Article Addendum

Deciphering the Possible Mechanism of GABA in Tobacco Pollen Tube Growth and Guidance

Guang-Hui Yu^{1,2}

Meng-Xiang Sun^{2,*}

¹College of Life Sciences; South-Central University for Nationalities; Wuhan, China

²Key Laboratory of MOE for Plant Developmental Biology; College of Life Sciences; Wuhan University; Wuhan, China

*Correspondence to: Meng-Xiang Sun; Key Laboratory of MOE for Plant Developmental Biology; College of Life Sciences; Wuhan University, Wuhan 430072 China; Tel.: +0086.27.68752378; Fax: 0086.27.68756010; Email: mxsun@whu.edu.cn

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Addendum to:

Quantum Dot-Mediated Detection of γ -Aminobutyric Acid Binding Sites on the Surface of Living Pollen Protoplasts in Tobacco

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ABSTRACT

γ -Aminobutyric acid (GABA) is an inhibitory transmitter in animal central and peripheral nervous systems, and also plays an important role in pollen tube growth and guidance. However, the mechanisms underlying these effects in plants are poorly understood, mainly because the GABA receptor in plants has not been elucidated. To address this issue, we recently created quantum dot probes to identify possible GABA receptors on the membrane surfaces of pollen protoplasts. We found that GABA bound to cell membranes and regulated downstream Ca^{2+} oscillation in the cells. These results provide important clues to further specifying the nature of the binding sites and deciphering the role of GABA as a signal molecule in pollen tube growth and orientation.

PLEIOTROPIC ROLE OF GABA IN PLANT DEVELOPMENT

γ -Aminobutyric acid (GABA) is a four-carbon nonprotein amino acid that is omnipresent in all organisms from prokaryotes to eukaryotes. Studies with vertebrates have shown that GABA is an inhibitory transmitter in the central and peripheral nervous systems. However, its role in plants is still a mystery. Previous studies have demonstrated its role as an osmotic adaptation to various abiotic and biotic stresses.^{1,2} It may also function as an extracellular and intracellular signaling molecule involved in regulating certain plant physiological processes. GABA may stimulate ethylene biosynthesis in sunflowers as part of the senescence process.³ It specifically upregulates the uptake of nitrate and expression of the nitrate transporter gene (*BnNrt 2*) in *Brassica napus*⁴ and regulates the expression of 14-3-3 gene family members in Arabidopsis seedlings.⁵ Palanivelu et al. revealed that GABA also plays a critical role in pollen tube growth and orientation.⁶ However, whether it acts via a pathway similar to that in animal cells by binding to specific receptors and thereby triggering downstream signaling, such as the Ca^{2+} /CAM pathway, is still unknown. Corresponding genes of GABA receptors have not yet been cloned, and the Arabidopsis gene bank contains no sequences homologous to the animal counterparts. Thus, the mechanisms underlying the regulation of pollen tube growth by GABA are far from being understood.

POSSIBLE GABA RECEPTOR ON TOBACCO POLLEN PROTOPLAST MEMBRANES

As pollen tube growth and guidance are key events in angiosperm fertilization, GABA has received much more attention since the discovery that this enigmatic neurotransmitter is involved in regulating pollen tube growth and guidance. The mechanism by which this molecule regulates and guides pollen tube growth needs to be explored. One speculation is that GABA receptors located on pollen cell membranes may be involved.⁷⁻⁹

To investigate the GABA receptors in plants, we turned to quantum dots (QDs), which have recently been applied to biological systems,¹⁰ including protein localization studies.¹¹⁻¹³ We coupled the amino group of GABA to water-soluble QDs in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) to create a novel fluorescence probe. Using this probe, we detected GABA binding sites on the membrane of tobacco pollen protoplasts. The probe also regulated Ca^{2+} oscillation in the cells tested.¹⁴ These findings provide important clues to further deciphering the role of GABA as a signal molecule in pollen tube growth, as well as demonstrate a technique to determine whether the GABA binding sites on the cell membrane are GABA receptors or transporters. Screening for GABA transporter mutants in higher plants and testing the ability of GABA to bind to cell membranes will help to further elucidate the mechanism for GABA. Our preliminary work showed that the application of GABA or

Figure 1. Working model of the role of GABA in regulating pollen tube growth.

GABA-QD resulted in different patterns of Ca^{2+} dynamics, implying that the binding sites may be one type of GABA receptors. Although still technically challenging, the isolation of the binding molecules is necessary to finally characterize their nature and function.

MECHANISMS CONTROL THE GABA GRADIENT IN THE FLOWER PISTIL

The gradients of gene expression production are usually important biological factors regulating cell fate. For example, several morphogens form gradients that control early *Drosophila* embryo pattern formation.¹⁵ Generally, gradients form and develop in one of two basic ways: gene products are either propagated by passive diffusion in intercellular spaces or actively by morphogen transmission from cell to cell. A gradient may also be formed by the ordinal degradation of gene products along tissues.

In plants, GABA is synthesized from glutamate through catalysis of glutamate decarboxylase (GAD; EC4.1.1.15), which is the limiting enzyme in GABA production.² In our preliminary investigation using high-performance liquid chromatography and cryosection, combined with immunofluorescence analysis, we confirmed a GABA gradient from the top to the bottom of tobacco pistils and the distribution of GAD was mainly in the parenchyma cells and vascular bundles of the stigma and style, through which pollen tubes may pass. Furthermore, with the aid of an *in vivo/in vitro* culture system, we found that tobacco pollen tubes grow preferentially toward the GABA source (unpublished data), which suggests that GABA synthesis is correlated with GABA transport, most likely contributing to GABA gradient formation. Palanivelu et al.⁵ reported that successful pollen tube growth and orientation may involve the interaction of the pollen tube with the pistil and that even the formation of the GABA gradient in the pistil may depend on pollen tube activity, such as the degradation of GABA within pollen tubes. However, which of the mechanisms for gradient formation is operative in the plant pistil still requires extensive investigation.

CLUES TO THE MECHANISM OF GABA IN POLLEN TUBE GROWTH GUIDANCE

The tip growth of pollen tubes is a complex system,¹⁶ requiring the participation of multiple signal molecules, such as the Rac/Rop protein,¹⁷⁻¹⁹ F-actin,²⁰ mitogen-activated protein kinase (MAPK),²¹ calmodulin-like domain protein kinase (CDPK)²² and the 14-3-3 protein,²³ which are all involved in the growth and orientation of pollen tubes. New data are providing valuable clues to decipher the role of GABA in this intricate signaling network and its possible connection with the signal components.

In eukaryotes, ligand-mediated signaling via G-protein-coupled receptors (GPCRs) is a conserved mechanism for extracellular signal perception at the plasma membrane. The GPCR-mediated signaling pathway plays a central role in vital processes, such as taste, olfaction, and chemotaxis in animals.²⁴ Among the complex components, the heterotrimeric G protein (consisting of α , β , and γ subunits) is also the conserved transduction molecule. It is a molecular switch that relays the extracellular signal perception to the downstream active reaction. The candidate effecting molecules of the α subunit are ion channels, adenylate cyclase (AC), phospholipase C (PLC), phospholipase D (PLD), and so on.²⁵ Interestingly, the roles of all these molecular components have been studied in pollen tubes.²⁶⁻²⁹ It is important to investigate whether these signal components are regulated by GABA via GPCRs in plants, as they are in animals.³⁰ In our preliminary study, we found that exogenous GABA could regulate the level of Ca^{2+} in tobacco pollen tubes.¹⁴ But we do not know if this occurs through regulation of the Ca^{2+} channel on the membrane or some other side effect, such as the increase in IP_3 (inositol 1,4,5-trisphosphate) caused by PLC activation.

These possibilities are summarized in the Figure 1 model, in which the putative GABA receptor is present on pollen tube cell membranes, possibly belongs to GPCRs. When pollen tubes grow through the flower pistil, the extracellular GABA may activate GPCRs, triggering an increase in Ca^{2+} levels; Ca^{2+} downstream response element, such as CDPKs then further affect the phosphorylation of actin regulatory proteins to regulate the dynamics of actin,³¹ and thus, eventually affects actin organization and vesicle trafficking. GABA may also permeate into cells via the GABA transporter to regulate Ca^{2+} levels and other downstream signal molecules, such as PLC, PLD or Rac/Rop, which regulate pollen tube orientation through activating the G protein α subunit. MAPKs may be the downstream effectors of Rac/Rop to participate in the regulation of pollen tube growth, functioning in the same way like its role in the root hair cells.³² Obviously, much more work remains to be done to elucidate the relationship between GABA and other signal molecules.

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