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Active Oxygen Species (AOS) Generating Enzyme Inhibition upon Generation of AOS in Plant by Single-Molecule Signaling of Ca^{2+}-Dependent Protein Kinase (CDPK) to Suppressor from *Phytophthora infestans*

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Introduction

Plants are exposed to pathogen attack in their environment and have developed mechanisms to respond to biotic elicitors and toxins from pathogens. Among the earliest host cell responses to such attack and stimuli are the production of superoxide anion [1-4], the hypersensitive response (HR) [5-8] and phytoalexin (PA) production [7,9] and a specific calcium signature is often reported [7,10-12]. It is well documented that pathogens produce either glucans or peptide-oligosaccharides that suppress HR and PA production [6,9,13-15]. Suppressor glucans inhibit the generation of AOS during an early period of Pi infection [9,16], yet the receptor that mediates the inhibition of HR in plant cells has not been reported.

The Pi suppressor glucan controls the production of phytoalexin and host cell death in compatible interactions between Pi and potato cultivars [9,15,17]. CDPKs, Ca^{2+}-binding Ser/Thr protein kinases, may function as sensors that decode and translate elevated calcium levels that enhance CDPK activity and subsequent downstream signaling events [13,18-22].

Upon elicitor stimulation of host cells, NADPH oxidase produces AOS as a defensive response [16,23-26]. Superoxide anion (O_2^-) is generated during incompatible interactions between potato and Pi and the production of cellular AOS may constitute the earliest event of the plant defence response and a signal for the induction of hypersensitive cell death, HR [1,26,27]. To date, CDPKs have not been identified in yeast or animal cells, although the mammalian calmodulin-dependent protein kinases also are important calcium-dependent signaling molecules [20]. Plant CDPKs comprise a large gene family (34 members in Arabidopsis) [28], suggesting that individual isoforms have different functions and participate in multiple but distinct signaling pathways. The current challenge in the CDPK field is not only to assign biological functions to specific CDPK isoforms but also to integrate CDPK signaling into the HR response and phytoalexin accumulation.

We have report that downstream CDPK-regulated processes control AOS production during HR. Glucan from the oomycete pathogen, *Phytophthora infestans* (Pi), represent the suppressor for hypersensitive cell death (HR) in plants and have been reported to inhibit the accumulation of PA.

To evaluate the activation of plant Ca^{2+}-dependent protein kinase (CDPK) after the binding of this Pi suppressor, we applied cross-correlation analysis to individual potato culture cells. We constructed a chimeric CDPK tandemly fused to green fluorescent protein (smGFP)-CDPK and the suppressor linked to Alexa-labeled antibodies. Dual-color cross-correlation spectroscopy yielded spectral information on the coincidence of the two fluorescent molecules at the single-molecule level.

Furuichi et al. have reported [29,30] that the suppressor binds CDPK, which phosphorylates NADPH oxidase, thereby inhibiting its ability to generate active oxygen species (AOS).

These results show that hypersensitive cell death (HR) inhibits the action of plant pathogen toxins to control superoxide radical formation by signal transduction via CDPK-mediated phosphorylation.

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