Mapping out the roles of MAP kinases in plant defense

Roger W. Innes

Arabidopsis contains 20 MAP kinase genes, but their roles in plant physiology have remained largely unknown because of a lack of mutants. Recent papers from two groups have shed new light on the function of two different MAP kinases. The Arabidopsis MPK4 gene appears to negatively regulate salicylic acid-mediated defense responses and positively regulate jasmonic acid-induced responses. The tobacco SIKP gene (orthologous to Arabidopsis MPK6) appears to positively regulate programmed cell death.

Mitogen-activated protein kinases (MAPKs) are signal transduction proteins and are found in all eukaryotes analyzed to date. They are typically involved in transducing extracellular signals. Although MAPKs have been studied in great detail in animal systems and in yeast, little is known about their functions in plants. In animal and yeast systems, MAPKs are activated by a kinase relay consisting of an MAPKK and an MAPKKK (Ref. 1). This also appears to be true in plants.  

First MAPK mutant in plants

The Arabidopsis genome contains 20 MAPK-like genes, but until recently no mutations had been reported. That has changed with the publication of a transposon insertion mutation in the Arabidopsis MPK4 gene by Morten Petersen et al.. The mpk4 mutant was identified in a standard forward-genetic screen as a severe dwarf. It was only after the disrupted gene was identified that the role of the disrupted gene could be elucidated. That role was to determine why the mpk4 mutant behaved like wild-type plants in response to these stresses. This forced Petersen et al. to search for new mechanisms to explain the abiotic stress connection and think more broadly about the possible causes of the dwarf phenotype.

One well-known cause of dwarfism in plants is constitutive expression of defense responses associated with elevated levels of salicylic acid (Ref. 8). A quick check of defense gene mRNA levels in the mpk4 mutant revealed constitutively high levels, thus suggesting that MPK4 might directly or indirectly regulate salicylic acid-mediated defenses. This led Petersen et al. into a thorough genetic and physiological analysis of defense response pathways in the mpk4 mutant. This work yielded several important conclusions. Foremost among these was that the mpk4 mutation causes a greatly elevated level of salicylic acid, and that...
the majority of the mpk4 mutant phenotypes (e.g. enhanced resistance to bacterial and oomycete pathogens, enhanced defense gene expression) can be alleviated simply by reducing the endogenous levels of salicylic acid via the expression of a bacterial salicylate hydroxylase gene (NahG).

Significantly, NahG only partially suppressed the dwarf phenotype of the mpk4 mutant. Furthermore, induction of the defense genes PDF 1.2 and TH11 by jasmonic acid is blocked in the mpk4 mutant, even in the NahG-containing line. Thus, MPK4 must regulate more than just salicylic acid levels. It is not clear whether these three phenotypes (dwarfism, jasmonic acid insensitivity and high salicylic acid levels) are independent effects of the mpk4 mutation, or whether the defect in jasmonic acid signaling leads indirectly to elevated levels of salicylic acid and dwarfism. In support of the hypothesis the three phenotypes are independent effects of the mpk4 mutation, there are precedents from yeast and animal systems for single MAPKs functioning in two or more independent signal transduction pathways, presumably via independent protein complexes.

Additionally, a single MAPK pathway can have multiple targets, thus the multiple effects of the mpk4 mutation are not particularly surprising.

Although mpk4 mutant plants are dwarfed, there appears to be no gross disruption of cellular homeostasis. This is seen in the lack of lesions in mpk4 plants and by the relatively small number of genes whose expression is altered. In addition, expression of a kinase inactive form of the MPK4 protein in the mpk4 mutant failed to rescue any of the mutant phenotypes, which argues against the possibility of ‘promiscuous’ cross-talk occurring among MAPK pathways in the absence of MPK4 protein. These observations indicate that MPK4 plays a specific role in the regulation of defense responses, and that the mpk4 mutant phenotype is not simply the result of pleiotropic effects caused by gross disruption of cellular functions.

To fully understand the role of the MPK4 protein, the upstream and downstream components of this MAPK pathway need to be identified. There are already yeast two-hybrid and in vitro data that point to the MAPKK and MAPKKK components that probably regulate MPK4 (Fig. 1), but the upstream receptor(s) and downstream targets are unknown. Likely candidates for the downstream targets would be transcription factors that bind to the PDF 1.2 promoter. Although these have not been identified, the fact that PDF 1.2 induction requires the ethylene signal transduction pathway points to the EIN3 and ERF transcription factors. These two proteins are known to constitute a transcription factor cascade leading to the induction of ethylene inducible genes. However, it is worth noting in this context that mpk4 mutants do not display developmental defects associated with the ein3 mutation, such as lack of a triple response, nor does mpk4 suppress the constitutive triple response of ctr1 mutants. It seems to rule out EIN3 as a likely target, but leave open the possibility that ERF1 and/or related transcription factors are targets.

Fig. 1. Representative examples from Arabidopsis of the three general classes of proposed plant mitogen-activated protein kinase (MAPK) cascades. Blue arrows indicate positive regulation and red Ts indicate negative regulation. Abbreviations: CTR1, constitutive triple response factor; EIN3, ethylene insensitive 3 protein; ERF1, ethylene response factor 1; ETR1, ethylene receptor; PDF1.2, plant defensin protein; SA, salicylic acid.

**Potential role for tobacco SIPK**

In contrast with MPK4, the tobacco SIPK protein has many known activators, including avirulent pathogens. Unfortunately, no one has been able to identify an inserional mutation in the Arabidopsis ortholog, MPK6, in spite of efforts by multiple laboratories. SIPK was first identified because it is strongly activated by wounding and by salicylic acid, and can be easily detected by in-gel protein kinase assays. Using a transient expression assay in Arabidopsis protoplasts, Jen Sheen and colleagues recently identified a family of closely related MAPKKs (ANP1, ANP2 and ANP3) that appear to function at the top of the SIPK/MPK6 pathway (Fig. 1). Perhaps significantly, these MAPKKs also appear to regulate auxin responses and cell division. This observation suggests that the failure to isolate...
mutations in MPK6 might be because it is an essential regulator of the plant cell cycle.

This possibility is important to keep in mind when interpreting the recent paper from Shuqun Zhang and colleagues, who propose that SIPK can participate directly in the induction of programmed cell death in tobacco leaves in response to pathogen elicitors. In this work, a constitutively activated mutant version of the MAPKK protein, NtMEK2, was shown to activate SIPK in vitro and in vivo. Activation of SIPK in tobacco leaves preceded induction of several known defense genes and induction of cell death. This cell death response was apparently not dependent on salicylic acid because it was not suppressed by expression of the NahG transgene. Although it is possible that SIPK is directly responsible for induction of cell death during a pathogen-induced hypersensitive response (HR), one must be cautious in interpreting the above data. Sustained activation of SIPK by the mutant MAPKK could be causing pleiotropic effects (e.g. activation of inappropriate cell division), which then cause activation of programmed cell death via a pathway independent of the pathogen-induced HR.

However, even with this caution, this work is significant because it convincingly shows that NtMEK2 activates SIPK in vivo. Given the data mentioned above from Sheen and colleagues, one would predict that NPK1 (tobacco ortholog of the Arabidopsis ANP genes) functions as the MAPKKK that activates NtMEK2 (Fig. 1). Thus, it should now be possible to assemble the full MAPKK–MAPKK–MAPK cascade for SIPK/MPK6, which would be a first for any MAPK pathway in plants. Based on two-hybrid and complementation analyses performed in yeast, the full triad for MPK4 can also be predicted (Fig. 1). Significantly, the MAPKKKs predicted to regulate MPK4 and MPK6 belong to distinct subclasses.

Three general classes of MAPK cascades in plants

Three subclasses of MAPKKKs have been defined in plants, the MEKK1 class, the ANP class and the Raf class. Members of all three subclasses have now been implicated in the regulation of disease resistance because the Raf-like MAPKK gene EDR1 has recently been shown to negatively regulate plant defense responses, including programmed cell death. In addition, a second Raf-like gene CTR1 functions to negatively regulate ethylene inducible genes, including the PDF1.2 gene. Thus the picture that emerges is one of competing MAPK pathways that positively and negatively regulate plant defenses (Fig. 1).

Acknowledgements

I thank John Mundy and his laboratory members for permission to discuss unpublished results. Work discussed from my laboratory was supported by the National Institutes of Health grant R01-GM64511 and a grant from Novartis Agricultural Biotechnology Research, Inc.

References

3 Huang, Y. et al. (2000) ATMPK4, an Arabidopsis homolog of mitogen-activated protein kinase, is activated in vitro by ATM1 through threonine phosphorylation. Plant Physiol. 122, 1301–1310
15 Zhang, S. et al. (1998) Activation of the tobacco SIP kinase by both a cell wall-derived carbohydrate elicitor and purified proteinaceous elicitors from Phytophthora spp. Plant Cell 10, 435–450
22 Kieber, J. et al. (1993) CTR1, a negative regulator of the ethylene response pathway in Arabidopsis, encodes a member of the Raf family of protein kinases. Cell 72, 427–441

Rogier W. Innes
Dept of Biology, Indiana University, Bloomington, IN 47405-3700, USA.
e-mail: rinnes@bio.indiana.edu

Pictures in Plant Science

Have you generated images that are not only visually stunning, but also provide a real insight into the molecular understanding of plant science? Please send these images*, plus a short explanation of the background to the work, to our editorial office:
plants@current-trends.com

*Please contact the editorial office for details of the correct electronic format before sending any images.