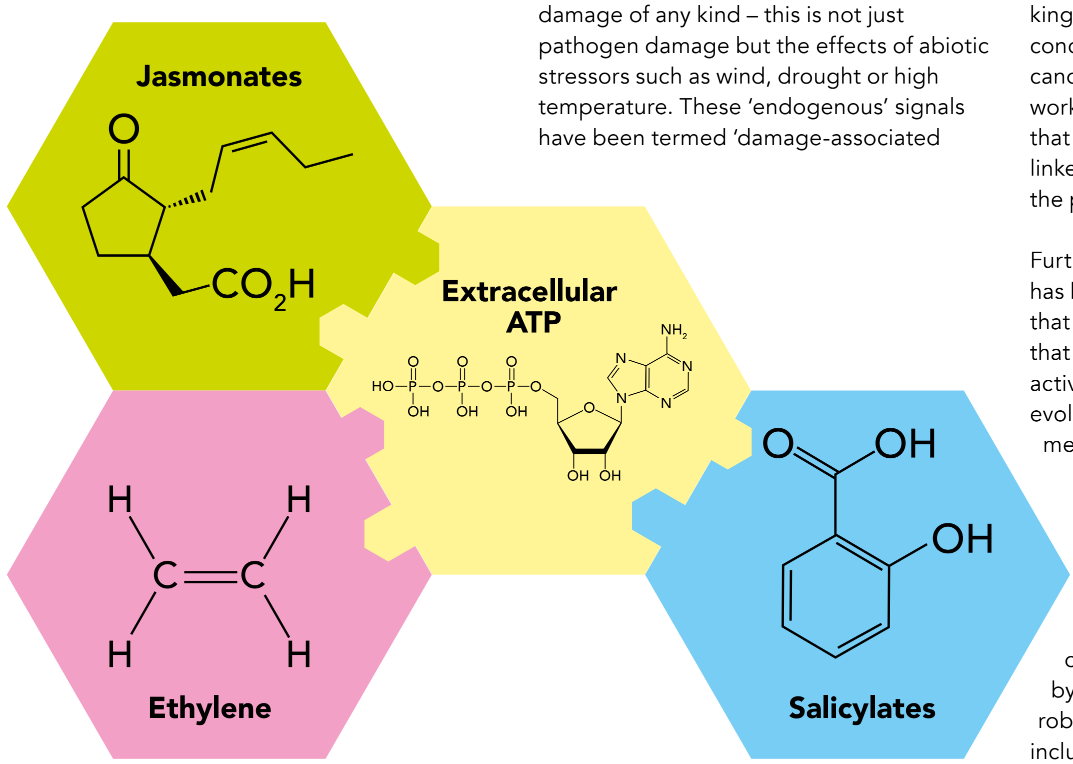
An energetic molecule DAMPens plant disease by boosting defence mechanisms

How do plants, which are, by definition, rooted to the spot, identify and respond to potential threats? One previously overlooked mechanism makes use of a molecule found throughout living things – the energy transporter ATP. Research led by **Dr Kiwamu Tanaka**, of Washington State University, USA, aims to elucidate how ATP is released in response to danger, how it is sensed by plant cells, and how this signal is translated into defensive mechanisms that could one day be harnessed to produce more robust crops.

It's a dog-eat-dog world out there, or, to put it another way, an insect-eat-plant world. But while dogs can run away from one another, plants have no option but to stay put in the face of attack. Plants are therefore continuously exposed to stresses in the form of both changing environmental conditions and attack from other organisms, from microbes to large grazing mammals. To survive, plants need a sophisticated early warning system to allow them to detect and respond to these stressors before too much damage is done.



DETECTING DANGER

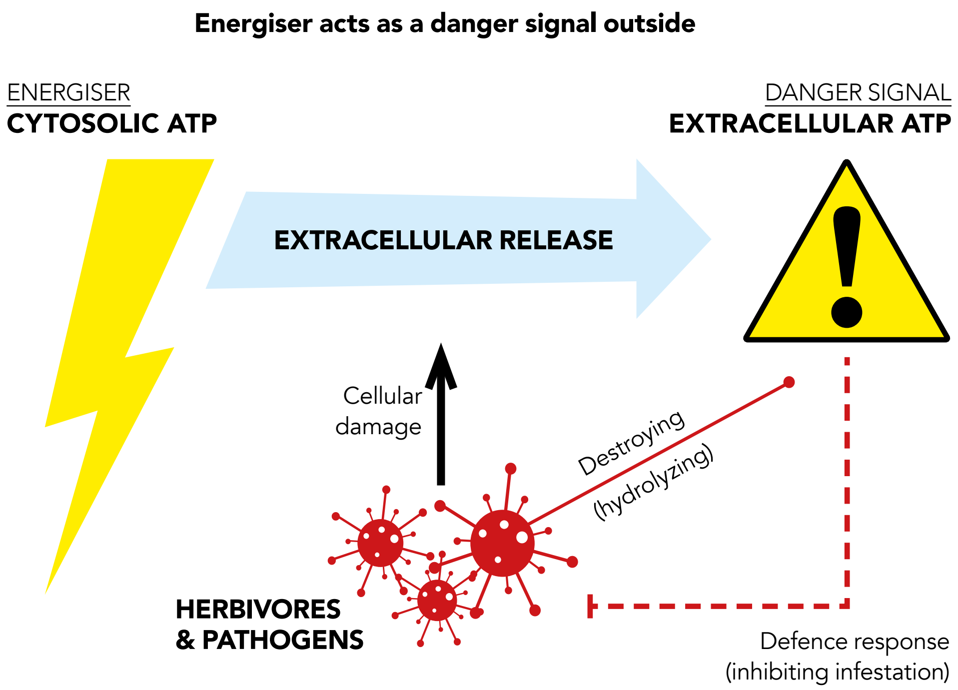
There are two potential sources of signals that warn plants of danger. These include molecules produced by attackers such as pathogens, and molecules released by the plants' own systems when they experience damage of any kind – this is not just pathogen damage but the effects of abiotic stressors such as wind, drought or high temperature. These 'endogenous' signals have been termed 'damage-associated molecular patterns', or 'DAMPs'.
In contrast to the 'exogenous' molecules produced by pathogens (called PAMPs), DAMPs have been little studied. Potential DAMPs identified so far include DNA, sucrose, and small pieces of protein known as peptides. In essence, any molecule that should be found inside a plant cell, when found on the outside of the cell (extracellularly) could potentially indicate a breach of the cell's protective membrane. These molecules can therefore be harnessed as DAMPs.

One molecule that is ubiquitous and vital to plant and animal cells is adenosine triphosphate (ATP), often referred to as the 'energy currency' of the cell. ATP contains a high-energy phosphate bond, which can be broken to release energy wherever it is needed in a cell. Researchers are only just beginning to realise that ATP may have as crucial a role outside the cell as it does inside, and across both plant and animal kingdoms. Because ATP is found in high concentrations in all cells, it is an ideal candidate DAMP, and indeed in plants, the work of Dr Tanaka and others has shown that the presence of extracellular ATP is linked to enhanced stress responses within the plant.

Further evidence that ATP acts as a DAMP has been provided by studies showing that herbivorous insects produce enzymes that break down ATP during their feeding activities – suggesting a counter-defence evolved by these attackers against an ATP-mediated DAMP response in plants.

BREACHING THE WALL

Since it is the presence of ATP outside a plant cell that signals 'danger,' plant cells need some way to identify the molecule on their outer surface. Plant cells are protected by both a thin membrane and a more robust, protective wall. In 2014, a team including Dr Tanaka identified a receptor



Plant researchers are only beginning to realise that ATP may have just as crucial a role outside cells as it does inside

molecule, P2K (aka DORN1/LecRK-I.9), located in the plasma membrane of plant cells, which recognises ATP.

Interestingly, the P2K receptor turns out to be unique regarding its protein structure, which has an extracellular lectin domain and an intracellular kinase domain – P2K is structurally very different from the animal ATP receptors, P2X and P2Y. Generally, lectin binds to sugar molecules; ATP has a pentose sugar in its molecular structure and the ATP binding pocket in the lectin domain of P2K is structurally similar in location to the sugar binding pocket of the canonical lectins. Moreover, P2K has a type of enzyme known as a 'kinase', which mediates the transfer of phosphate groups from one molecule to another. Such an enzyme-linked receptor was not seen in the membrane of animal ATP receptors. Found only in plants, P2K represents an ancient innovation that has been conserved throughout evolutionary time in plant kingdom. The extracellular ATP signalling mechanism has evolved independently but differentially between sessile plants and movable animals.

Dr Tanaka's team have already used specially-developed mutant plants to demonstrate the significant role of ATP and P2K in plant responses. Mutants, in which P2K is inactive, are both unable to recognise ATP, and unable to produce normal responses to cell damage. In contrast, plants producing an excess of P2K are over-responsive to the presence of ATP and over-reactive to damage. These findings strongly suggest that extracellular ATP is involved in a plant stress response, mediated by the cell membrane-located receptor molecule P2K.

But these findings appear to raise more questions than they answer. How exactly does P2K identify the presence of ATP? What happens after ATP is recognised by P2K? How is this translated into a functional plant defence response? Which molecular pathways are involved and how do they interact with other plant defence pathways? Dr Tanaka's current work, funded by the US National Science Foundation, aims to answer these fundamental questions regarding how plants respond to this DAMP signal.

AN IN-DEPTH UNDERSTANDING

The long-term goal of Dr Tanaka's research is to develop a comprehensive, in-depth understanding of how different kinds of molecules in plant cells interact to stimulate a defence response to wounding or other damage. Using genetic, biological and biochemical approaches, his team aim to explore the mechanism by which ATP is sensed by the P2K receptor, identifying the key components of the downstream signalling pathway that is triggered by recognition of extracellular ATP.

Finally, Dr Tanaka and his team aim to explore the broader signalling networks that link ATP with better-known plant defence pathways – providing an interplay of precisely-controlled responses to stress. Interplay between ATP and another DAMP generated by cell wall degradation, oligogalacturonic acid (OGA), has already been suggested, and it is now becoming apparent that the two interact with the defence pathways mediated by plant stress hormones, e.g., ethylene, jasmonates, and salicylates.

In the long term, Dr Tanaka's research may enable us to harness DAMPs, their receptors, and downstream signalling molecules through genetic engineering to produce plants that are better able to respond to environmental conditions and protect themselves against predators and pests. This will have knock-on environmental benefits in terms of reducing the need to apply pesticides, lowering pollution, increasing crop yields and decreasing costs. In a world where the environment is changing more rapidly than ever before, Dr Tanaka's research promises to give plants a helping hand to keep up.

Q&A

What first gave you the idea that ATP might have another role in addition to its well-known function as the energy currency of cells?

In mammals, extracellular ATP has already been identified as a very important signal, which functions via canonical P2 receptors in neurotransmission, inflammation, and innate immunity. In plants, many studies have reported that ATP treatment can cause various physiological changes during growth and stress responses. However, since its mode of action was unexplained for a long time, there has been significant skepticism in the plant community about another such role of ATP. Those evidences were enough to motivate us for the receptor quest with a hypothesis that ATP also had a significant role in the extracellular region in plants.

How did you go about identifying P2K as a potential receptor of ATP?

Extracellular ATP induces elevation of the cytosolic free calcium that is an essential second messenger for many cellular activities. By monitoring the ATP-induced calcium response, we have identified an insensitive mutant from a randomly mutageniased population. After careful genomic analysis of the insensitive mutant, we identified the gene, P2K, responsible for the ATP insensitive phenotype. We then found that the extracellular domain of P2K directly binds to ATP based on the biochemical and computational analyses, demonstrating that P2K acts as a bona fide receptor for extracellular ATP in plants.

The Tanaka lab – (left to right) Sonika, Joseph DeShields, Tumelo Moyo, Matthew Marcec, Diwaker Tripathi, Natalia Moroz, Jeremy Jewell, and Kiwamu Tanaka

Can you outline the likely steps or components in the pathway from ATP and P2K to the plant defence response?

Since we are only at the beginning of discoveries about extracellular ATP and its receptor P2K in plants, the downstream pathway after ATP recognition is completely opaque. Based on our research, together with a wealth of published data, we hypothesise that extracellular ATP and its receptor P2K play a central role in mediating the plant's innate immunity. Our expectation is that ATP orchestrates plant immunity by crosstalk with other stress hormones, e.g., ethylene, salicylates, and jasmonates. To test this hypothesis, we are now working on omics-based systems biology approaches to identify such a complex signalling network.

Which steps in the pathway are most likely to be suitable targets for crop improvement, do you think?

If a plant response is too slow, it allows the offending pathogens and pests to infest easily. If a response is too fast and strong, that would impair cell fitness in a given environment. Achieving a desirable level of host resistance is therefore a challenge. As extracellular ATP plays a key role in fine tuning the plant immune response, identification of how this signal is translated into defensive responses will be a primary target for improving stress-resistant crops.

It seems we are just scraping the surface in terms of knowledge of plant defence responses and DAMPs. What will be the most exciting developments in this field over the next decade or so?

If the mechanism of DAMP-based plant defence responses is unveiled, we will understand exactly how plants sense self damage when they get injured or infected, and how plants, due to their sessile nature, have evolved differently from movable animals. This is exciting because the knowledge gained will eventually be leveraged to make agricultural crops robust in terms of preventing disease and pests and/or therapeutic treatment of devastated fields. Considering recent adverse environmental changes, increasing food demand, and predicted population growth, we urgently need to design more stress-resistant crops.

Detail

RESEARCH OBJECTIVES

Dr Tanaka's research focuses on investigating and improving scientific understanding of plant defence mechanisms. His recent work has looked at endogenous danger signals to further this knowledge.

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COLLABORATORS

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BIO

Dr Kiwamu Tanaka obtained his PhD from Kagoshima University, Japan. He was a Research Scientist and Post-Doctoral Fellow in Division of Plant Sciences at University of Missouri, USA. He joined Washington State University in 2014, where he is currently teaching Molecular Genetics of Plant and Pathogen Interactions.

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