

Extracellular ATP: a Potential Molecule Regulating the Defence Response of Plants to Biotic Stresses – a Review

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Abstract

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Although adenosine 5'-triphosphate (ATP) is commonly considered as an intracellular energy currency molecule, animal, plant, and microbial cells can secrete ATP from the cytosol into the extracellular matrix. In plant cells, extracellular ATP (eATP) is found to play important roles in regulating several physiological processes, such as cell growth, development, and death. Interestingly, recent studies suggest that eATP could be a potential molecule required for the regulation of the defence responses of plants to pathogen infection and herbivore attack. This review article summarises the preliminary studies that have been conducted regarding the possible involvement of eATP in plant defence responses to biotic stress. And, we also attempt to address some speculations and theoretical discussions to aid future research in this area.

Keywords: calcium; extracellular adenosine 5'-triphosphate; herbivore; pathogen; salicylic acid

Adenosine 5'-triphosphate (ATP) is mainly produced by intracellular organelles (including the mitochondria and chloroplasts) in eukaryotes or it is produced by plasma membrane located ATPases in bacteria. It is widely known that this molecule serves as energy currency to support the energy-requiring biochemical reactions.

The existence of extracellular ATP (eATP) was first revealed in animal cells. It was found that animal cells can secrete ATP from the cytosol into the extracellular matrix through anion channels, vesicular exocytosis, gap junction hemichannels, or ATP-binding cassette (ABC) transporters (BODIN & BURNSTOCK 2001; DUTTA *et al.* 2002; LAZAROWSKI *et al.* 2003). Animal cells also have the ability to directly produce eATP via a novel plasma membrane F_0F_1 -ATP synthase complex (MOSER *et al.* 1999; MARTINEZ *et al.* 2003; MANGIULLO *et al.* 2008). In addition to the release or production of eATP, animal cells can hydrolyse eATP by ATP hydrolytic enzymes localised in the

extracellular matrix, such as ecto-nucleotidases and ecto-apyrases (YEGUTKIN *et al.* 2000; MIZUMOTO *et al.* 2002). Changes in the level of eATP are found to affect neurotransmission, growth, immune responses, and muscle contraction of animal cells (LUSTIG *et al.* 1993; KHA KH & BURNSTOCK 2009). And, the studies in animal cells have revealed that the membrane-associated P2-type purinoceptor proteins, including P2Y (G protein-coupled receptors) and P2X (ligand-gated ion channels), are responsible for the perception of eATP and the eATP-mediated physiological processes (DICHMANN *et al.* 2000; KHA KH & NORTH 2006; ABBRACCHIO *et al.* 2006).

Similar to animal cells, plant cells can also release ATP from the cytosol (via ABC transporters or exocytosis) and hydrolyse eATP (by apoplastic nucleotidases or apyrases) (THOMAS *et al.* 2000; KIM *et al.* 2006; RIEWE *et al.* 2008; TANAKA *et al.* 2010a, 2014; CAO *et al.* 2014; CHOI *et al.* 2014; SHEPPARD 2014). However, no study has shown the existence

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of ATP synthase in the plant cell surface or plasma membrane, indicating that intracellular ATP (iATP) could be the major, even sole, resource of plant eATP.

Some studies have revealed that artificially changing the levels of plant eATP can influence the growth, development, and survival of plant cells (TANAKA *et al.* 2010a, 2014; CAO *et al.* 2014; CHOI *et al.* 2014; SHEPPARD 2014). And, eATP appears to regulate some unique physiological processes of plants, such as thigmotropism and gravitropism of roots (TANAKA *et al.* 2010a, 2014; and references cited therein). Previous studies have shown that calcium (Ca^{2+}), nitric oxide (NO), reactive oxygen species (ROS), salicylic acid (SA), jasmonates (JA), cyclic nucleotides, polyphosphoinositides, sugars, abscisic acid (ABA), and polyamines play important roles as central signalling molecules during a variety of plant stress responses (TUTEJA & SOPORY 2008). An elevation of eATP levels can induce the expression of many genes involved in stress responses (LIM *et al.* 2014). And, exogenous application of ATP can obviously affect the production of Ca^{2+} , NO, ROS, and SA (see details below). Therefore, eATP is considered as one of the central signalling molecules in stress responses of plants.

Although genomic sequence-based surveys to identify plant eATP receptor homology to animal purinoceptors failed to find any suitable candidate proteins, it is still believed that plants possess membrane-associated receptor proteins to perceive eATP. This is supported by the recent study by CHOI *et al.* (2014), who revealed that the DORN1 protein (Does not Respond to Nucleotides 1, a lectin receptor kinase I.9) in *Arabidopsis* binds eATP with high affinity and is required for some eATP-induced physiological responses.

Intriguingly, recent experimental advances about plant eATP suggest that eATP could be an important component to affect the defence responses of plants to different types of biotic stresses (see details below). Understanding such roles of eATP in plants is seemingly helpful in greatly expanding the current knowledge of regarding the functions of plant ATP and the mechanisms of plant defence responses.

Signalling molecules involved in plant defence responses are affected by eATP

Treatments of the plant with exogenous ATP can increase the contents of cytosolic free calcium ($[\text{Ca}^{2+}]_{\text{cyt}}$),

ROS, and NO, all of which are well known as important intracellular signalling molecules (KIM *et al.* 2006; SONG *et al.* 2006; FORESI *et al.* 2007; WU & WU 2008; DEMIDCHIK *et al.* 2009; REICHLER *et al.* 2009; CLARK *et al.* 2010; TANAKA *et al.* 2010b; SUN *et al.* 2012a; LIM *et al.* 2014; MÖHLMANN *et al.* 2014). The increase of $[\text{Ca}^{2+}]_{\text{cyt}}$ induced by eATP is considered to come from an influx of Ca^{2+} from the extracellular Ca^{2+} and a release of Ca^{2+} from the intracellular Ca^{2+} storages including the vacuole, endoplasmic reticulum or mitochondria (DEMIDCHIK *et al.* 2009; TANAKA *et al.* 2010b). The increased production of ROS induced by eATP is considered to come from the plasma membrane NADPH oxidases or mitochondria (DEMIDCHIK *et al.* 2009; SUN *et al.* 2012a). Further studies showed that the eATP-induced productions of NO and ROS are dependent on the elevation of $[\text{Ca}^{2+}]_{\text{cyt}}$ (WU & WU 2008; DEMIDCHIK *et al.* 2009; SUELDO *et al.* 2010; SUN *et al.* 2012a; MÖHLMANN *et al.* 2014). In turn, the production of either ROS or NO may also act as an important event for further elevation of $[\text{Ca}^{2+}]_{\text{cyt}}$ upon eATP stimulation (WU & WU 2008; DEMIDCHIK *et al.* 2009). These observations indicate that there exists a synergistic interaction among Ca^{2+} , ROS, and NO during eATP perception.

SONG *et al.* (2006) reported that in *Arabidopsis* leaves the genes encoding the enzymes for JA and ET (ethylene) biosynthesis were induced by exogenous ATP, implying that eATP could also have the ability to increase the content of JA and ET (although this has not been confirmed by any scientific reports). CHIVASA *et al.* (2009) revealed that treatment with exogenous ATP caused a decrease of SA content of tobacco leaves.

Furthermore, the eATP-mediated signal transduction network could be more complex than expected. For example, it is reported that the release of ATP from the roots of *Medicago truncatula* can be blocked by Ca^{2+} chelator (KIM *et al.* 2006), while the eATP level of tobacco leaves is found to be decreased by SA application (CHIVASA *et al.* 2009). These observations indicate that, as the downstream signalling molecules of eATP, Ca^{2+} , and SA can also affect the eATP level.

Another noteworthy point in studying the eATP-initiating signalling pathways is the interrelationship between eATP and iATP. As mentioned above, plant eATP mainly originates from the iATP pool. Thus, in principle, the level of plant iATP could affect the eATP level to a large extent. If this is the case and considering that a mutual characteristic of

Ca^{2+} and SA is that they can affect the production of iATP [i.e., Ca^{2+} can positively mediate the iATP level (JOUAVILLE *et al.* 1999), and SA can decrease iATP (NORMAN *et al.* 2004)], one may assume that changes in the eATP level by Ca^{2+} or SA could be attributed to the effects of Ca^{2+} and SA on iATP production. However, such a hypothesis is merely speculative and could be too simple. For example, the effect of Ca^{2+} on iATP production is determined by the balance between cytosolic and mitochondrial Ca^{2+} levels, rather than only by a change of the cellular Ca^{2+} level (GRIFFITHS & RUTTER 2009). Thus, the actual mechanism may be more complex than the hypothesised one.

It is well known that plant defences against pathogens and herbivores are conferred by either SA or JA/ET signalling pathways, depending on the infection or herbivore grazing strategy (MUR *et al.* 2013). Ca^{2+} , ROS, and NO have emerged as inducers or suppressors of the SA and JA/ET signalling pathways (COCA & SAN-SEGUNDO 2010; MUR *et al.* 2013; and references cited therein). Thus, it is reasonable to assume that changes of eATP could affect the plant defences against pathogens or herbivores by regulating these downstream signalling molecules. However, few studies have investigated this issue.

Potential role of eATP in the defence responses to herbivores

Short-term (0.5–3 h) treatment with exogenous ATP (1, 5, 50, or 100 μM) can trigger some physiological events that happen when plants experience wounding, herbivore attack, or pathogen infection, such as the accumulation of ROS and the expression of some defence response genes including *PAL1* (a well-known gene marker of phenolic-based defence responses), *LOX2* (encoding the enzyme for JA synthesis), and *ACS6* (encoding the enzyme for ET synthesis) (SONG *et al.* 2006). CHOI *et al.* (2014) revealed that treatment with exogenous 100 μM ATP for 5–60 min increased the expression of mitogen-activated protein kinases, which have been reported to be induced by wounding, herbivore attack, or pathogen infection and be involved in herbivore and pathogen resistance (ZHANG & KLESSIG 1998; MENKE *et al.* 2004; WU *et al.* 2008; HEINRICH *et al.* 2011). Correspondingly, by suppressing the expression of apyrase that functions in hydrolysing eATP, it is found that a slight enhancement of the eATP concentration

for 3–6 days induced the accumulation of ROS, biosynthesis of lignin, and expression of several genes that are involved in the defence responses to insect herbivores or pathogens (LIM *et al.* 2014).

Cell wounding is a common result of herbivore attack (HEIL 2009), and cell wounding can also afford a passive route of ATP release (TANAKA *et al.* 2010a). Therefore, although there has yet been no experimental evidence to show whether the eATP level of plants would be actually increased upon herbivore attack, it is expected that herbivore attack could cause an elevation of eATP levels by wounding. SONG *et al.* (2006) reported that in the leaves of *Arabidopsis*, eATP levels released at wounding sites can reach 40 μM . CHOI *et al.* (2014) reported that about sixty percent of the eATP-induced genes were also induced by wounding, and ectopic expression of DORN1 at a higher level increased the responses of *Arabidopsis* to wounding. In animal cells, ATP is released from the inflamed, damaged, or metabolically impaired cells into the extracellular space and functions as a danger signal to trigger inflammatory and other immune-related processes of the adjacent cells (HANLEY *et al.* 2004). HEIL (2009) proposed that eATP release in plants may also be a damaged-self signal to trigger the defence response against herbivore attack. This seems to be supported by a recent work by HEIL *et al.* (2012), who demonstrated that the application of ATP to slightly wounded lima bean (*Phaseolus lunatus*) leaves resulted in an enhancement of the level of *extrafloral nectar* (EFN) secretion, which is known as an indirect defence response of plants to herbivorous insects (ELIAS 1983; KESSLER & BALDWIN 2002).

More interestingly, herbivorous insects appear to evolve the genetic capacity to terminate this “damaged-self signalling” of plants. Treatment with oral secretions of *Helicoverpa zea* caused a degradation of eATP in tomato leaves, and a high level of ATPase activity and three ATP hydrolysing enzymes were detected in the saliva and salivary glands of this herbivore. The application of these purified ATP-hydrolysing enzymes suppressed the expression of the JA/ET-mediated defensive genes of tomato leaves in response to herbivore attack (WU *et al.* 2012). Hence, it seems that *Helicoverpa zea* can use these ATP-hydrolysing enzymes to limit the release of eATP during plant cell wounding, thereby counteracting the defence response of host plants for the herbivore’s own benefit. However, similar phenomena have not been reported in other herbivore-plant interactions.

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Thus, more research is expected to reveal whether eATP could be a potential determinant of the survival of both the herbivore and the host plant during their interaction.

Plant defence responses to pathogens and eATP

As mentioned above, short-term or slight enhancements of eATP level seem to induce some physiological responses that are also induced by or are associated with the defence responses to pathogen (SONG *et al.* 2006; LIM *et al.* 2014). Thus, eATP could serve as an early signal of plant defence responses to pathogen infection. However, the treatment of tobacco leaves with exogenous ATP at a higher level ($\geq 800 \mu\text{M}$) or with prolonged term (days) has been shown to compromise the basal resistance of tobacco leaves to viral and bacterial infection (CHIVASA *et al.* 2009), consistent with the further observations that the long-term (for days) reduction or competition of eATP using pharmacological agents induced the accumulation of pathogenesis-related (PR) protein and increased the resistance of tobacco leaves to bacterial and viral pathogens (CHIVASA *et al.* 2009, 2010).

It is well known that SA can induce the expression of PR genes and enhance the resistance of plants to bacterial (or viral) pathogens (GAFFNEY *et al.* 1993; DELANEY *et al.* 1995). Thus, the responses triggered by eATP reduction appear very similar to those induced by SA. In addition, when an increase in the eATP concentrations compromised the resistance of tobacco leaves to viral and bacterial infection, the SA content was obviously decreased (CHIVASA *et al.* 2009). Furthermore, CHIVASA *et al.* (2009) found that the treatment of tobacco leaves with SA decreased the level of eATP, which preceded the induction of PR by SA, and blocking of this reduction in the eATP level suppressed the SA-induced expression of PR genes. These observations imply that the eATP-mediated plant defence responses may be associated with SA, and the ability of SA to induce defence responses could also be associated with eATP. However, the relationship between the SA- and eATP-regulated defence responses was explored only in tobacco by CHIVASA *et al.* (2009; 2010). More scientific works are expected to investigate whether such a relationship could widely exist in the plant kingdom.

Another study by CHIVASA *et al.* (2009) found that the eATP reduction in fact occurs when tobacco leaves are infected with the bacterial pathogen

Pseudomonas syringae pv. *tabaci* (*P.s.t.*), whereas this reduction was not observed when using mutant *P.s.t.* strains that lack the *hrp* gene (hypersensitive reaction and pathogenicity). This observation suggests that the product of the *hrp* gene can invoke a reduction of eATP.

The exact mechanism through which the product of the *hrp* gene can cause the reduction of eATP of tobacco leaves has not yet been elucidated. Previous studies showed that a fungal pathogen elicitor from *Mycosphaerella pinodes* stimulated the ecto-ATPases of pea (KAWAHARA *et al.* 2003). ROBIN *et al.* (2014) reported that adenylate kinases secreted by *Xanthomonas oryzae* use eATP of host plant as a phosphate donor. Thus, it is likely that the product of the *hrp* gene directly consumes plant eATP or stimulates eATP hydrolysis. Otherwise, it is also likely that the product of the *hrp* gene could impair iATP production and subsequently decrease the eATP level. For example, hairpin, a bacteria-derived elicitor protein that is encoded by the *hrp* gene cluster, is found to inhibit mitochondrial ATP production (KRAUSE & DURNER 2004).

It should also be noted that an increase in eATP promotes the stomatal opening of *Arabidopsis* leaves and a depletion of eATP by apyrase inhibits stomatal opening (HAO *et al.* 2012). Because stomatal opening is a major route of pathogen entry into plant tissues (ZENG *et al.* 2012), it is worth considering whether the eATP-regulated stomatal opening (or closing) could be involved in the effects of eATP on the defence responses to pathogens.

In particular, in addition to a change in the eATP level of the host plants in response to pathogen infection, some studies have shown that the microbial cell itself can also release substantial amounts of eATP (THOMAS *et al.* 2000; IVANOVA *et al.* 2006). Although there is no evidence to show whether eATP production from bacterial pathogens has a potential function in mediating the defence responses of host plants during the host-pathogen interaction, a theoretical possibility still exists. Future research is expected to reveal the roles of pathogen-produced eATP during the plant-pathogen interaction.

Systemic acquired resistance (SAR) can be regulated by eATP

After the formation of a necrotic lesion by an earlier localised infection by the pathogen (either as

part of the hypersensitive response or as a symptom of disease), systemic acquired resistance (SAR) in uninfected tissues is activated via long-distance signal transmission from the infected tissue (RYALS *et al.* 1996; SHAH & ZEIER 2013). In the last decades, several candidates for this long-distance signal transmission, including SA, methyl salicylate, dicarboxylic acid, azelaic acid, JA, and the amino acid-derivative pipecolic acid, have been identified (DEMPSEY & KLESSIG 2012; SHAH & ZEIER 2013). Interestingly, it has been revealed that eATP depletion has the potential to trigger a response similar to SAR. CHIVASA *et al.* (2009, 2010) observed that the treatment of one half of a tobacco leaf with an eATP inhibitor (AMP-PCP or a mixture of glucose and hexokinase) enhanced the resistance to pathogens and PR protein accumulation in the untreated half of the same leaf. Furthermore, Chivasa and co-workers also excluded the possibility that this systemic effect of eATP depletion is due to the leakage or movement of eATP inhibitors to the untreated half.

One of the assumptions regarding this phenomenon is that eATP can affect those known candidates for the signal transmission of SAR. Otherwise, as an extracel-

lular molecule, eATP itself is a mutual component of adjacent cells in multicellular organisms, which means that the ATP released from one cell can directly affect adjacent cells. In animal cells, eATP has been proved to play a role in cell-to-cell communication (COTRINA *et al.* 2000). Based on these characteristics of eATP, it is also possible that when the treatment with eATP inhibitor decreased the eATP level in the cells of the treated tissue, the reduced secretion of eATP from the cells of the treated tissue would lead to a decrease in eATP that can be perceived by the cells adjacent to the treated tissue, and thus induce defence responses in the adjacent tissue. However, the phenomenon that eATP depletion can trigger a SAR-like response was reported only by CHIVASA *et al.* (2009, 2010). Moreover, the concrete interpretation of this phenomenon has not been clear, either.

Conclusions and perspectives

The current literature summarised in this review demonstrates that under biotic stresses, changes in the eATP level could play important roles in regulat-

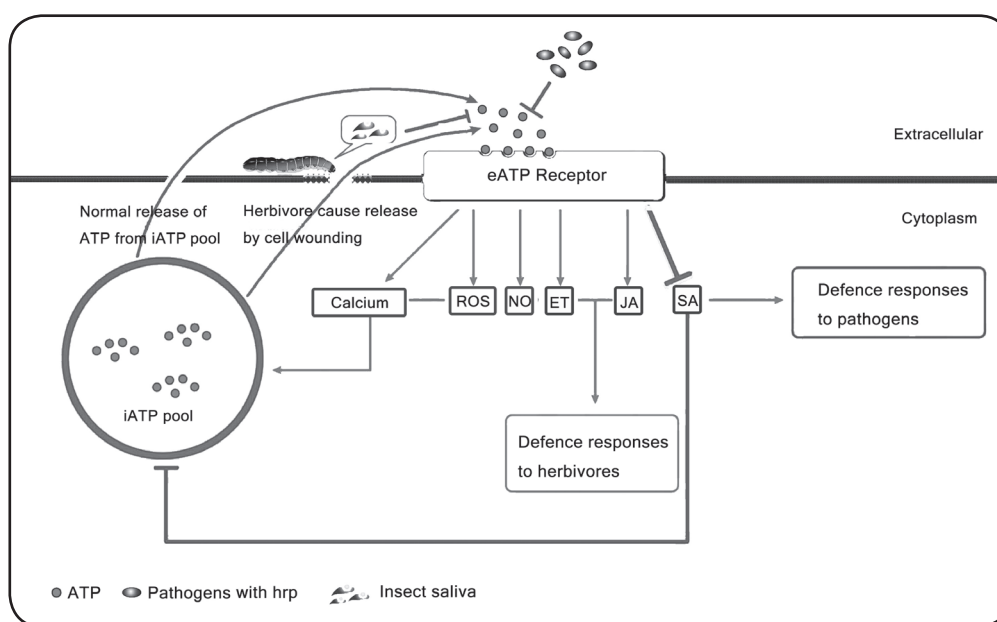


Figure 1. Potential signalling pathways mediated by eATP in the mediation of defence responses to biotic stresses. Through binding to membrane-associated receptor proteins, an increase in eATP causes increases in the production of calcium (Ca^{2+}), reactive oxygen species (ROS), nitric oxide (NO), jasmonic acid (JA), and ethylene (ET). JA and ET can act as signalling molecules for the induction of plant defence responses to herbivorous insects. Salicylic acid (SA), as a key signalling molecule for the activation of defence responses to pathogens, is suppressed by an increase in the eATP level. In turn, calcium and SA could affect the eATP level by affecting the iATP level. In natural environments, pathogenic bacteria with the *hrp* gene (hypersensitive reaction and pathogenicity) can decrease eATP. Herbivore attack may increase eATP by cell wounding or decrease eATP through the secretion of ATPase.

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ing the defence responses of plants to different types of biotic stresses (pathogen infection or herbivore attack). Similarly, some abiotic stress can also affect the release of ATP and the change of eATP level plays important roles in regulating the biotic stress responses. For example, SUN *et al.* (2012b) found that salt shock induced a transient elevation in the eATP level, and eATP regulated a wide range of cellular processes required for salt adaptation. Thus, it seems likely that eATP is a central signal involved in the plant response to a variety of environmental stresses. We make an attempt to illustrate how the eATP-mediated signalling pathways are involved in the mediation of the defence responses to biotic stresses (Figure 1). This scheme is only initial, since research concerning plant eATP is still at its early stages. Particularly, most of the studies about the effects of eATP on plant defence responses used pharmacological approaches. An alternative interpretation of the result obtained by such approaches is that the responses of plants to eATP changes might be attributed to the changes of the eATP-dependent metabolic processes in the extracellular matrix, rather than to signalling changes mediated by eATP receptors. Fortunately, the identification of the extracellular receptor targets of plant eATP by the CHOI *et al.* (2014) would help precisely reveal the mechanisms for the effects eATP on plant defence responses.

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