

- 10 Smith, L.G. *et al.* (2001) Tangled1: a microtubule binding protein required for the spatial control of cytokinesis in maize. *J. Cell Biol.* 152, 231–236
- 11 Bichet, A. *et al.* (2001) BOTERO1 is required for normal orientation of cortical microtubules and anisotropic cell expansion in *Arabidopsis*. *Plant J.* 25, 137–148
- 12 Burk, D.H. *et al.* (2001) A katanin-like protein regulates normal cell wall biosynthesis and cell elongation. *Plant Cell* 13, 807–827
- 13 Furutani, I. *et al.* (2000) The SPIRAL genes are required for directional control of cell elongation in *Arabidopsis thaliana*. *Development* 127, 4443–4453
- 14 Whittington, A.T. *et al.* (2001) MOR1 is essential for organizing cortical microtubules in plants. *Nature* 411, 610–613
- 15 Tournebise, R. *et al.* (2000) Control of microtubule dynamics by the antagonistic activities of XMAP215 and XKCM1 in *Xenopus* egg extracts. *Nat. Cell Biol.* 2, 13–19
- 16 Charasse, S. *et al.* (1995) Characterization of the cDNA and pattern of expression of a new gene over-expressed in human hepatomas and colonic tumours. *Eur. J. Biochem.* 234, 406–413
- 17 Andrade, M.A. and Bork, P. (1995) HEAT repeats in the Huntingtons-disease protein. *Nat. Genet.* 11, 115–116
- 18 Groves, M.R. *et al.* (1999) The structure of the protein phosphatase 2A PR65/A subunit reveals the conformation of its 15 tandemly repeated HEAT motifs. *Cell* 96, 99–110
- 19 Vasquez, R.J. *et al.* (1994) XMAP from *Xenopus* eggs promotes rapid plus end assembly of microtubules and rapid microtubule polymer turnover. *J. Cell Biol.* 127, 985–993
- 20 Vasquez, R.J. *et al.* (1999) Phosphorylation by CDK1 regulates XMAP215 function *in vitro*. *Cell Motil. Cytoskeleton* 43, 310–321
- 21 Walczak, C.E. *et al.* (1996) XKCM1: a *Xenopus* kinesin-related protein that regulates microtubule dynamics during mitotic spindle assembly. *Cell* 84, 37–47
- 22 Desai, A. *et al.* (1999) Kin I kinesins are microtubule-destabilizing enzymes. *Cell* 96, 69–78
- 23 Popov, A.V. *et al.* (2001) XMAP215 regulates microtubule dynamics through two distinct domains. *EMBO J.* 20, 397–410
- 24 Spittle, C. *et al.* (2000) The interaction of TOGp with microtubules and tubulin. *J. Biol. Chem.* 275, 20748–20753
- 25 Heald, R. (1999) A dynamic duo of microtubule modulators. *Nat. Cell Biol.* 2, E11–E12
- 26 Kemp, B.E. and Pearson, R.B. (1990) Protein-kinase recognition sequence motifs. *Trends. Biochem. Sci.* 15, 342–346

Patrick J. Hussey*

Timothy J. Hawkins

Dept of Biological Sciences, University of Durham, South Road, Durham, UK DH1 3LE.

*e-mail: p.j.hussey@durham.ac.uk

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 *SIPK* 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 signals¹. 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^{2,3} (Fig. 1). Activation of MAPKKKs in animals and yeast is typically controlled by transmembrane receptors, although numerous other proteins are known to regulate MAPKKK activity¹.

Piecing together a complete pathway from a receptor to a MAPK target has not been accomplished in plants. Indeed, no

one has shown a complete MAPKKK–MAPKK–MAPK assembly other than by heterologous assays in yeast^{4,5}. Equally significant, no one has identified a direct target of a plant MAPK, or determined their biological functions. The two papers I will discuss here represent important advances in our understanding of plant MAPKs in that they provide our first insights into the biological functions of two distinct MAPK family members, *Arabidopsis* *MPK4* and tobacco *SIPK*.

First MAPK mutant in plants

The *Arabidopsis* genome contains 20 MAPK-like genes⁶, 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.*⁷ 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 researchers knew that they had found an MAPK mutant.

Dwarfism in plants can be caused by many different things; thus the challenge for Petersen *et al.* was to determine why disruption of *MPK4* resulted in growth defects. Previous work had indicated that *MPK4* is activated by multiple abiotic stresses, including cold, low humidity, hyper-osmolarity, touch and wounding,

suggesting that its primary role might be in regulating adaptation to environmental stress⁸. Surprisingly, the *mpk4* mutant behaved like wild-type plants in response to these stresses⁷. This forced Petersen *et al.* to scrap the abiotic stress connection and think more broadly about the possible causes of the dwarfed phenotype.

'...the mpk4 mutation causes a greatly elevated level of salicylic acid and...the majority of the mpk4 mutant phenotypes... can be alleviated simply by reducing the endogenous levels of salicylic acid via the expression of a bacterial salicylate hydroxylase gene...'

One well known cause of dwarfism in plants is constitutive expression of defense responses associated with elevated levels of salicylic acid^{9,10}. 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

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 elicitors²⁰. 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* transgene²⁰. 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^{17,18}, 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 MAPKKK–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^{4,5}, the full triad for MPK4 can also be predicted (Fig. 1). Significantly, the MAPKKKs predicted to regulate MPK4 and MPK6 belong to distinct subclasses¹⁷.

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 class¹⁷. Members of all three subclasses have now been implicated in the regulation of disease resistance because the *Raf*-like

MAPKKK gene *EDR1* has recently been shown to negatively regulate plant defense responses, including programmed cell death²¹. In addition, a second *Raf*-like gene *CTR1* functions to negatively regulate ethylene inducible genes²², 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 National Institutes of Health grant R01-GM46451 and a grant from Novartis Agricultural Biotechnology Research, Inc.

References

- Widmann, C. *et al.* (1999) Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. *Physiol. Rev.* 79, 143–180
- Ligterink, W. (2000) MAP kinases in plant signal transduction: how many, and what for? In *MAP Kinases in Plant Signal Transduction* (Vol. 27) (Hirt, H., ed.), pp. 11–27, Springer-Verlag
- Huang, Y. *et al.* (2000) ATMPK4, an *Arabidopsis* homolog of mitogen-activated protein kinase, is activated *in vitro* by AtMEK1 through threonine phosphorylation. *Plant Physiol.* 122, 1301–1310
- Ichimura, K. *et al.* (1998) Isolation of ATMEK1 (a MAP kinase kinase kinase)-interacting proteins and analysis of a MAP kinase cascade in *Arabidopsis*. *Biochem. Biophys. Res. Commun.* 253, 532–543
- Mizoguchi, T. *et al.* (1998) Identification of a possible MAP kinase cascade in *Arabidopsis thaliana* based on pairwise yeast two-hybrid analysis and functional complementation tests of yeast mutants. *FEBS Lett.* 437, 56–60
- The *Arabidopsis* Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 108, 796–815
- Petersen, M. *et al.* (2000) *Arabidopsis* map kinase 4 negatively regulates systemic acquired resistance. *Cell* 103, 1111–1120
- Ichimura, K. *et al.* (2000) Various abiotic stresses rapidly activate *Arabidopsis* MAP kinases ATMPK4 and ATMPK6. *Plant J.* 24, 655–665
- Bowling, S.A. *et al.* (1994) A mutation in *Arabidopsis* that leads to constitutive expression of systemic acquired resistance. *Plant Cell* 6, 1845–1857
- Bowling, S.A. *et al.* (1997) The *cpr5* mutant of *Arabidopsis* expresses both NPR1-dependent and NPR1-independent resistance. *Plant Cell* 9, 1573–1584
- Roberts, C.J. *et al.* (2000) Signaling and circuitry of multiple MAPK pathways revealed by a matrix of global gene expression profiles. *Science* 287, 873–880
- Bent, A.F. (2001) Plant mitogen-activated protein kinase cascades: negative regulatory roles turn out positive. *Proc. Natl. Acad. Sci. U. S. A.* 98, 784–786
- Penninckx, I.A. *et al.* (1998) Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defense gene in *Arabidopsis*. *Plant Cell* 10, 2103–2113
- Solano, R. *et al.* (1998) Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. *Genes Dev.* 12, 3703–3714
- 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
- Zhang, S. and Klessig, D.F. (1997) Salicylic acid activates a 48-kD MAP kinase in tobacco. *Plant Cell* 9, 809–824
- Kovtun, Y. *et al.* (2000) Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. *Proc. Natl. Acad. Sci. U. S. A.* 97, 2940–2945
- Kovtun, Y. *et al.* (1998) Suppression of auxin signal transduction by a MAPK cascade in higher plants. *Nature* 395, 716–720
- Nishihama, R. and Machida, Y. (2000) The MAP kinase cascade that includes MAPKKK-related protein kinase NPK1 controls a mitotic process in plant cells. *Results Probl. Cell Differ.* 27, 119–130
- Yang, K.-Y. *et al.* (2001) Activation of mitogen-activated protein kinase pathway is involved in disease resistance in tobacco. *Proc. Natl. Acad. Sci. U. S. A.* 98, 741–746
- Frye, C.A. *et al.* (2001) Negative regulation of defense responses in plants by a conserved MAPKK kinase. *Proc. Natl. Acad. Sci. U. S. A.* 98, 373–378
- Kieber, J.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

Roger 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.