

## Addendum

# Manipulation of Plant Programmed Cell Death Pathways During Plant-Pathogen Interactions

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## KEY WORDS

Adi3, Protein kinase B, Akt, 3-phosphoinositide-dependent protein kinase-1, disease resistance, disease susceptibility, programmed cell death.

## Addendum to:

*Adi3 is a Pdk1-interacting AGC kinase that negatively regulates plant cell death.*

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## ABSTRACT

The interaction of plants with bacterial pathogens involves the manipulation of programmed cell death (PCD) pathways. During a resistance interaction PCD is induced in a process termed the hypersensitive response (HR) which may function to limit pathogen spread. In a susceptible plant-pathogen interaction the pathogen both inhibits and induces host PCD depending on the infection stage. Genes/pathways regulating PCD in plants have been difficult to identify due to a lack of homologous sequences in plants for mammalian genes that control apoptosis and possibly due to functional redundancy. Our labs study plant PCD pathways and bacterial speck disease in tomato which is caused by *Pseudomonas syringae* pv. *tomato* (*Pst*). We recently identified the tomato protein kinases Pdk1 and Adi3 as negative regulators of plant PCD. The plant Pdk1/Adi3 pathway appears to function similarly to the Pdk1/PKB (Akt) pathway in mammals which functions as a major apoptosis negative regulation pathway. Here we discuss regulation of Pdk1/Adi3 and targeting of this pathway during the tomato-*Pst* interaction for modulation of host PCD.

Plant pathogenic bacteria, such as *Pseudomonas syringae* pv. *tomato* (*Pst*), promote disease in hosts by injecting effector proteins into the plant cell using a type III secretion system (TTSS). Once inside the plant cell, these effector proteins suppress immunity pathways and promote certain host susceptibility factors.<sup>1,2</sup> While the direct targets of most effector proteins are unknown, recently some have been shown to act as suppressors of immunity-associated programmed cell death (PCD).<sup>3-5</sup> Plants have evolved resistance (R) proteins to recognize some pathogen effector proteins. This recognition leads to massive changes in gene expression and resistance to the pathogen.<sup>6,7</sup> The hypersensitive response (HR) is a part of resistance and includes rapid cell death and associated cell wall thickening at the site of attempted infection that appears to limit pathogen spread and access to nutrients. The host cell death which occurs during the HR is a form of PCD and occurs within 12 hours of pathogen recognition.<sup>8</sup> From this knowledge it is obvious that manipulation of host PCD pathways plays a very important role during both immunity and disease. However, it has been difficult to identify plant genes involved in regulating PCD let alone determining if they are specifically manipulated during plant-pathogen interactions.

Bacterial speck disease of tomato is caused by *Pst* and the *Pst* effector protein AvrPto is known to promote disease.<sup>6</sup> However, tomato lines containing the Ser/Thr protein kinase R protein Pto are resistant to *Pst* through recognition of AvrPto by Pto.<sup>6</sup> We are studying genes involved in regulating PCD pathways in tomato and how they are affected during the *Pst*-tomato interaction.

## PLANT PCD GENES

The search for plant genes involved in PCD has mostly revealed genes that have an indirect role in regulating PCD<sup>9</sup> and not direct pathway regulators. Searching for direct plant PCD regulating genes based on sequence identity to mammalian apoptosis regulators (e.g.: PKB/Akt and Bcl-2 family members) indicates such sequences are not obviously present in plants. However, advanced bioinformatic searches has revealed the presence of some of these types of sequences and functions in plants.<sup>10</sup> It remains to be determined if these methods will identify more mammalian-like PCD regulators.

We recently used a combination of methods to identify two protein kinases, Adi3 and Pdk1, from tomato that act as negative regulators of plant PCD.<sup>11</sup> Adi3, a Ser/Thr protein kinase, was isolated based on its interaction with the tomato R protein Pto and the *Pst* effector protein AvrPto.<sup>12</sup> Adi3 belongs to the AGC family of protein kinases. A central regulator of AGC kinases, the Ser/Thr protein kinase 3-phosphoinositide-dependent protein

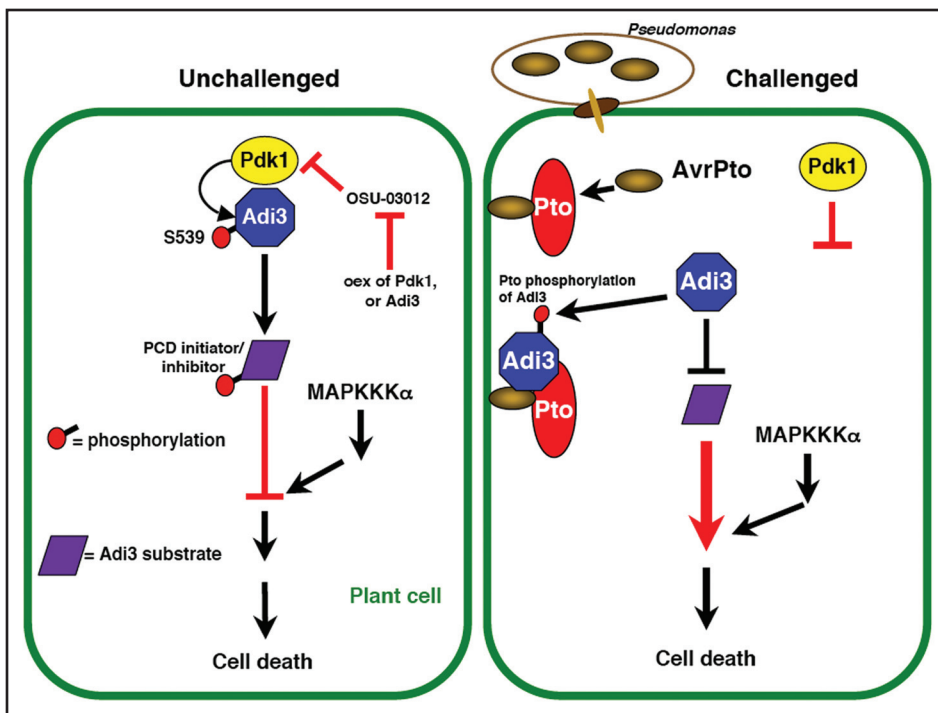


Figure 1. Model for manipulation of Pdk1/Adi3 cell death regulation during pathogen interaction. In unchallenged plant cells, left, Adi3 is activated by Pdk1 phosphorylation at S539. Adi3 then acts to negatively regulate cell death by activating PCD inhibitors or inactivating PCD initiators. MAPKKKα acts downstream in a parallel connected pathway.<sup>11</sup> The Pdk1 inhibitor OSU-03012 induces cell death that can be attenuated by Pdk1 or Adi3 overexpression.<sup>11</sup> In *Pst* challenged cells, right, Adi3 interaction with Pto/AvrPto changes Adi3 cellular localization eliminating Pdk1 activation of Adi3. Thus, Adi3 can not act on downstream substrates leading to loss of cell death control and bringing about cell death. Pto phosphorylation of Adi3 may contribute to this inactivation of Adi3.

kinase-1 (Pdk1), was shown to activate Adi3 by phosphorylation at Ser539.<sup>11</sup> Using both virus induced gene silencing (VIGS) and an inhibitor of Pdk1, Adi3 and Pdk1 were shown to be negative regulators of PCD.<sup>11</sup> Additionally, Adi3 was able to suppress HR cell death induced by a constitutive form of Pto.<sup>11</sup>

Adi3 was also connected to pathogen-associated host cell death pathways. Using VIGS, Adi3 activity was shown to signal through MAPKKKα,<sup>11</sup> a MAPKKK known to regulate PCD during both resistant and susceptible plant-pathogen interactions.<sup>13</sup> Additionally, Pto was shown to phosphorylate Adi3 independent of AvrPto.<sup>11</sup> The interaction of Adi3 with Pto/AvrPto and the connection of its function with pathogen-associated cell death suggests the Pdk1/Adi3 cell death regulation pathway is manipulated during pathogen interactions to bring about cell death.

## REGULATION OF THE Pdk1/Adi3 PATHWAY

In mammalian systems, Pdk1 regulates substrates in both a stimulated and constitutive manner.<sup>14</sup> In mammalian systems, stimulated production of phosphatidylinositol 3,4,5-triphosphate (PtdIns(3,4,5)P<sub>3</sub>) localizes Pdk1 to the plasma membrane where it can activate substrates that also bind PtdIns(3,4,5)P<sub>3</sub> such as PKB.<sup>15,16</sup> The pleckstrin homology (PH) domain of Pdk1 and PKB is responsible for PtdIns(3,4,5)P<sub>3</sub> binding.<sup>16,17</sup> Recently it has been shown that for plant Pdk1 phosphatidic acid (PA), not PtdIns(3,4,5)P<sub>3</sub>, binds the PH domain and acts as the lipid activator.<sup>18</sup> Pdk1 also activates cytoplasmically localized substrates and is thought to be anchored in the cytoplasm by PH domain binding to inositol

phosphates.<sup>17</sup> Thus, PA activation of Adi3 through Pdk1 phosphorylation seems unlikely since Adi3 is predicted to be soluble and does not contain a PH domain. It is yet to be determined if plant Pdk1 can bind inositol phosphates for cytoplasmic localization. Cytoplasmically localized Pdk1 may constitutively activate Adi3 and control of Adi3 activity would most likely be at the level of phosphatases, as has been shown for other proteins that are constitutively phosphorylated by Pdk1.<sup>14</sup>

## A MODEL FOR DIRECTED LOSS OF Adi3 FUNCTION IN PATHOGEN-ASSOCIATED CELL DEATH

Our data support a model (Fig. 1) that when cells are challenged by *Pst* the interaction of Adi3 with Pto/AvrPto interferes with the negative regulatory function of Adi3 leading to cell death. Pto phosphorylates Adi3<sup>11</sup> and it is possible this event inhibits Adi3 function. Our in vitro data indicate this phosphorylation does not require AvrPto indicating that the role of AvrPto, which is known to be localized to the plasma membrane<sup>6</sup> might be to recruit Pto/Adi3 to that cellular location. Thus, a change in the localization of Adi3 to the plasma membrane would not allow for cytoplasmically localized Pdk1 to activate Adi3. This, in combination with Pto phosphorylation of Adi3 may lead to a loss of Adi3 function and bring about cell death.

Our data leave unresolved the question of whether Adi3 inactivation during pathogen interactions underlies cell death associated with immunity or with disease. The association of MAPKKKα with Adi3 suggests that Adi3 could be a target during both resistance and disease. However, recent evidence indicates that kinase activity of Pto is not required for HR-associated cell death.<sup>19</sup> This suggests that if Pto phosphorylation of Adi3 is biologically significant it may play a role in disease-associated cell death.

The discovery of the Pdk1/Adi3 pathway provides new perspectives on PCD regulation and manipulation during plant-pathogen interactions and indicates that mammalian-like PCD regulators are present in plants. Our studies indicate that Adi3 is the functional, not sequence, homolog of mammalian PKB, a Pdk1 activated negative regulator of apoptosis,<sup>20</sup> even though the two proteins share only 21.4% identity.<sup>11</sup> This may indicate that other mammalian-like PCD regulators exist in plants but their discovery will require functional studies and not sequence-based analysis.

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