 and 2; AA generated the 3D structures and the models of Fig. 1; MCB wrote specific parts of the article text; and AC conceived the project and wrote the article with feedback from all authors.

## ORCID

Andrea Alfieri https://orcid.org/0000-0002-9716-0339
Alex Costa https://orcid.org/0000-0002-2628-1176
Maria Cristina Bonza https://orcid.org/0000-0001-7096-2967

## References

**Alfieri A, Doccula FG, Pederzoli R, Grenzi M, Bonza MC, Luoni L, Candeo A, Armada NR, Barbiroli A, Valentini G *et al.* 2020.** The structural bases for agonist diversity in an *Arabidopsis thaliana* glutamate receptor-like channel. *Proceedings of the National Academy of Sciences, USA* **117**: 752–760.

**Castro-Rodríguez V, Kleist TJ, Gappel NM, Okumoto S, Machado M, Denyer T, Timmermans MCP, Frommer WB, Wudick MM. 2020.** Noxious effects of cell surface display glutamate sensors on plant growth and development. *bioRxiv*. doi: 10.1101/2020.03.24.005223.

**Choi WG, Hilleary R, Swanson SJ, Kim SH, Gilroy S. 2016.** Rapid, long-distance electrical and calcium signaling in plants. *Annual Review of Plant Biology* **67**: 287–307.

**Dzubay JA, Jahr CE. 1999.** The concentration of synaptically released glutamate outside of the climbing fiber-Purkinje cell synaptic cleft. *Journal of Neuroscience* **19**: 5265–5274.

**Farmer EE, Gao YQ, Lenzoni G, Wolfender JL, Wu Q. 2020.** Wound- and mechano-stimulated electrical signals control hormone responses. *The New Phytologist* **227**: 1037–1050.

**Farmer EE, Gasperini D, Acosta IF. 2014.** The squeeze cell hypothesis for the activation of jasmonate synthesis in response to wounding. *New Phytologist* **204**: 282–288.

**Felle HH, Zimmermann MR. 2007.** Systemic signalling in barley through action potentials. *Planta* **226**: 203–214.

**Gangwar SP, Green MN, Michard E, Simon AA, Feijó JA, Sobolevsky AI. 2020.** Structure of the *Arabidopsis* glutamate receptor-like channel GLR3.2 ligand-binding domain. *Structure* doi: 10.1016/j.str.2020.09.006.

**Gilroy S, Białasek M, Suzuki N, Górecka M, Devireddy AR, Karpiński S, Mittler R. 2016.** ROS, calcium, and electric signals: key mediators of rapid systemic signaling in plants. *Plant Physiology* **171**: 1606–1615.

**Goto Y, Maki N, Ichihashi Y, Kitazawa D, Igarashi D, Kadota Y, Shirasu K. 2020.** Exogenous treatment with glutamate induces immune responses in Arabidopsis. *Molecular Plant Microbe Interaction* **33**: 474–487.

**Hedrich R, Salvador-Recatalà V, Dreyer I. 2016.** Electrical wiring and long-distance plant communication. *Trends in Plant Science* **21**: 376–387.

**Keinath NF, Waadt R, Brugman R, Schroeder JI, Grossmann G, Schumacher K, Krebs M. 2015.** Live cell imaging with R-GECO1 sheds light on flg22- and chitin-induced transient $[Ca^{(2+)}]_{cyt}$ patterns in Arabidopsis. *Molecular Plant* **8**: 1188–1200.

**Kiep V, Vadassery J, Lattke J, Maaß J-P, Boland W, Peiter E, Mithöfer A. 2015.** Systemic cytosolic $Ca^{2+}$ elevation is activated upon wounding and herbivory in Arabidopsis. *New Phytologist* **207**: 996–1004.

**Kong D, Hu H-C, Okuma E, Lee Y, Lee HS, Munemasa S, Cho D, Ju C, Pedoeim L, Rodriguez B *et al.* 2016.** L-Met activates Arabidopsis GLR $Ca^{2+}$ channels upstream of ROS production and regulates stomatal movement. *Cell Reports* **17**: 2553–2561.

**Kudla J, Becker D, Grill E, Hedrich R, Hippler M, Kummer U, Parniske M, Romeis T, Schumacher K. 2018.** Advances and current challenges in calcium signaling. *New Phytologist* **218**: 414–431.

**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.

**Marvin JS, Borghuis BG, Tian L, Cichon J, Harnett MT, Akerboom J, Gordus A, Renninger SL, Chen TW, Bargmann CI *et al.* 2013.** An optimized fluorescent probe for visualizing glutamate neurotransmission. *Nature Methods* **10**: 162–170.

**Mayer ML. 2020.** Glutamate receptors from diverse animal species exhibit unexpected structural and functional diversity. *Journal of Physiology*. doi: 10.1113/JP279026.

**Michard E, Lima PT, Borges F, Silva AC, Portes MT, Carvalho JE, Gilliham M, Liu L-H, Obermeyer G, Feijó JA. 2011.** Glutamate receptor-like genes form $Ca^{2+}$ channels in pollen tubes and are regulated by pistil D-serine. *Science* **332**: 434–437.

**Mou W, Kao YT, Michard E, Simon AA, Li D, Wudick MM, Lizzio MA, Feijó JA, Chang C. 2020.** Ethylene-independent signaling by the ethylene precursor ACC in Arabidopsis ovular pollen tube attraction. *Nature Communications* **11**: 4082.

**Mousavi SAR, Chauvin A, Pascaud F, Kellenberger S, Farmer EE. 2013.** GLUTAMATE RECEPTOR-LIKE genes mediate leaf-to-leaf wound signalling. *Nature* **500**: 422–426.

**Nguyen CT, Kurenda A, Stolz S, Chételat A, Farmer EE. 2018.** Identification of cell populations necessary for leaf-to-leaf electrical signaling in a wounded plant. *Proceedings of the National Academy of Sciences, USA* **115**: 10178–10183.

**Ni J, Yu Z, Du G, Zhang Y, Taylor JL, Shen C, Xu J, Liu X, Wang Y, Wu Y. 2016.** Heterologous expression and functional analysis of rice GLUTAMATE RECEPTOR-LIKE family indicates its role in glutamate triggered calcium flux in rice roots. *Rice* **9**: 9.

**Ortiz-Ramírez C, Michard E, Simon AA, Damineli DSC, Hernández-Coronado M, Becker JD, Feijó JA. 2017.** GLUTAMATE RECEPTOR-LIKE channels are essential for chemotaxis and reproduction in mosses. *Nature* **549**: 91–95.

**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.

**Shao Q, Gao Q, Lhamo D, Zhan H, Luan S. 2020.** Two glutamate- and pH-regulated $Ca^{2+}$ channels are required for systemic wound signaling in Arabidopsis. *Science Signaling* **13**: eaba1453.

**Singh SK, Chien C-T, Chang I-F. 2016.** The Arabidopsis glutamate receptor-like gene GLR3.6 controls root development by repressing the Kip-related protein gene KRP4. *Journal of Experimental Botany* **67**: 1853–1869.

**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.

**Tapken D, Anschütz U, Liu L-H, Huelsken T, Seebohm 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* **11**: ra47.

**Tegeder M, Hammes U. 2018.** The way out and in phloem loading and unloading of amino acids. *Current Opinion in Plant Biology* **43**: 16–21.

**Toyota M, Spencer D, Sawai-Toyota S, Jiaqi W, Zhang T, Koo AJ, Howe GA, Gilroy S. 2018.** Glutamate triggers long-distance, calcium-based plant defense signaling. *Science* **361**: 1112–1115.

**Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, Hansen KB, Yuan H, Myers SJ, Dingledine R. 2010.** Glutamate receptor ion channels: structure, regulation, and function. *Pharmacological Reviews* **62**: 405–496.

**Vincent TR, Avramova M, Canham J, Higgins P, Bilkey N, Mugford ST, Pitino M, Toyota M, Gilroy S, Miller AJ *et al.* 2017.** Interplay of plasma membrane and vacuolar ion channels, together with BAK1, elicits rapid cytosolic calcium elevations in Arabidopsis during aphid feeding. *Plant Cell* **29**: 1460–1479.

**Vincill ED, Bieck AM, Spalding EP. 2012.** $Ca^{2+}$ conduction by an amino acid-gated ion channel related to glutamate receptors. *Plant Physiology* **159**: 40–46.

**Vincill ED, Clarin AE, Molenda JN, Spalding EP. 2013.** Interacting glutamate receptor-like proteins in phloem regulate lateral root initiation in Arabidopsis. *Plant Cell* **25**: 1304–1313.

**Wang G, Hu C, Zhou J, Liu Y, Cai J, Pan C, Wang Y, Wu X, Shi K, Xia X *et al.* 2019.** Systemic root-shoot signaling drives jasmonate-based root defense against nematodes. *Current Biology* **29**: 3430–3438.

**Wildon DC, Thain JF, Minchin PEH, Gubb IR, Reilly AJ, Skipper YD, Doherty HM, O'Donnell PJ, Bowles DJ. 1992.** Electrical signalling and systemic proteinase inhibitor induction in the wounded plant. *Nature* **360**: 62–65.

**Wudick MM, Michard E, Oliveira Nunes C, Feijó JA. 2018a.** Comparing plant and animal glutamate receptors: common traits but different fates? *Journal of Experimental Botany* **69**: 4151–4163.

**Wudick MM, Portes MT, Michard E, Rosas-Santiago P, Lizzio MA, Nunes CO, Campos C, Santa Cruz Damineli D, Carvalho JC, Lima PT *et al.* 2018b.** CORNICHON sorting and regulation of GLR channels underlie pollen tube $Ca^{2+}$ homeostasis. *Science* **360**: 533–536.

**Yan C, Fan M, Yang M, Zhao J, Zhang W, Su Y, Xiao L, Deng H, Xie D. 2018.** Injury activates $Ca^{2+}$/calmodulin-dependent phosphorylation of JAV1-JAZ8-WRKY51 complex for jasmonate biosynthesis. *Molecular Cell* **70**: 136–149.

**Zimmermann MR, Felle HH. 2009.** Dissection of heat-induced systemic signals: superiority of ion fluxes to voltage changes in substomatal cavities. *Planta* **229**: 539–547.