# GABA signaling: a conserved and ubiquitous mechanism

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**In plants, research on γ-aminobutyric acid (GABA) has focused on its role as a metabolite, mainly in the context of responses to biotic and abiotic stresses. By contrast, studies of GABA in vertebrates have concentrated mainly on its role as a neurotransmitter and signaling molecule. Here, we discuss recent findings that point towards a possible role for GABA as a signaling molecule in plants.**

γ-Aminobutyric acid (GABA) is a non-protein amino acid that is conserved from bacteria through yeast to vertebrates and was discovered in plants over half-a-century ago [1]. It is mainly metabolized through a short pathway called the GABA shunt, because it bypasses two steps of the tricarboxylic-acid (TCA) cycle (Figure 1). The pathway is composed of three enzymes: the cytosolic glutamate decarboxylase (GAD) and the mitochondrial enzymes GABA transaminase (GABA-T) and succinic semialdehyde dehydrogenase (SSADH), although differences in the subcellular localization of GABA-shunt enzymes in different organisms have been reported (e.g. in yeast, SSADH is in the cytosol [2]). In an alternative reaction, succinic semialdehyde can be converted to GHB (γ-hydroxybutyric acid) through a GHB dehydrogenase (GHBDH) present in animals and recently identified in plants [3]. Interestingly, whereas research of GABA in vertebrates has focused mainly on its role in the context of plant responses to stress, because of its rapid and dramatic production in response to biotic and abiotic stresses (reviewed in [4,5]). as a signaling molecule and neurotransmitter, in plants, GABA was mainly considered to be a metabolite For example, disruption of the unique *SSADH* gene in *Arabidopsis* results in plants undergoing necrotic cell death caused by the accumulation of reactive oxygen intermediates (ROIs) when they are exposed to environmental stresses [6]. A recent article [7] reports that a gradient of GABA concentration is essential for the growth and guidance of pollen tubes and suggests that this amino acid plays a role in intercellular signaling in plants, possibly similar to its role in animals. The *pop2* mutant cannot produce a transaminase that degrades GABA (Figure 1), which results in the lack of a GABA concentration gradient, and this ultimately leads to growth inhibition and misguidance of *pop2* pollen tubes in *pop2* pistils. The main question raised by these recent findings is whether GABA itself serves as a signaling molecule in plants. If so, this would imply that GABA is capable of mediating developmental changes and cell guidance by interacting with specialized plant receptors. Discussion of these recent findings is the main purpose of this review.

## The role of GABA in development

Genetic studies show that modification of the GABA concentration can have severe consequences on development of both humans and plants. In humans, disruption of SSADH or GABA-T causes severe clinical manifestations associated with the accumulation of neurotransmitters in physiological fluids [8,9]. In addition, another genetic disease characterized by generalized seizures in the first hours of life is thought to be linked with a deficiency in GAD [10]. Similarly, transgenic plants that ectopically express a constitutively active GAD enzyme have a higher content of GABA and a lower content of glutamate, resulting in abnormal growth and development [11]. Additionally, knockout plants with a disruption in one of the five *GAD* genes present in *Arabidopsis* have roots that

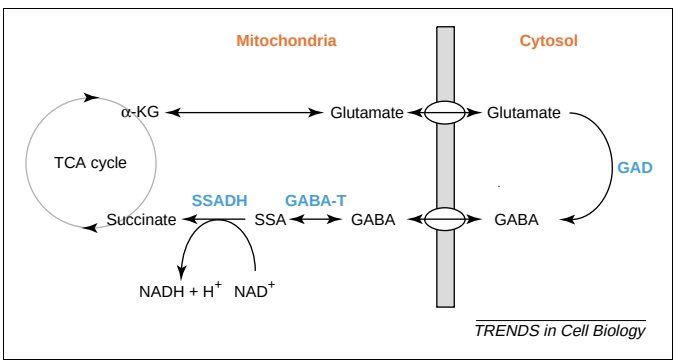


**Figure 1**. Schematic representation of the γ-aminobutyric acid (GABA)-shunt metabolic pathway. The GABA shunt is composed of three enzymes (blue). The glutamate decarboxylase (GAD) is a cytosolic enzyme that catalyzes the irreversible decarboxylation of glutamate to produce GABA. GABA is transported into the mitochondria where it is converted into succinic semialdehyde (SSA) by a GABA transaminase (GABA-T/POP2). SSA is then reduced by a succinic semialdehyde dehydrogenase (SSADH) to form succinate, which enters the tricarboxylic acid (TCA) cycle. α-KG, α-ketoglutarate.

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are less developed than those of wild-type (WT) plants and have lower levels of GABA (N. Bouché, A. Fait, M. Zik and H. Fromm; unpublished). Furthermore, disruption of the unique *SSADH* gene in *Arabidopsis* results in plants with high levels of ROIs [6]. In the study by Preuss and colleagues [7], inactivation of a gene encoding GABA transaminase resulted in higher GABA content in *Arabidopsis* flowers and, to a lesser extent, in the rest of the plant (e.g. in the leaves). Interestingly, there is a correlation between sterility and GABA content – the *pop2* mutants accumulating most GABA in flowers produced fewer seeds. Therefore, modification of GABA levels in plants can affect the development of the whole organism. However, whether this effect is caused by metabolic imbalance or modified signaling pathways mediated by GABA receptors is still unknown.

In addition to its neurotransmitter function in mature neurons, GABA is involved in the development of the nervous system, promoting neuronal migration, proliferation and differentiation (for a recent review, see [12]). These effects are mediated by the activation of GABA receptors, which provoke depolarization of the membrane in the immature brain, where, contrary to the adult brain, GABA is excitatory. Consequently, voltage-gated $Ca^{2+}$ channels and then $Ca^{2+}$ signaling pathways are activated. Indeed, GABA is a chemoattractant that can influence neuronal growth *in vitro*. During cortical development, GABA can promote DNA synthesis and cell proliferation. Neurons become assembled into functional networks by growing axons and dendrites, collectively called neurites. GABA regulates neuronal differentiation by promoting outgrowth of neurites. Interestingly, the work reported by Preuss and coworkers establishes a new parallel between the role of GABA in development of the central nervous system and in the growth of pollen tubes in *Arabidopsis*. Using antibodies raised against GABA and the direct measurement of GABA levels *in situ* in different flower cells, the authors elegantly show that levels of GABA increase along the path through which pollen tubes travel to female tissues. GABA concentration is 20 μM in the stigma and up to 500 μM in the integument cells that are part of the ovule and localized near the embryo sac where the target egg cell resides (Figure 2). In *pop2* mutants, this GABA gradient is disturbed, first along the pollen path, because all tissues have a similar GABA concentration, and second in the ovule itself, because GABA accumulates not only in the integument cells but also in most of the cells present in the ovule. Consequently, the growth of most of the *pop2* pollen tubes is arrested before reaching the *pop2* ovules, and the fraction of tubes that extend to the ovule are no longer targeted to the correct cell but are misguided. Crosses between WT and *pop2* mutants reveal that the growth and guidance of pollen tubes are restored if either the pistil or the pollen grain is WT. Therefore, an important factor for maintaining the guidance and targeting of the tube is the ability to degrade GABA; that is, the activity of POP2 (a functional GABA-T) has to be present in one of the parental tissues. Although this study clearly demonstrates the role of GABA in the development of pollen tubes, the mechanism of action of GABA is still vague. The *pop2* phenotype might result

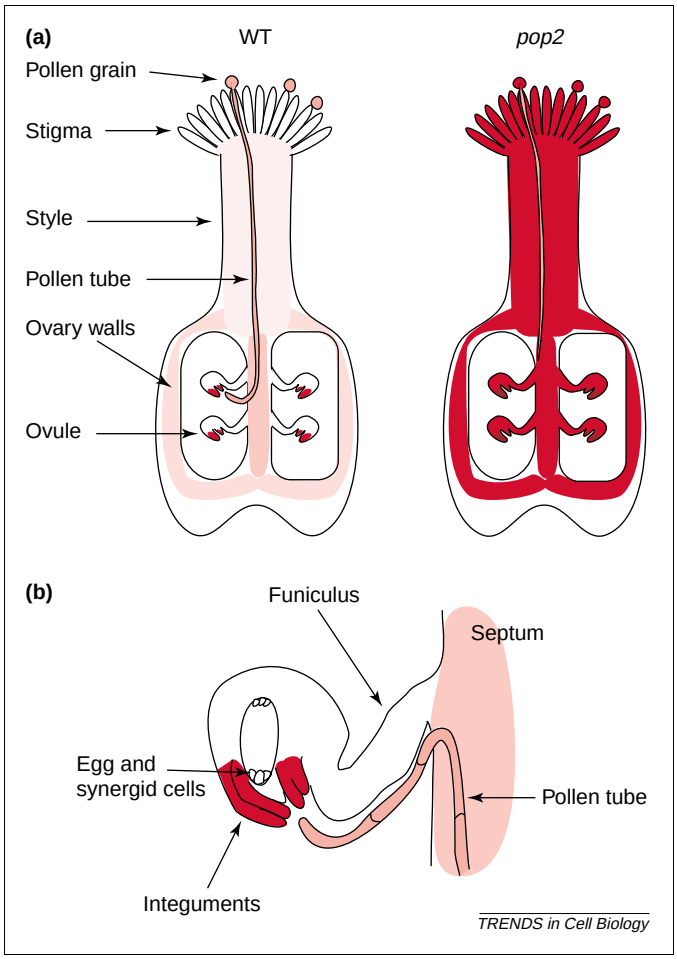


**Figure 2**. A γ-aminobutyric acid (GABA) gradient in *Arabidopsis* pistils is essential for the growth and guidance of pollen tube to the female gametophyte. **(a)** A pollen tube is emerging from a pollen grain and is growing into the pistil, through the stigma and the style to reach the ovule **(b)** where the pollen tube is guided to the egg cell of the target female. Concentrations of GABA in the tissues are represented as a gradient of color going from white (low) to red (high), according to the data of Palanivelu, R. *et al.* [7]. In the wild-type (WT) plants, GABA concentrations slowly increase along the pollen tube path, going from 20 μM in the stigma, 60 μM in the style, 110 μM in ovary walls and 160 μM in the septum to 500 μM in integument cells. Concentrations of GABA in pollen grains are ~270 μM, and are probably the same in pollen tubes, although this is yet to be determined. *pop2* mutants lack the ability to degrade GABA and have similar concentrations of GABA in the pistil and the ovule (~1500 μM), where integuments are still accumulating more GABA (24 000 μM).

from changes in the function of GABA receptors or from the modification of the metabolism, such as the toxicity of an overproduced metabolite. The mechanisms involved in the release and uptake of GABA in pollen tubes and cells close to the growing tube also remains to be identified. In neurons, GABA is stored in synaptic vesicles and released by exocytosis. The uptake of GABA is coupled to cotransport of $Na^{+}$ and is mediated by transporters of the neurotransmitter superfamily (NTS). Such a mechanism has never been demonstrated in plants, and no homolog to NTS members was detected in plant genomes. However, members of the amino-acid transporter superfamily might be putative candidates for the transport of GABA, whereby some of them transport GABA and are expressed in pollen (reviewed in [13]).

Interestingly, Heger *et al.* [14] have recently examined the development of neurons that synthesize and secrete the gonadotropin-releasing hormone (GnRH). These cells

originate outside the brain in the olfactory placode and must then migrate to the brain. Using transgenic mice that overproduce GABA specifically in GnRH neurons, the authors show that some of these cells migrate into aberrant locations in the cortex, and that during embryonic development fewer neurons find their way to appropriate sites in the hypothalamus. The similarities between the sensitivity of GnRH neurons and pollen tubes to GABA is intriguing and might involve parallel mechanisms of action, such as receptor-mediated signaling pathways.

## Receptors of the GABA-signaling system

The initial event leading to the activation of a cellular signaling pathway is the binding of a ligand, such as a hormone, to a specific receptor. If GABA could activate signaling pathways in a broad range of organisms, then GABA receptors should be present in these organisms. In the central nervous system of mammals, GABA is the principal neurotransmitter, mediating inhibitory synaptic currents by binding to receptors localized in pre- or postsynaptic membranes. Two types of receptors exist in brain cells: ionotropic receptors ($GABA_A$ and $GABA_C$ receptors), which are ligand-gated ion channels, and metabotropic receptors ($GABA_B$ receptors), which are coupled to G proteins. Although the GABA neurotransmitter system is very specific for synapses, recent studies reveal that GABA receptors are also expressed in nonexcitable cells and in a variety of human tissues, such as heart, liver, lung, ovary and testis [15]. For instance, GABA regulates the secretion of hormones through receptors [16]. Moreover, in rodent and human testis, a complete GABA system is present [17]. These findings imply that GABA could be a signaling molecule not only in brain but also in other organs. GABA receptors are also found in lower organisms such as *Caenorhabditis elegans* [18]. Thus, GABA receptors seem to be more widely distributed than was previously thought.

Although genes that are highly homologous to the animal GABA receptors are not present in the *Arabidopsis* genome, similarity searches reveal a new family of 20 genes (AtGLRs) [19] that share sequence and structural homology with ionotropic glutamate receptors of mammals (iGluRs). As described in Figure 3, AtGLRs contain transmembrane segments, a pore region and two extracellular domains predicted to bind to amino acids because of their homology with the bacterial periplasmic amino-acid-binding protein (PBP), namely a LIVBP-like domain (leucine-, isoleucine-, valine-binding protein) located in the long part of the N-terminal of the protein and a LAOBP-like domain (lysine-, arginine-, ornithine-binding protein) formed by S1 and S2 extracellular domains (Figure 3). In iGluRs of mammals, the LAOBP-like domain binds glutamate (and glycine) [20], whereas the role of the LIVBP-like domain is not yet fully understood. Nevertheless, this domain is known to bind modulatory ligand molecules, such as ifenprodil, an antagonist of iGluR [21], and it displays structural homology with several receptors, including the $GABA_B$ receptors [22,23]. It is, therefore, tempting to speculate that GABA could bind to this domain and modulate the activity of some of the AtGLRs. When GABA was applied as a directional source *in vitro*, it did not modify the orientation of pollen tubes [7],

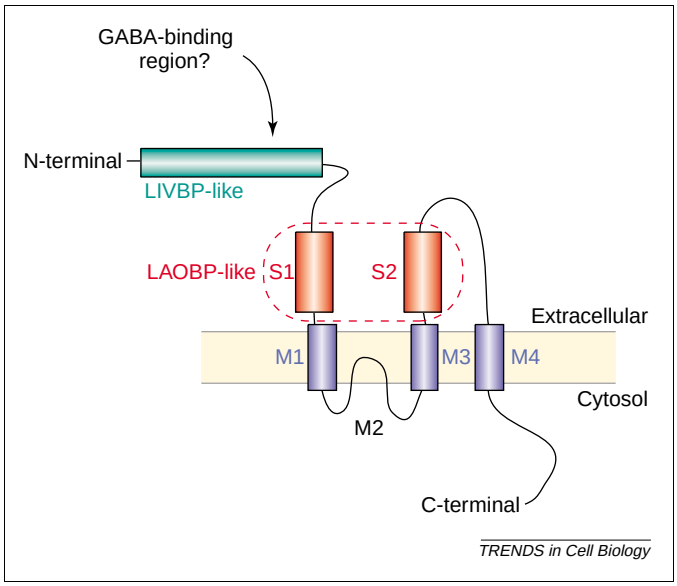


**Figure 3**. The structure of the glutamate receptors of *Arabidopsis* (AtGLRs). AtGLRs are membrane proteins with a large N-terminal extracellular region, three transmembrane segments (M1, M3 and M4; blue), a P-loop region (M2, initially thought to be transmembrane segment) and a C-terminal cytoplasmic end. Two bacterial periplasmic amino-acid-binding protein (PBP) domains are found at the extracellular side: a LIVBP-like domain (leucine-, isoleucine-, valine-binding protein; green) within the first 400 amino acids, and a LAOBP-like domain (lysine-, arginine-, ornithine-binding protein) formed by the S1 and S2 regions (red). LAOBP-like domains, at least in mammals, are involved in glutamate binding [20]. LIVBP-like domains could bind modulatory ligands, such as GABA.

contrary to the effect of some second messengers, such as cAMP [24]. An explanation could be that GABA has no effect alone, with the main ligand of AtGLRs (e.g. glutamate) being absent in the assay. Recent data suggest that AtGLRs are involved in the transport of cations and probably mediate $Ca^{2+}$ entry into plant cells [25,26]. Interestingly, the involvement of transmembrane ion fluxes, in particular $Ca^{2+}$ fluxes, in the growth of pollen tubes is well documented [27,28]. Whereas glutamate or glycine provoke a rapid cytosolic $Ca^{2+}$ burst in plant cells [26,29], no effect of GABA (used alone) on cytosolic $Ca^{2+}$ was recorded (B. Kaplan and H. Fromm; unpublished). Nevertheless, glutamate and glycine can also act synergistically to modulate the cytosolic concentration of $Ca^{2+}$ [29], implying that AtGLRs can interact with a variety of ligands. Moreover, the contrasting effects of known agonists and antagonists of GABA receptors on growth of the duckweed (*Lemna minor L*) water plant suggest the presence of $GABA_B$-responsive receptors in plants [30]. The identification of AtGLRs that are specifically expressed in pollen tubes could contribute to an understanding of the possible role of these proteins in mediating the effect of GABA on the growth of pollen tubes.

## Concluding remarks

The metabolic pathway involved in GABA synthesis is common to most, if not all, eukaryotic and prokaryotic cells and is not restricted to neurons. Therefore, components of the GABA system, both putative receptors and metabolic pathways, are present in a broad range of cells, raising the possibility that activation of signaling cascades through this amino acid could be a phylogenetically conserved ubiquitous mechanism. However, although GABA receptors mediate the effect of GABA on the development of neurons, their

involvement in developmental processes in plants still has to be demonstrated. It is tempting to compare the growth of neurites with that of pollen tubes; however, similarities between the two should be taken with caution because these are different structures operating under different conditions. Future studies require combined molecular, biochemical, electrophysiological and genetic approaches to identify the components of GABA signaling in plants. For example, the role of GABA could be studied in polarized cells that are similar to pollen tubes, such as root hairs. Proving that GABA is a signaling molecule in plants will require intensive and exciting research in the next few years.

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# Detection of peptidoglycans by NOD proteins


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**Mechanisms of innate immune defense are based on the recognition of invariant microbial molecular patterns by specific receptors, followed by the activation of signaling pathways and the expression of effector molecules that will defeat the invading microorganism. Two recent reports add to the growing list of these pattern-recognition receptors by showing that the intracellular nucleotide-binding oligomerization domain 1 (NOD1) protein recognizes a diaminopimelate-containing muropeptide, a cell-wall component of Gram-negative bacteria.**



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