

# Does diacylglycerol serve as a signaling molecule in plants?

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Diacylglycerol (DAG) is an important signaling phospholipid in animals, specifically binding to the C1 domain of proteins such as protein kinase C. In most plant species, however, DAG is present at low abundance, and no interacting proteins have yet been identified. As a result, it has been proposed that the signaling function of DAG has been discarded by plants during their evolution. In this mini-review, we summarize the accumulating experimental evidence which supports that notion that changes in DAG content in response to particular cues are a feature of plant cells. This behavior suggests that DAG does indeed act as a signaling molecule during plant development and in response to certain environmental stimuli.

## Introduction

The cytoplasmic membrane comprises a phospholipid bilayer. In addition to their role as major structural components of the membrane, phospholipid molecules also act as secondary messengers since the discovery of the phosphoinositide/phospholipase C (PI/PLC) pathway.<sup>1</sup> In this pathway, phosphoinositide is metabolized to phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) by two catalytic steps, and PIP<sub>2</sub> is converted to inositol 1,4,5-triphosphate (IP<sub>3</sub>) and diacylglycerol (DAG) by PLC. DAG can also be formed from the hydrolysis of glycerophospholipid (mainly phosphatidylcholine, PC), through the action of phospholipase C (PC/PLC) (also known as non-specific PC, or NPC). DAG is later phosphorylated by diacylglycerol kinase (DGK) to form phosphatidic acid (PA), a molecule which can also be produced by PLD via the hydrolysis of structural phospholipids such as PC. PA can be converted to DAG by lipid phosphate phosphatase (LPP), phosphatidic acid phosphatase (PAP) and PA hydrolase (PAH), and specifically in plants to DAG pyrophosphate (DGPP) by PA kinase (PAK). DGPP can be converted to PA by LPPs such as diacylglycerol pyrophosphate phosphatase. The pathway involving these molecules is shown in **Figure 1**.

Certain phospholipids, in particular PA and IP<sub>3</sub>, play important modulating roles in plants. PA is a key lipid signaling

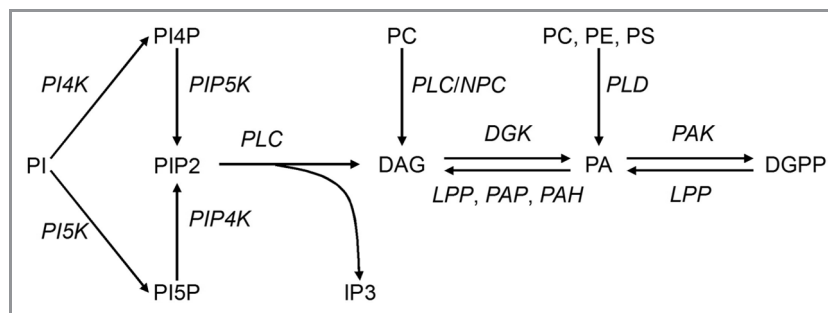
molecule, and its involvement in the stress response, in metabolism and in development has been recently reviewed.<sup>2</sup> IP<sub>3</sub> participates in the response to various abiotic stresses, gravitropism, phototropism and auxin transport.<sup>3–5</sup> Besides these two ones, other phospholipids have been also increasingly concerned about, and their roles are found to be fantastic.

## DAG is Prevalently Believed to be Out of Plant Phospholipid Signaling

The mechanics of phospholipids in signaling machinery in animals are reasonably well understood. In animal cells, IP<sub>3</sub> binds to its receptor ligand-gated calcium channel, which triggers the release of Ca<sup>2+</sup> from the intracellular Ca<sup>2+</sup> reservoir to the cytoplasm.<sup>3</sup> However, no functional plant receptor of IP<sub>3</sub> has yet been identified, and no homologs of the animal receptor genes are present in the genomes of either *Arabidopsis thaliana*, rice or poplar, or in any of the publicly accessible plant EST libraries.<sup>6</sup> As both the green alga *Chlamydomonas* sp and the ciliate *Paramecium* sp do possess such a receptor, the indication is that the IP<sub>3</sub> receptor has been discarded during plant evolution.<sup>7</sup> Nevertheless, IP<sub>3</sub> accumulates in plant cells following their exposure to environmental stimuli, and its accumulation has been correlated with the mobilization of intracellular calcium.<sup>3,8,9</sup> Perhaps, therefore, IP<sub>3</sub> receptors differing from ligand-gated calcium channels have evolved in plants.

DAG binds specifically to its target C1 domain, a small (~50 residue) cysteine-rich structural unit originally described as a protein kinase C (PKC) lipid-binding module.<sup>10</sup> In animal cells, DAG recruits the C1 domain-containing PKCs and PKDs onto the cytoplasmic membrane, and activates PKC via its phosphorylation; phosphorylated PKC activates PKD and triggers certain downstream signaling pathways.<sup>10</sup> In an effort to isolate plant PKC homologs, the PKC inhibitor 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H-7) was shown to inhibit light-stimulated stomatal opening, as well as enhancing dark-induced stomatal closure in *Commelina communis*; at the same time, treatment with both DAG analogs and the synthetic diacylglycerols 1,2-dihexanoylglycerol and 1,2-dioctanoylglycerol had the opposite effect on stomatal closure, thereby providing some experimental evidence for the existence of PKC in plants.<sup>11</sup> When a *Brassica campestris* enzyme exhibiting the properties of a conventional mammalian PKC was activated by DAG or its analog phorbol

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**Figure 1.** A simplified representation of phospholipid metabolism. PI, phosphoinositide; PI4P, phosphoinositide 4-phosphate; PI5P, phosphoinositide 5-phosphate; PIP2, phosphoinositide 4,5-bisphosphate; DAG, diacylglycerol; IP3, inositol 1,4,5-triphosphate; PA, phosphatidic acid; DGPP, diacylglycerol pyrophosphate; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI4K, phosphoinositide 4-kinase; PI5K, phosphoinositide 5-kinase; PIP4K, phosphoinositide phosphate 4-kinase; PIP5K, phosphoinositide phosphate 5-kinase; PLC, phospholipase C; NPC, non-specific phospholipase C; PLD, phospholipase D; DGK, diacylglycerol kinase; PAK, phosphatidic acid kinase; LPP, lipid phosphate phosphatase; PAP, phosphatidic acid phosphatase; PAH, PA hydrolase.

ester and other cofactors, it was able to in vitro phosphorylate the PKC-specific substrate  $\alpha$ -peptide.<sup>12,13</sup> Furthermore, a potato kinase having properties similar to conventional PKC isoenzymes was restricted by the presence of PKC inhibitors in its function during pathogenesis, and the PKC activator 12-O-tetradecanoylphorbol 13-acetate promoted this effect.<sup>13</sup> Meanwhile the maize protein ZmcPKC70 proved able to bind phorbol 12-myristate 13-acetate and to possess some of the properties of a conventional PKC.<sup>14</sup> However, subsequent studies have shown that the genomes of plants, including lower plants, lack PKC encoding gene, and the effect of these PKC-specific inhibitors is most likely achieved through their interaction with protein kinases such as calcium/calmodulin-dependent protein kinase, calcineurin B-like proteins and AGC kinases.<sup>15</sup>

As DAG is a precursor for glycolipids, storage lipids and the major structural phospholipids, which together account for about 90% of all plant lipids, it is not considered to be a plausible membrane-localized secondary messenger.<sup>15</sup> The reason that the DAG content of plant cells is relatively low is that PLC-generated DAG is rapidly phosphorylated to PA by DGK. Thus PA (rather than DAG) has typically been implicated as a major plant secondary messenger.<sup>16-18</sup> Thus, these findings bring about an opinion that DAG is possibly not a signaling messenger in plants.

### Increasing Evidence for DAG Acting as a Signaling Molecule in Plants

The DAG content of the plant cell is low, but its presence is necessary for certain developmental processes and the response to particular environmental stimuli. DAG accumulates strongly in the apical domain of the plasma membrane at the tip of elongating tobacco pollen tube. This accumulation is abolished by treatment with U-73122, a specific inhibitor of PLC, with the result that pollen tube elongation is inhibited. The inference from this observation is that DAG probably acts as a signaling molecule in the regulation of pollen tube tip growth.<sup>19</sup> DAG also accumulates via the PC/PLC (NPC) pathway under stressful conditions. In *A. thaliana* plants subjected to salinity stress, the

activity of NPC was increased, promoting the production of DAG by 4-fold.<sup>20</sup> Phosphate starvation of *A. thaliana* upregulates *AtNPC4* and *AtNPC5*, which is for the accumulation of DAG and the supply of inorganic phosphate;<sup>21,22</sup> Later during phosphate starvation, the PC content is transiently increased, before its rapid decrease which coincides with an increase in DAG content, indicating most of the newly synthesized DAG is derived via the PC/PLC pathway.<sup>23</sup> In tobacco cell cultures, treatment with brassinolide raises the DAG content within 15 min through the elevation of PC/PLC activity in a concentration-dependent manner; at the same time the size of the PA pool is not significantly increased.<sup>24</sup> The DAG content of *Dunaliella salina* cells is rather high in comparison with most animal tissues, particularly in the chloroplast and plasma membrane. When confronted with osmotic shock, the plasma membrane DAG content increases markedly to a level sufficient to consider DAG to be a genuine potential secondary messenger in PLC-mediated signal transduction.<sup>25</sup>

DAG is rapidly converted to PA in plant cells, but some reports have suggested that the decrease in DAG content is not accompanied by any increase in that of PA. In parsley and tobacco cell suspensions elicited by fungal glycoprotein or *Phytophthora cryptogea* cryptogein, the DAG pool declines within 15 min and PC/PLC activity is also reduced, while the content of PA rises slightly from a low background level.<sup>26,27</sup> This observation has been taken to indicate that the decrease in DAG content is largely the result of the downregulation of the PC/PLC pathway rather than to its conversion to PA. In  $\text{AlCl}_3$  treated tobacco BY-2 cells, the activity of PC/PLC is restricted, with the result that their DAG content is rapidly and greatly reduced, while at the same time there is no observable effect on the activity of any of the enzymes involved in the catalysis of DAG to PA and other products, and the contents of these products are also not altered.<sup>28</sup> Elongating tobacco pollen tubes incubated in vitro in the presence of various concentrations of  $\text{AlCl}_3$  suffer a growth restriction, and their DAG content is also significantly reduced in a concentration-dependent fashion. The exogenous supply of DAG can relieve this growth restriction, but the supply of exogenous PA has

a much smaller effect.<sup>28</sup> Along with the observations of Helling et al.,<sup>19</sup> this result demonstrates that DAG itself most likely serves as a signaling molecule.

Environmental stress can induce the transcription of a number of genes encoding enzymes involved in the catalysis from PA to DAG in some plant species. The *A. thaliana* gene *AtLPP1* encodes a lipid phosphate phosphatase which catalyzes the conversion of DGPP to PA, and then of PA to DAG. Its transcription can be rapidly (though transiently) induced by  $\gamma$  or UV-B irradiation, and can also be elicited by the presence of harpin, a molecule associated with oxidative stress.<sup>29</sup> Transcript of the two *Vigna unguiculata* phosphatidic acid phosphatase genes *VuPAP $\alpha$*  and *VuPAP $\beta$*  accumulates in the leaf in response either to the progressive dehydration of the whole plant or the rapid desiccation of detached tissue, and the expression of *VuPAP $\beta$*  can also be induced by the supply of abscisic acid.<sup>30</sup> Because the increases in PA and DGPP levels in response to stress are transient, PAK and PAP appear to be important attenuators for the regulation of PA signaling events.<sup>16,31</sup> For example, *AtLPP2* functions as a negative regulator of PA-mediated ABA signaling during germination.<sup>32</sup> However, apart from the attenuating effect of PAP, there is the intriguing possibility that DAG acts antagonistically to PA.

The role of DAG as a secondary messenger is achieved through its binding to target proteins, except for DGK functioning in conversion DAG to PA. Although higher plants lack genes encoding PKCs, they do possess genes encoding a variety of C1 domain containing proteins. The *A. thaliana* genome, for example, includes 164 such genes (www.arabidopsis.org), while 22 are known in the rice genome (rice.plantbiology.msu.edu). This indicates that although it has been assumed by default that PKCs are the sole target of DAG, it is possible that other proteins—possibly even some lacking kinase activity—could represent the prime DAG targets in the plant cells. A number of proteins unrelated to PKC are capable of high affinity binding with the DAG analog phorbol ester, which suggests a measure of complexity in the signaling pathways activated by DAG.<sup>33</sup> The bread wheat gene *TaCHP*, for example, which confers an

enhanced level of both salinity and drought tolerance,<sup>34</sup> encodes a protein carrying three C1 domains. Its gene product has transactivation activity but no PKC activity.<sup>35</sup>

A body of experimental evidence supports the idea that DAG does indeed function as a signaling molecule in plants, although convincing proof of this idea is not yet forthcoming. At the same time, DAG represents a major component of the structure and dynamics of plant membranes, and any excessive accumulation can induce the formation of unstable and asymmetric regions, which are required for membrane fusion and division.<sup>36,37</sup> Membrane fusion is involved in a range of physiological processes, the most notable of which is cell division. Thus the supply of exogenous DAG or its endogenous PLC-induced production promotes membrane fusion,<sup>38,39</sup> and in this way regulates pollen tube growth via the acceleration of cell division.<sup>19,28</sup>

## Conclusions and Perspectives

The question as to whether or not DAG serves as a signaling molecule in plants remains unresolved. The DAG content of plant cells is typically low, and no target plant protein participating in signal transduction has yet been identified. However, DAG content is known to fluctuate in response to a variety of developmental and environmental cues. Therefore, it would be academically significant to decode its acting mechanisms, and a critical requirement in establishing DAG's role as a signaling molecule is to identify its binding target(s). Besides, the plant/animal kingdom divergence with respect to the DAG-mediated phospholipids signaling pathway remains an intriguing puzzle.

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