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Review

Extracellular ATP: a potential regulator of plant cell death

HANQING FENG*, DONGDONG GUAN, JINGYUE BAI, KUN SUN AND LINGYUN JIA

College of Life Science, Northwest Normal University, Lanzhou 730070, China

SUMMARY

Adenosine 5'-triphosphate (ATP) has been regarded as an intracellular energy currency molecule for many years. In recent decades, it has been determined that ATP is released into the extracellular milieu by animal, plant and microbial cells. In animal cells, this extracellular ATP (eATP) functions as a signalling compound to mediate many cellular processes through its interaction with membrane-associated receptor proteins. It has also been reported that eATP is a signalling molecule required for the regulation of plant growth, development and responses to environmental stimuli. Recently, the first plant receptor for eATP was identified in *Arabidopsis thaliana*. Interestingly, some studies have shown that eATP is of particular importance in the control of plant cell death. In this review article, we summarize and discuss the theoretical and experimental advances that have been made with regard to the roles and mechanisms of eATP in plant cell death. We also make an attempt to address some speculative aspects to help develop and expand future research in this area.

Keywords: cell death, extracellular ATP.

INTRODUCTION

All cells use adenosine 5'-triphosphate (ATP) as energy currency to drive and fuel energy-requiring biochemical reactions. Although ATP is usually considered to be localized in intracellular organelles, such as the mitochondria, chloroplasts and cytoplasm, many studies have revealed that this molecule is secreted from the cytosol into the extracellular matrix by animal, plant and microbial cells (Boyum and Guidotti, 1997; Parish and Weibel, 1980; Thomas *et al*., 2000).

In animal cells, the release of ATP into the extracellular matrix is mediated by anion channels, gap-junction hemichannels, ATP-binding cassette (ABC) transporters and vesicular exocytosis (Bodin and Burnstock, 2001; Dutta *et al*., 2002; Lazarowski *et al*., 2003). In addition to the release of ATP from the cytosol, extracellular ATP (eATP) of animal cells can be produced directly via a novel plasma membrane F_0F_1-ATP synthase complex (Mangiullo *et al*., 2008; Martinez *et al*., 2003; Moser *et al*., 1999). Simultaneously, some ATP hydrolytic enzymes in the extracellular matrix, such as ecto-nucleotidases and ecto-apyrases, can hydrolyse eATP (Mizumoto *et al*., 2002; Yegutkin *et al*., 2000). Thus, the level of eATP is highly controlled by a dynamic balance of eATP generation and hydrolysis. Furthermore, eATP is found to be an absolute requirement for several physiological processes of animal cells, including neurotransmission, cell growth and death, immune responses and muscle contraction (Khakh and Burnstock, 2009; Lustig *et al*., 1993). Some studies have reported that eATP can stimulate an increase in cytosolic free calcium ($[Ca^{2+}]_{cyt}$), nitric oxide (NO) or reactive oxygen species (ROS), all of which are well known as important cellular signalling molecules (Dichmann *et al*., 2000; Silva *et al*., 2006). In addition, the eATP of animal cells has been confirmed to trigger these physiological events or increases in signalling molecules by binding and activating membrane-associated receptor proteins, including P2Y (G-protein-coupled receptors) and P2X (ligand-gated ion channels) (Abbracchio *et al*., 2006; Khakh and North, 2006).

In recent decades, eATP has also been found to exist in the extracellular matrix of plant cells (Cao *et al*., 2014; Choi *et al*., 2014b; Sheppard, 2014; Tanaka *et al*., 2010a; 2014; and references cited therein). Intracellular ATP (iATP) may be a major resource of plant eATP because the existence of ATP synthase at the cell surface or plasma membrane of plant cells has not been reported. Previous studies have revealed that plant cells can release ATP from the iATP pool via either ABC transporters or exocytosis (Kim *et al*., 2006; Thomas *et al*., 2000). Moreover, the level of eATP may be increased by certain environmental stimuli, such as wounding, touch, hypertonic stress and pathogen infection (Cao *et al*., 2014; Choi *et al*., 2014b; Sheppard, 2014; Tanaka *et al*., 2010a; 2014; and references cited therein). Similar to animal cells, plant cells also possess apoplastic nucleotidases and apyrases to hydrolyse eATP (Riewe *et al*., 2008). Some studies have demonstrated that artificially changing the level of plant eATP can affect cell growth, development, biotic and abiotic stress responses, cell viability, thigmotropism and gravitropism, indicating that eATP plays important physiological roles in the plant (Cao *et al*., 2014; Choi *et al*., 2014b; Sheppard, 2014; Tanaka *et al*., 2010a; 2014; and references cited therein). It is believed that the effects of eATP on these plant physiological processes are dependent on a membrane-associated receptor protein(s) because eATP cannot freely diffuse across the plasma membrane (Choi *et al*.,

**Correspondence*: Email: hanqing_feng@hotmail.com

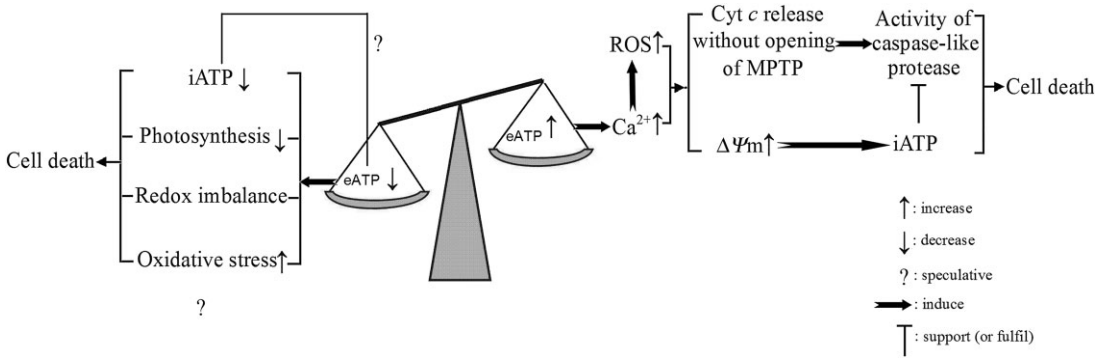

Fig. 1 The effects of extracellular adenosine 5'-triphosphate (eATP) levels on cell death (an extremely low or high level of eATP causes cell death), and a possible model for the potential signalling pathways or physiological events mediated by eATP involved in the induction of cell death. Cyt *c*, cytochrome *c*; iATP, intracellular ATP; MPTP, mitochondrial permeability transition pore; ROS, reactive oxygen species.

2014b; Sheppard, 2014; Tanaka *et al*., 2010a). Recently, DORN1 (Does not Respond to Nucleotides), a lectin receptor kinase, has been found to recognize eATP in *Arabidopsis* by mutant screening, and is required for some eATP-induced cellular responses (Choi *et al*., 2014a).

A most intriguing and complex phenomenon in plant eATP studies is that drastic changes in plant eATP level, either increases or decreases, can cause cell death (Fig. 1; see details in Table 1 and references cited therein). This demonstrates that the homeostasis of the eATP level is vital for plant cell survival. Although the roles and mechanisms of eATP in plant cell death have not been fully elucidated, such behaviour of eATP provides a highly attractive paradigm for increasing our knowledge of how plants govern the cell death process through an extracellular molecular regulator.

PLANT SIGNAL TRANSDUCTION MEDIATED BY eATP

Downstream signalling molecules of eATP

In animal cells, it has been demonstrated that eATP, through the activation of its receptor proteins located in the plasma membrane, initiates an increase in $[Ca^{2+}]_{cyt}$ level, which acts as an early signalling step for the eATP-mediated physiological events (Dichmann *et al*., 2000). Treatment with exogenous ATP can also result in a specific increase in $[Ca^{2+}]_{cyt}$ in plant cells (Demidchik *et al*., 2009; Möhlmann, 2014; Tanaka *et al*., 2010b). Choi *et al*. (2014a) revealed that, in *Arabidopsis*, DORN1 protein binds eATP with high affinity and is required for the eATP-induced increase in the $[Ca^{2+}]_{cyt}$ level. Currently, it is believed that the eATP-induced increase in $[Ca^{2+}]_{cyt}$ originates from an influx of Ca^{2+} from the extracellular space through plasma membrane Ca^{2+}-permeable channels and a release of Ca^{2+} from the internal Ca^{2+} pools, including the vacuole, endoplasmic reticulum and mitochondria (Demidchik *et al*., 2009; Tanaka *et al*., 2010b).

The perception of eATP by plant cells also causes an increased production of both extracellular and intracellular ROS (Clark *et al*., 2010; Demidchik *et al*., 2009; Kim *et al*., 2006; Lim *et al*., 2014; Möhlmann, 2014; Song *et al*., 2006). Demidchik *et al*. (2009) have proposed that eATP primarily induces the production of extracellular superoxide anion through the activation of plasma membrane NADPH oxidases. Then, part of the superoxide anion is converted to other forms of ROS (such as H_2O_2), which have the ability to enter the cytosol, subsequently leading to the accumulation of cytosolic ROS. It has also been noted that the treatment of *Populus euphratica* cell cultures with exogenous ATP leads to the generation of mitochondrial ROS (mtROS) (Sun *et al*., 2012a).

Stimulation with exogenous ATP also induces an increase in the content of NO in addition to $[Ca^{2+}]_{cyt}$ and ROS (Clark *et al*., 2010; Foresi *et al*., 2007; Möhlmann, 2014; Reichler *et al*., 2009; Salmi *et al*., 2013; Wu and Wu, 2008). In addition, it has been reported that exogenous ATP can activate the transcription of the genes encoding the enzymes in the jasmonic acid (JA) and ethylene (ET) biosynthetic pathways (Song *et al*., 2006). The content of salicylic acid (SA) has been shown to be decreased in tobacco leaves after exogenous ATP treatment (Chivasa *et al*., 2009).

Interactions of the eATP-initiating signalling molecules

Some studies have shown that the elevation of $[Ca^{2+}]_{cyt}$ acts as an upstream signalling event for the eATP-induced production of NO and ROS (including extracellular, cytosolic and mitochondrial ROS) (Demidchik *et al*., 2009; Möhlmann, 2014; Sueldo *et al*., 2010; Sun *et al*., 2012a; Wu and Wu, 2008). In turn, as the downstream signalling molecules of $[Ca^{2+}]_{cyt}$, ROS and NO may contribute to a further elevation of $[Ca^{2+}]_{cyt}$ on eATP stimulation. Demidchik *et al*. (2009) have proposed that the increase in ROS level by the elevation of $[Ca^{2+}]_{cyt}$ can further enhance the $[Ca^{2+}]_{cyt}$ level by activating an ROS-sensitive Ca^{2+}-permeable channel at the plasma membrane. NO has also been reported to participate in the elevation of $[Ca^{2+}]_{cyt}$ induced by eATP (Wu and Wu, 2008). These observations

Table 1 Pharmacological analyses of the effects of extracellular adenosine 5'-triphosphate (eATP) on cell death. The pharmacological agents used include eATP traps, a specific competitive inhibitor of eATP and exogenous ATP.

Plant material	Pharmacological agents and characteristics	Events	References
Tobacco leaves	AMP-PCP (specific competitive inhibitor of the eATP pool)	At 7 days after treatment, 2000 μM AMP-PCP activated cell death in leaves grown under a 16-h photoperiod at 100 $\mu mol/m^2/s$, and 400–800 μM AMP-PCP was sufficient to activate cell death in leaves grown in a 16-h photoperiod at 200 $\mu mol/m^2/s$	Chivasa *et al*. (2009, 2010)
Tobacco leaves	Mixture of glucose and hexokinase (catalyses a reaction that consumes ATP during the phosphorylation of glucose to glucose-6-phosphate)	At 7 days after treatment, 100 mM glucose plus 0.1 or 0.5 units/μL hexokinase activated cell death in leaves grown in a 16-h photoperiod at 200 $\mu mol/m^2/s$	Chivasa *et al*. (2009, 2010)
Arabidopsis cell cultures	Apyrase (hydrolase that breaks down ATP to AMP via ADP)	Treatment with 50 or 100 units/mL apyrase for 72 h decreased the viability of the cell cultures grown in darkness	Chivasa *et al*. (2005)
Arabidopsis cell cultures	Mixture of glucose and hexokinase	Treatment with 100 mM glucose plus 20, 50, 100 or 200 units/mL hexokinase for 72 h decreased the viability of the cell cultures grown in darkness or with a 16-h photoperiod	Chivasa *et al*. (2005)
Arabidopsis cell cultures	AMP-PCP	Treatment with 0.5, 1.0 or 0.5 mM AMP-PCP for 72 h decreased the viability of the cell cultures grown in darkness	Chivasa *et al*. (2005)
Arabidopsis leaves	AMP-PCP, apyrase and mixture of glucose and hexokinase	Between 2 and 4 days after treatment, 0.5 units/mL apyrase, 1.85 units/mL hexokinase plus 50 mM glucose, or 5 mM AMP-PCP, activated cell death in leaves grown in a 16-h photoperiod at 250 $\mu mol/m^2/s$	Chivasa *et al*. (2005)
Arabidopsis roots and shoots	Mixture of glucose and hexokinase	1.85 units/mL hexokinase plus 50 mM glucose applied to the growth medium of hydroponic plants for 7 days caused death of the roots and some of the shoots grown in a 16-h photoperiod	Chivasa *et al*. (2005)
Whole *Arabidopsis* seedlings	AMP-PCP	Death was triggered in plants grown on normal nutrient agar and transferred to nutrient agar supplemented with 1 mM AMP-PCP for 4 weeks	Chivasa *et al*. (2005)
Tobacco leaves	AMP-PCP, apyrase and mixture of glucose and hexokinase	Between 2 and 4 days after treatment, 0.5 units/mL apyrase, 1.85 units/mL hexokinase plus 50 mM glucose, or 1 mM AMP-PCP, activated cell death in leaves grown in a 16-h photoperiod at 250 $\mu mol/m^2/s$. Treatment with a higher concentration (5 mM) of AMP-PCP activated cell death in the upper systemic leaves which had not received the primary treatment	Chivasa *et al*. (2005)
Bean leaves	Apyrase and a mixture of glucose and hexokinase	Between 2 and 4 days after treatment, 0.5 units/mL apyrase or 1.85 units/mL hexokinase plus 50 mM glucose activated cell death in leaves grown in a 16-h photoperiod at 250 $\mu mol/m^2/s$.	Chivasa *et al*. (2005)
Maize cell cultures	AMP-PCP, apyrase	Treatment with 1 mM AMP-PCP or 100 units/mL apyrase for 72 h decreased the viability of cell cultures grown in darkness	Chivasa *et al*. (2005)
Maize cell cultures	Mixture of glucose and hexokinase	Treatment with 50 mM glucose plus 200 units/mL hexokinase for 72 h decreased the viability of cell cultures grown in darkness or with a 16-h photoperiod	Chivasa *et al*. (2005)
Cell cultures of *Populus euphratica*	ATP	Stimulation with 1000 or 2000 μM ATP caused cell death after 30 min Treatment with 500, 1000 or 2000 μM ATP for 12 or 24 h activated cell death, whereas lower doses (10–200 μM) did not	Sun *et al*. (2012a)

ADP, adenosine 5'-diphosphate; AMP, adenosine 5'-monophosphate; AMP-PCP, β,γ-methyleneadenosine 5'-triphosphate.

suggest that, during the perception of eATP by plant cells, Ca^{2+}, ROS and NO are interconnected. However, the development of a precise placement of these signalling molecules with respect to each other is still relatively difficult. For example, in *Salvia miltiorrhiza* hairy roots, eATP-induced NO production can stimulate ROS production, whereas eATP-induced ROS cannot induce NO synthesis (Wu and Wu, 2008). This observation contrasts with that found by Sun *et al*. (2012a), who reported that the eATP-induced production of NO in *P. euphratica* cell cultures is dependent on the production of ROS. These different data, which appear contradictory at first, could result from inherent differences in metabolism among the different plant materials or species used. However, these data could also reflect the flexibility of the cross-talk among the eATP-initiating signalling molecules. In recent decades, Ca^{2+}, ROS, SA, NO, JA and ET have emerged as inducers or suppressors of plant cell death (Gadjev *et al*., 2008; Tuominen *et al*., 2004; and references cited therein). Thus, as downstream signals of eATP, these signalling molecules may play important roles in eATP-mediated cell death. In *P. euphratica* cell cultures, Sun *et al*. (2012a) have revealed that increases in $[Ca^{2+}]_{cyt}$ and ROS are necessary for eATP-induced cell death (see details below). However, the lack of knowledge about how the downstream signals of eATP are involved in eATP-mediated plant cell death is still remarkable, and is considered to be an important issue that needs to be studied extensively in the near future.

eATP AND CELL DEATH

Cell death in response to an extremely high level of eATP

As determined by ultrastructural studies, there are two distinct forms of cell death: necrosis and apoptosis (Kerr *et al*., 1972). The term 'necrosis' is generally described as a chaotic and uncontrolled mode of death, whereas the term 'apoptosis' is specifically used to describe controlled cell death (Reape *et al*., 2008; and references cited therein).

In plant cells, eATP is secreted into the surrounding medium in the nanomolar or higher range (Sun *et al*., 2012b; Tanaka *et al*., 2010a, 2014). From *P. euphratica* cell suspensions, it was found that the incubation of cells with a high concentration of exogenous ATP (≥0.5 mM) for 12 or 24 h caused apoptotic-like cell death, which shared many characteristics with apoptosis in animals and plants (Sun *et al*., 2012a). A higher concentration of eATP (≥1.0 mM) seemed to accelerate this process (exogenous ATP at 1.0 or 2.0 mM caused apoptotic-like cell death after only 30 min of incubation). Although it remains unknown whether such a high level of eATP can occur under natural conditions, this observation implies that eATP is a potent trigger of apoptosis in plants.

Over the years, the mechanism of apoptotic-like cell death has been studied extensively in animal and plant cells. Currently, it is widely accepted that the central event that drives this form of cell death is the release of cytochrome (Cyt) *c* from the mitochondria into the cytoplasm (Bras *et al*., 2005; Green and Reed, 1998; Reape *et al*., 2008; Vianello *et al*., 2007). In the cytoplasm, the released Cyt *c* complexes with apoptosis protease-activating factor-1 (APAF-1) and pro-caspase-9 in the presence of dATP to form the apoptosome. This complex then activates downstream effector caspases (such as caspase-3), which dictate the apoptotic response (Bras *et al*., 2005; and references cited therein). During the eATP-induced apoptosis process of *P. euphratica* cells, Sun *et al*. (2012a) observed an obvious release of Cyt *c* from the mitochondria and a subsequent increase in the activity of caspase-like proteases, indicating that a high level of eATP can induce plant apoptosis via a mechanism similar to that described above.

Furthermore, Sun *et al*. (2012a) revealed that the elevation of $[Ca^{2+}]_{cyt}$ and the accumulation of ROS are necessary for the eATP-induced release of Cyt *c* and subsequent apoptosis. This observation is consistent with a common generalization that the release of Cyt *c* can be induced by Ca^{2+} elevation and ROS accumulation in animal and plant cells (Gunter *et al*., 2004; Hajnoczky *et al*., 2003; Simon *et al*., 2000; Szabadkai and Rizzuto, 2004; Vacca *et al*., 2006). In detail, it should be noted that there are multiple mechanisms for the Ca^{2+}- or ROS-induced Cyt *c* release. One of the characterized mechanisms is that Ca^{2+} and ROS can cause the opening of the mitochondrial permeability transition pore (MPTP), which can lead to the nonspecific release of Cyt *c* via matrix swelling and rupture of the mitochondrial membrane (Amirsadeghi *et al*., 2007; Crompton, 1999; Kanno *et al*., 2004). Alternatively, some studies have reported that Ca^{2+} or ROS can induce an MPTP-independent release of Cyt *c* through the selective permeabilization of the outer mitochondrial membrane via interaction with certain channels or pores in the mitochondrial outer membrane, such as the voltage-dependent anion channels (VDACs) or the pores formed by the pro-apoptotic Bcl-2 family proteins (Amirsadeghi *et al*., 2007; Bras *et al*., 2005; Petrosillo *et al*., 2004). On the basis of the data available, the latter mechanism appears to be the most likely to occur during the eATP-induced release of Cyt *c* and apoptosis, because no apparent MPTP opening was observed during this process (Sun *et al*., 2012a).

Another noteworthy point during eATP-induced Cyt *c* release and apoptosis is the change in $\Delta\Psi_m$ (mitochondrial transmembrane potential) and iATP level. In animal and plant mitochondria, Cyt *c* is essential for the generation of $\Delta\Psi_m$, and $\Delta\Psi_m$ is required for mitochondrial ATP production. Thus, in principle, the generation of $\Delta\Psi_m$ and iATP may be severely disrupted by the release of Cyt *c*. Conversely, during the eATP-induced release of Cyt *c*, significant increases in both $\Delta\Psi_m$ and the iATP level were observed, and these increases were demonstrated to facilitate eATP-induced activation of caspase-like proteases (Sun *et al*., 2012a). Although this observation is consistent with the previous finding that an increase in iATP production may serve as a certain

step of the apoptosis process to fulfil the energy requirement for the activation of caspase-like proteases (Skulachev, 2006), the central question is how the increases in $\Delta\Psi_m$ and iATP can occur when Cyt *c* is released on eATP stimulation. One possible model, which has been proposed in some forms of animal apoptosis, is that mitochondria can only release a proportion of the total Cyt *c* to trigger cell death responses, whereas the rest of the Cyt *c* pool remains associated with the inner membrane to sustain $\Delta\Psi_m$ and iATP production (Jürgensmeier *et al*., 1998; Waterhouse *et al*., 2001). However, whether such a model also applies to the eATP-induced apoptosis of plant cells is still a matter of discussion.

Further observations have shown that the incubation of *P. euphratica* cell suspensions with high concentrations (≥2.0 mM) of the products of ATP hydrolysis, including adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate (AMP), adenosine and inorganic phosphate, does not induce apoptosis during a 24-h incubation. Moreover, in contrast with ATP, although ADP also induces an increase in $[Ca^{2+}]_{cyt}$, the effect of ADP on the $[Ca^{2+}]_{cyt}$ level is less pronounced than that of ATP at the same concentration. More importantly, ADP exposure does not lead to an increase in both $\Delta\Psi_m$ and intracellular ROS (Demidchik *et al*., 2011; Sun *et al*., 2012a). Thus, the eATP-induced apoptosis is not attributable to ATP metabolites. However, a more elaborate study is still required to exclude other possibilities. For instance, early work in animal cells has reported that eATP can be transformed into extracellular adenosine 3',5'-cyclic monophosphate (cAMP) (Gentile *et al*., 1988), and it was found that extracellular cAMP could be involved in the initiation of the cell death programme of stalk cells in the developmental cycle of the primitive eukaryote *Dictyostelium* (Cornillon *et al*., 1994).

Cell death in response to the depletion of eATP

By using some enzymes that can consume or hydrolyse eATP, Chivasa *et al*. (2005, 2009, 2010) revealed that the removal of eATP triggered cell death in both cell cultures and whole plants (Table 1). Moreover, neither AMP nor ADP, the products of ATP hydrolysis, can trigger cell death, indicating that cell death is a specific response to the depletion of ATP rather than to the accumulation of ADP or AMP (Chivasa *et al*., 2005). This was further demonstrated by the observation that specific competitive inhibitors of the eATP pool can also cause plant cell death (Chivasa *et al*., 2005, 2009, 2010). Moreover, it seems that only a drastic decrease in eATP can trigger cell death, because these pharmacological agents at relatively lower concentrations induced defence responses to pathogens (Chivasa *et al*., 2009).

The mechanism of cell death in response to eATP depletion has not been investigated in detail. It is even unclear whether the observed cell death is similar to apoptosis or necrosis. Recently, Chivasa *et al*. (2010) have revealed that application of β,γ-methyleneadenosine 5'-triphosphate (AMP-PCP, a specific competitive inhibitor of eATP), at a dose that does not trigger cell death, causes a marked suppression of the expression of several subunits of the mitochondrial and chloroplast ATP synthase proteins of tobacco leaves. Theoretically, the suppressed expression of these protein subunits would inevitably lead to a decline in iATP generation. As a decline in intracellular energy has been found to be an early event in the process of some types of plant cell death (Azad *et al*., 2008; Comelli *et al*., 2003), it is possible that the cell death in response to eATP depletion is associated with a decline in iATP. If this is the case, and considering that plant eATP mainly originates from iATP (Cao *et al*., 2014; Choi *et al*., 2014b; Tanaka *et al*., 2010a, 2014), eATP depletion could trigger a self-amplifying cycle, in which a decline in eATP leads to decreased iATP production, resulting in a further decrease in the eATP level. The consequence of this amplification loop may ensure the onset of cell death in response to eATP depletion (Chivasa *et al*., 2011).

In addition to the suppression of the expression of ATP synthase proteins, eATP depletion also massively suppresses the expression of many proteins that perform important physiological functions, including photosynthesis, maintenance of the cellular redox state and protection against oxidative stress (Chivasa *et al*., 2010). In some studies, a decrease in photosynthesis, imbalance of redox status or increase in oxidative stress may occur upstream and act as important factors in the induction of plant cell death (Bi *et al*., 2009; Trachootham *et al*., 2008). Thus, the cell death induced by the depletion of eATP could also be attributed to the disruption of these physiological functions, although direct evidence is still lacking.

Moreover, Chivasa *et al*. (2009) found that the addition of the Ca^{2+} chelator accelerated the cell death induced by eATP depletion. Combining this observation with the previous finding that the perturbation of Ca^{2+} homeostasis is responsible for many forms of plant cell death (Demaurex and Poburko, 2009; Kudla *et al*., 2010), we can assume that the disruption of Ca^{2+} homeostasis could be involved in the cell death induced by eATP depletion. However, Kim *et al*. (2006) have reported that the Ca^{2+} chelator can block the eATP release of plant cells. Correspondingly, the release of eATP in animal cells has been demonstrated to be a Ca^{2+}-dependent process (Burkeen *et al*., 2011). Thus, an alternative explanation for the observation by Chivasa *et al*. (2009) is that the Ca^{2+} chelator could further decrease the eATP level through the blocking of ATP release, and thus accelerate the cell death induced by eATP depletion, rather than through a direct effect on Ca^{2+} homeostasis.

It is also noteworthy that the cell death induced by eATP depletion only was observed by long-term (several days) application of the eATP inhibitor or hydrolytic enzymes of eATP (Table 1). In contrast, the effects of eATP on Ca^{2+} and other signalling molecules seem to be rapid and transient (several seconds or minutes) (see Demidchik *et al*., 2003, 2009, 2011; Jeter *et al*., 2004). This kinetic difference makes it likely that cell death in response to

eATP depletion may be partly a result of the metabolic changes under the condition of longer term depletion of eATP, e.g. a decrease in the activity of the ATP-dependent enzymes in the extracellular matrix. Thus, careful attention must be paid to the interpretation of the results obtained using pharmacological approaches for the study of eATP.

Nevertheless, the finding that eATP depletion triggers cell death is helpful in further understanding the mechanism underlying some forms of plant cell death. For example, a previous study has shown that fumonisin B1 (FB1, a fungus-derived cell death-activating elicitor) induces plant cell death through certain intracellular signalling pathways (Asai *et al*., 2000). Studies by Chivasa *et al*. (2005, 2011) have revealed that FB1 induces plant cell death by triggering the depletion of eATP, indicating that eATP, as an extracellular component, is also responsible for the induction of cell death by this fungus-derived cell death-activating elicitor.

CONCLUSIONS AND PERSPECTIVES

Although the main role of ATP is to provide energy for intracellular biochemical reactions, when it is released into (or produced in) the extracellular matrix, this molecule becomes an important signalling component for the regulation of a wide range of physiological processes. The current literature summarized in this review demonstrates that dramatic changes in plant eATP levels (decreases or increases) trigger plant cell death responses. To the best of our knowledge, few signalling molecules have similar features to eATP. We have also made an attempt to illustrate how the eATP-mediated signalling pathways or physiological events are involved in the induction of the plant cell death process (Fig. 1). However, this scheme is only preliminary, as research concerning plant eATP is still in its early stages. Nevertheless, future studies in this research area are expected to greatly expand the current knowledge regarding the functions of plant ATP and the mechanisms of plant cell death. Moreover, considering that eATP, as an extracellular molecule, is a mutual component of adjacent cells in multicellular organisms, changes in eATP release from cells experiencing certain environmental stimuli (for example, pathogen infection or wounding by herbivore attack) will directly affect the fate of adjacent cells. Fortunately, the identification of the extracellular receptor targets of plant eATP by Choi *et al*. (2014a) should greatly accelerate related research in the future.

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