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Article Addendum

PA, a stress-induced short cut to switch-on ethylene signalling by switching-off CTR1?

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Constitutive triple response 1 (CTR1) is a protein kinase that represses plant responses to ethylene. Recently, we have shown that CTR1 function is negatively regulated by the lipid second messenger phosphatidic acid (PA) in vitro.1 PA was shown to inhibit (1) CTR1's protein kinase activity, (2) the intramolecular interaction between N-terminus and kinase domain, and (3) the interaction of CTR1 with the ethylene receptor ETR1. PA typically accumulates within minutes in response to biotic or abiotic stresses, which are known to induce ethylene formation. Although long-term treatment with ethephon does stimulate PA accumulation, our results show no fast increase in PA in response to ethylene. A speculative model is presented which explains how stress-induced PA formation could switch on downstream ethylene responses via interaction of the lipid with CTR1.

Role of PA in CTR1 Kinase Regulation

The plant hormone ethylene regulates growth and development and responses to biotic and abiotic stress. By genetic screens, many of the players involved in perception and subsequent signal transduction have been identified.2 In Arabidopsis, ethylene is perceived by a family of receptors, represented by ETR1, ERS1, EIN4, ETR2 and ERS2. In air, these receptors serve to activate constitutive triple response 1 (CTR1), which is a negative regulator of the pathway. CTR1 actively represses EIN2 function, thus inhibiting all downstream ethylene responses.3,4 Upon binding of ethylene, the activity of the receptors is altered and CTR1 is deactivated, which results in activation of ethylene responses. While the genetic evidence is compelling, surprisingly little is known about the molecular mechanism of CTR1 activation. It has been shown that CTR1 has ser/thr kinase activity in vitro and that it can autophosphorylate as well as phosphorylate the artificial substrate myelin basic protein.5 A direct interaction with the ethylene receptors seems to be required for CTR1 function.6

CTR1 is a homolog of the mammalian Raf-1 kinase, a MAP kinase kinase kinase, which is involved in various cellular processes including proliferation and differentiation, both normal and pathological. In order to be activated, Raf-1 must be recruited to the membrane. This recruitment involves binding to the lipid second messenger phosphatidic acid (PA) through a specific lipid-binding site in the protein.7,9 In plants, PA plays a role in many different stress-signaling pathways as well as in normal plant development. Besides being an intermediate in glycerolipid synthesis at the ER, PA is transiently generated via two lipid signaling pathways, involving either phospholipase D (PLD) or phospholipase C, the latter coupled to diacylglycerol kinase activity.10,11

We found that CTR1, like Raf-1, directly interacts with PA.1 The binding site, however, is different than the region identified for Raf-1. In vitro protein kinase activity of CTR1 was shown to be inhibited by PA. Moreover, PA inhibited the intra-molecular interaction of the CTR1 kinase domain with the N-terminus as well as with the interaction with the ethylene receptor ETR1. Together, this biochemical evidence shows inhibition of the action of the negative regulator CTR1 and thus suggests a positive role of PA in ethylene signaling.1

A Model for the Role of Stress-Induced PA in Inducing Ethylene Responses via Inhibition of CTR1

Since PA affected CTR1 function in vitro, it was of interest to determine whether PA is part of the ethylene pathway. Thus, we investigated whether ethylene itself induces a PA response in Arabidopsis seedling and suspension-cultured cells. However, no rapid PA accumulation was observed in either cells or seedlings in response to ethephon or ACC (Fig. 1). As a positive control, cells were treated with NaCl, which induced accumulation of PA within minutes (Fig. 1C). As PA formation does not seem to occur as a direct response to ethylene perception, we propose the role of PA in ethylene signaling to be independent of ethylene recognition. Several stress stimuli, such as wounding, pathogenic elicitors and salt do...
give a rapid PA response and are known to trigger ethylene responses.\textsuperscript{11-15} It is possible that this increase in PA leads to inhibition of CTR1, which in turn would relieve repression of the EIN2 pathway and induce downstream ethylene signaling.

This hypothesis is the basis for our working model on the role of PA in ethylene signaling, which is presented in Figure 2. In the absence of ethylene, the ethylene receptors bind and activate the CTR1 kinase, resulting in suppression of downstream ethylene signaling. In the presence of ethylene, CTR1 dissociates from the receptors and becomes inactive, resulting in derepression of the downstream ethylene pathway\textsuperscript{3} (Fig. 2A). Figure 2B builds further on the existing model and suggests an alternative or complementary pathway to inhibit CTR1 kinase activity and to induce ethylene responses in the absence of ethylene but in the presence of stress-induced PA. The inhibition of CTR1 kinase by PA could be a fast way to switch on the ethylene pathway in response to stress, without the need to generate ethylene first. Alternatively, PA dependent inhibition of CTR1 could function synergistically with ethylene and work to promote rapid and/or heightened activation of ethylene responses. This mechanism could act in parallel with other recently described stress-activated pathways that induce ethylene production through induction of ACC synthase (ACS) gene expression\textsuperscript{16} and ACS activity.\textsuperscript{12} An advantage of the PA pathway would be its speed.

**Long-term Ethylene Mediated Responses**

Since ethephon treatment did give a PA response after 24 hours (Fig. 1B; also observed before by Lee et al.\textsuperscript{17}), this may be significant for the progression or maintenance of long-term ethylene-regulated responses. Many ethylene-mediated phenomena, including autocatalytic ethylene regulated senescence, e.g., fruit ripening and flower senescence, are long-term phenomena. PA could represent...
a secondary mechanism to ensure inactivation of CTR1 and the progression of developmental processes. In support of PA’s role, silencing of PLDα1, one of the enzymes that can produce PA, has been shown to delay ethylene-induced senescence.

Conclusions

Evidence is provided for a novel regulator of CTR1 activity; the signaling lipid PA. Its proposed function in vivo is fully consistent with both ethylene and PA signaling and their relation to stress. Our future work will focus on elucidating the implications of PA-binding for CTR1 function and ethylene responses.

References