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Extracellular ATP Signaling and Cell Death in Plants

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The role of ATP in Plant Cell Death (PCD) where a cell through signaling or detection, starts Apoptosis (planned cell death) of the cell and signals to other cells to start Apoptosis as well. PCD with its signaling role in the extracellular matrix is a topic of study that many people have taken up and researched. What effect ATP has on cell death and whether it can be triggered with ATP products, have been tested to see if they caused PCD. Also how ATP can affect the cell death it triggers and whether it can reverse this death with the use of FB1 which is a cell death pathway which is induced through mycotoxins. This leads to more study in the field of PCD and its link to ATP as how ATP reverses the death caused by pathways like that of FB1 or how ATP could be applied to plants in the throes of PCD and how and when is the best chance of reversal. This leads to applications that would help growers and humanity based on these studies.

Introduction

What is ATP's contribution in the extracellular matrix to PCD (Plant Cell Death)? The plant cell uses several molecules in the extracellular matrix, where ATP is used to talk to other cells through signaling. ATP reduces Cytosolic Calcium or Cs2+, as it is used to make ATP in the matrix and when Ca2+ in conjunction ATP is significantly increased, the cell starts to react by starting to die. This can be in response to infection, cell age, or other problems with the cell. How low the ATP needs to be to start this process and whether any of its products or other equivalent molecules can do the same job is an important avenue of study as our world population grows when crops would be well helped by a way of knowing when the plant is signaling PCD. ATP and its role in cell death help us to understand how it occurs and what triggers can be thrown in stopping it's effects in plants who would be best helped if what is infecting the plant could be eradicated while the cells around it are saved from the PCD being signaled through the local cells.

Recent Progress

ATP and its role in PCD is being studied in different ways, such as how PCD results from ATP signaling by examining metabolite products of ATP, extracellular cytosolic calcium (Ca 2+) and cell viability by ATP stimulation. Initially, cultures of *P.euphratica* were exposed to ATP solutions and cell death was observed at high concentrations of 0.5 to 2.0 mm, while low concentrations of 10 to 200 µm did not cause PCD. The high dosed cells displayed hallmark features of PCD, such as retraction of cytoplasm from the cell wall, shrinkage of the cytoplasm, DNA fragmentation, and condensed or stretched chromatin. ATP was hydrolyzed in light and dark conditions which initially raised the question of metabolite induced PCD as the observations in light caused faster PCD than that of in dark.

Cell suspensions were tested with the products of ATP hydrolysis (metabolites), which was that of ADP, AMP, adenosine, and inorganic phosphate (KH2PO4) to see if they had any effect on PCD in plants but the metabolites did not invoke PCD during the 24 hour period, which implied that ATP metabolites do not induce PCD. Extracellular Ca2+ was significantly decreased by ATP in liquid Murashige and Skoog medium (LMS) at concentrations of 3.0, 1.8, 1.5, and 0.8Mm, though Ca2+ had no real effect on cell viability after the observation period of 6 to 24 hours. ATP's early signaling occurred in minutes while crytogein exerted effects in less than three hours. When ATP was washed away, all concentrations tested led to increases in PCD after 30 minutes, compared to the control. This indicated that ATP signaling is so fast that half an hour is enough to start PCD. (Jian Sun)

Ca2+ has also been studied in *Arabidopsis* roots where the enzymatic wall was removed from mature

epidermis which still allowed ATP response. The ATP caused dose-dependent increase of Ca2+ in epidermal protoplasts. The experiment used a "higher than previously used" (Demidchik et al., 2009) Ca2+ concentration to drive Ca2+ and maximize the resulted signal. Elevations by ATP increased Ca2+ concentrations by about 19%, while αβme-ATP and ADP also caused elevation of protoplast Ca2+ which was inhibited by channel blockers like Gd3+ which blocks root cell Ca2+ permeable channels. This outcome of the experiment showed that extracellular APT is noticed near the plasma membrane.

In other results, ATP traps were studied on how they affect PCD by trapping ATP. Apyrase and glucose-hexokinase were the traps tested on Arabidopsis root cultures where the cell death was counted during a 24 to 28 hour period. Results show that dialyzed and nondialyzed apyrase had no overall effect on cell viability. Treating with a BSA equivalent to apyrase didn't cause PCD. AMD and ADP from apyrase and ATP did not cause PCD either. Glucose-hexokinase caused dose-dependent death while dialyzed hexokinase, denatured hexokinase, ADP, nor glucose -6-phosphate showed no cell death and these results enforced the conclusion that removing extracellular ATP promotes PCD in plants.

In contrast, the adding of extracellular ATP has also been studied in its conjunction with FB1 triggered cell death. FB1 which elicits programmed cell death with a mycotoxin that turns on plant defenses, lead to eventual PCD. FB1 was added to specimens to monitor the fate of ATP during treatment. ATP was traced with {32P} a florescent marker, with menthol as control. After 24 hours the treated cultures showed a degradation of plasma membrane integrity which was obvious 72 hours later when cells became chlorotic (chloroplast death or plant yellowing), and began to lose mitochondrial dehydrogenase activity. This placed ATP loss upstream of FB1 in PCD. Because of ATP's place before FB1 in cell death, the ability to fend off PCD by ATP was lastly studied. Treating with 1µm of FB1 caused decline in Arabidopsis cells and eventual total cell death. Addition of ATP cultures before cell death delayed it about 48 hours. Adding ATP in the first 40 hours after FB1 was added showed that ATP rescued cells from FB1 cell death but at 48 hours or later, cell death was irreversible. Lastly the study of dose-response to ATP from plants treated with FB1 showed that a minimum of ~400 µm was needed to save plant roots from PCD. Arabidopsis was sewn onto two agar plates and later moved to a plate of FB1 and a plate of FB1 mixed with ATP. Cotyledon (embryotic leaves) of both plants died but only the FB1 and ATP mix grew the first set of true leaves. This showed that ATP did not save the plants from FB1 stunting but did keep full plant death from occurring. (Chivasa et al., 2005)

Discussion

These recent results address how ATP is shown as an extracellular signal for plant cells. That ATP reduction signals for the cell to die and how ADP, AMP and apyrase which are products or precursors of ATP did not signal for cell death in plants. To date it is unclear why only ATP is favored in this signaling pathway, but all tests showed that only ATP was able to trigger this response. ATP and its effects on Ca2+ also indicated that the membrane of the cell is what senses the ATP which could be a way the cell knows to die when a signal comes in from itself or other neighbor cells confirming that they communicate through chemical messengers. FB1 which poisons the cells with mycotoxin and causes other effects like stunting or poor fruiting and eventual PCD was studied about what can be done to reverse or halt its effects with ATP showed fruitful results. ATP was shown to be a precursor of FB1 death and in a certain amount of time after FB1 was introduced, if ATP was introduced, the cells did not experience PCD. This test out of all the others has implications of treatment for plant cells poisoned by bacterial toxins or general PCD. Knowing how much and how early in the process of PCD that ATP is a part of could lead to testing and indication of the triggers of PCD in plants which allow early detection or more targeted detection of bacterial infections of crops. Additionally, the knowledge that ATP can be added to plants that are experiencing PCD could allow for a form of treatment for plants that are still in decline after the initial pathogen has been treated, thus saving the crop. This could be helpful in the future when food will be at such a premium as our planetary population grows.

References

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