



ARTICLE ADDENDUM



## A crosstalk between extracellular ATP and jasmonate signaling pathways for plant defense

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### ABSTRACT

Damage-associated molecular patterns (DAMPs), such as extracellular ATP, act as danger signals in response to biotic and abiotic stresses. Extracellular ATP is perceived by a plant purinoceptor, P2 receptor kinase 1 (P2K1), inducing downstream signaling for defense responses. How ATP induces these defense responses has not been well studied. A recent study by Tripathi et al. (*Plant Physiology*, 176: 511–523, 2018) revealed a synergistic interaction between extracellular ATP and jasmonate (JA) signaling during plant defense responses. This signaling crosstalk requires the formation of secondary messengers, i.e., cytosolic calcium, reactive oxygen species, and nitric oxide. This finding has given a new direction towards understanding the defense signals activated by DAMPs. In this addendum, we discuss possible insights into how extracellular ATP signaling interacts with the JA signaling pathway for plant defense responses.

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All organisms use adenosine-5-triphosphate (ATP) as an essential energy source and as a basic substrate in various intracellular biochemical and signaling reactions.<sup>1</sup> In plants, external stimuli, such as wounding or herbivore feeding, can cause the release of ATP from inside (the cytoplasm) to outside the cell (the extracellular matrix). The released ATP is recognized as a signal, a so-called damage-associated molecular pattern (DAMP), to activate plant defense responses.<sup>2</sup> Although the role of extracellular ATP as a DAMP in plants has not been well understood, after the discovery of the first purinoceptor in plants, P2 receptor kinase 1 (P2K1), which was originally identified based on the isolation of an ATP-insensitive mutant, *dorn1* (does not respond to nucleotide 1) by a forward genetic screening, it is now evident that extracellular ATP acts as an essential signal for plant life cycle activities.<sup>3,4</sup> Unlike the purinoceptors in animals, P2K1 is a lectin-receptor kinase (also known as LecRK-I.9). As highlighted in several reviews, accumulating evidence has suggested a role for extracellular ATP in plant growth, development, and stress responses.<sup>2,4-7</sup> Additionally, extracellular ATP is categorized as a classical DAMP, in contrast to secondary DAMPs or phytochemicals (e.g., elicitor peptides).<sup>8</sup>

There are several lines of evidence showing that extracellular ATP is involved in plant defense responses. For example, ATP induces the expression of defense-related genes, e.g., JA- and ethylene-induced genes.<sup>9,10</sup> ATP treatment of wounded areas of lima bean leaves mimicked JA-dependent responses.<sup>11</sup> Suppression of extracellular ATP-hydrolyzing enzymes, ectoapyrases, caused the accumulation of extracellular ATP, which consequently induced the expression of defense genes. Recent

transcriptomic study revealed that approximately 60% of ATP-induced genes are also induced by wounding.<sup>3</sup> In addition, gene Ontology enrichment analysis based on the ATP-induced transcriptome suggested that extracellular ATP is involved in a range of plant responses to abiotic and biotic stresses.<sup>12</sup> All these studies directly indicate that extracellular ATP acts as a central signal in many plant defense responses.

In contrast, there are several reports indirectly suggesting involvement of extracellular ATP in plant defense responses. For example, some insects (e.g., herbivorous caterpillars and whitefly larvae) were reported to secrete saliva containing ATP-hydrolyzing enzymes.<sup>13,14</sup> Application of the caterpillar apyrase blocked glandular trichome production (a plant defense reaction against herbivores) and the expression of defense-related genes at the wound site.<sup>14</sup> Furthermore, enhanced apyrase catalytic activity in globose insect galls on *Calliandra brevipes* was detected.<sup>15</sup> A clear explanation is that those apyrases dampen extracellular ATP, thus attenuating the plant defense response. Similar mechanisms for controlling extracellular ATP concentrations by parasitic worms or blood-sucking insects feeding on animals have been described<sup>16,17</sup> (see the review by Guiguet et al.<sup>18</sup> in detail). Interestingly, a reduction in extracellular ATP levels was observed following plant infection by a bacterial pathogen *P. syringae*, but not observed using its type III secretion system deficient *hrcV* mutant,<sup>19</sup> suggesting that there are effector proteins for extracellular ATP depletion. Taken together, these observations suggest that pathogens and insects always seek to reduce extracellular ATP levels to elude plant defenses, thereby enhancing subsequent infestation of the plant host.

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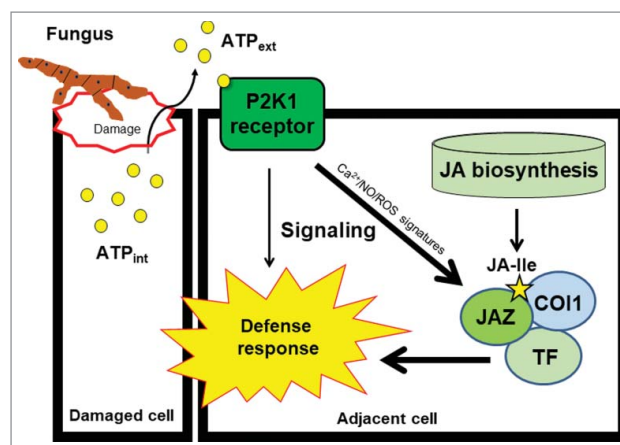
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Using knockout mutants and a P2K1 overexpression line, Balagué et al. showed that P2K1 was involved in increased resistance to a bacterial pathogen, *P. syringae*, through JA signaling.<sup>20</sup> Extracellular ATP is also reported to enhance stomatal immunity against bacterial pathogens,<sup>21</sup> in which P2K1 directly controls the activity of an NADPH oxidase, RBOHD, that catalyzes the production of superoxide, a type of reactive oxygen species (ROS). Ectopic expression of P2K1 in Arabidopsis, tobacco, and potato showed enhanced resistance to the oomycete pathogen *Phytophthora infestans* or *P. brassicae*.<sup>22,23</sup> In a recent study, Tripathi et al. showed the ATP induced resistance against a necrotrophic fungus, *Botrytis cinerea*, in a P2K1-dependent manner.<sup>24</sup> In this case, ATP and JA act synergistically to maximize the plant defense response. All these abovementioned data strongly suggest the essential role of ATP in mediating plant immunity against pathogens via P2K1. However, a dearth of information about the function of P2K1 has restricted our further understanding of ATP signaling.

Although a mechanism for extracellular ATP in plant immunity has not been explored in great detail, Tripathi et al. dissected the downstream signaling pathway of extracellular ATP, which interplays with other signaling pathways of plant defense hormones.<sup>24</sup> Data mining using publicly available software identified the P2K1-associated coexpression network containing a set of defense-related genes, some of which encode jasmonate ZIM-domain (JAZ) proteins, key regulators of JA signaling. A reporter-based approach measuring protein stability revealed that the stability of the JAZ1 protein is directly regulated by ATP addition in a proteasome-dependent manner. This protein stability change required the interaction of JAZ1 with a JA receptor, coronatine-insensitive 1 (COI1), which is indispensable for the induction of JA-specific primary transcription factors.<sup>25</sup> Interestingly, ATP-induced JAZ1 degradation was not attenuated upon the addition of JA biosynthesis inhibitors or in a JA biosynthesis mutant. These results were corroborated by the finding that ATP does not change the basal levels of JA content, showing the direct interaction of extracellular ATP signaling with JA signaling pathways. In addition, ATP-induced JAZ1 degradation requires the formation of secondary messengers. A synergistic effect of ATP and JA on plant defense is attributed to direct activation of JA signaling by ATP-induced changes in the signatures of secondary messengers. These results demonstrate that ATP activates stress hormone signaling, which eventually activates additional defense systems.

In summary, the proposed model (Fig. 1) indicates crosstalk between extracellular ATP and JA signaling for plant defense responses, where intracellular ATP molecules are released into the extracellular matrix upon damage caused by pathogen attack. The released ATP acts as a danger signal, which binds to its receptor P2K1, followed by the formation of secondary messenger signatures:  $\text{Ca}^{2+}$ , NO and ROS. This extracellular ATP signaling via P2K1 enhances the COI1-JAZ protein interaction and ultimately activates JA-signaling-mediated gene expression for plant defense responses. This event primes the JA-dependent defense responses prior to JA accumulation upon pathogen attack.

Further studies, e.g., identifying the modifications of COI1 and/or JAZ1 proteins upon ATP addition, can help determine



**Figure 1.** A proposed model of the interaction between extracellular ATP signaling and JA signaling in plants based on Tripathi et al. (2018).<sup>24</sup> Extracellular ATP, released after cellular damage by pathogen attack, binds to the P2K1 receptor on the plasma membrane. The activated P2K1 receptor turns on extracellular ATP signaling, which directly enhances JA signaling by changing the interaction of the COI1-JAZ1 complex. Eventually, P2K1-mediated ATP signaling induces the defense response against pathogens by crosstalk with the JA-mediated pathway in addition to the ATP-specific pathway. This ATP-activated JA signaling requires the formation of second messengers ( $\text{Ca}^{2+}$ , ROS, and NO) and is probably a bypass event that occurs before *bona fide* JA synthesis to prime plant defense responses. TF: transcription factor.

how the COI1-JAZ1 complex forms upon activation of extracellular ATP signaling, which depends on the formation of secondary messenger signatures.

Additionally, Tripathi et al. showed that salicylic acid (SA)-dependent genes were induced by extracellular ATP.<sup>24</sup> Given that ATP treatment does not alter SA levels,<sup>19,24</sup> there is the possibility of direct activation of SA signaling by extracellular ATP signaling via secondary messengers, as seen in the case of the crosstalk between extracellular ATP and JA signaling, which needs to be further explored in the future.

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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