PHOSPHORYLATION

The key to staying faithful

The cascades in which mitogen-activated protein kinases (MAPKs) play a part can have radically different outcomes, making promiscuity among MAPKs dangerous to the cell. Yet each pathway comprises closely related components. How do MAPKs remain faithful to their pathways? In the 1 February issue of *EMBO Journal*, Takuji Tanoue and colleagues explain one mechanism. Remarkably, this relies on the identity of just two amino acids.

We know of three types of MAPKs, each with their own cascade. The extracellular-signal regulated kinases (ERKs) are activated by growth factors, generally leading to proliferation, whereas p38s and Jun-N-terminal kinases (JNKs) are activated by stress signals, usually causing cell-cycle arrest or apoptosis. Each MAPK has to interact with the kinases that activate it (MAPKKs), the phosphatases that inactivate it (MKPs) and its substrates, which are also kinases (MAPKAPKs). A single acidic site outside the active site — the common docking or CD site — interacts with all these molecules, but it doesn't explain the different binding specificities of the MAPKs. Could there be another site that regulates specificity?

Mutation of the CD in p38 reduced, but didn't completely prevent, binding of the p38-specific MAPKAPK 3pk, implying that another docking site exists. By searching for charged residues and systematically mutating them, the authors identified a pair of residues on p38, Glu 160 and Asp 161, that account for this residual binding. Mutation of the CD site and this second site, which they dubbed the ED site, markedly reduced the ability of p38 to phosphorylate 3pk.

The corresponding residues in ERK2 are two threonine residues. Mutation of these to Glu and Asp enabled ERK2 to bind 3pk, and mutation of ERK2's CD site to make it identical to p38's improved the interaction further. Extending these studies to other MAPKAPKs and MKPs revealed that, although the CD is necessary for binding, the nature of the ED regulates specificity. Together, the two sites form a groove with two pins in it. Only if it can interact with both pins can a MAPK-interacting protein do its business.

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References and links

ORIGINAL RESEARCH PAPER Tanoue, T. *et al*. Identification of a docking groove on ERK and p38 MAP kinases that regulates the specificity of docking interactions. *EMBO J.* **20**, 466–479 (2001)

FURTHER READING Tanoue, T. *et al*. A conserved docking motif in MAP kinases common to substrates, activators and regulators. *Nature Cell Biol.* **2**, 110–116 (2000)

WEB SITE Mammalian MAPK signalling pathways

IN BRIEF

TECHNOLOGY

A high signal-to-noise Ca^{2+} probe composed of a single green fluorescent protein.

Nakai, J., Ohkura, M. & Imoto, K. *Nature Biotechnol*. **19**, 137–141 (2001)

Currently available Ca^{2+} probes based on green fluorescent protein (GFP) have a low signal-to-noise ratio, limiting their use. Nagai *et al.* have built a GFP with a Ca^{2+}–calmodulin-sensitive enzyme tagged on one end and calmodulin on the other. Ca^{2+}-induced conformational changes cause a large increase in fluorescence.

CELL SIGNALLING

Regulation of a novel human phospholipase C, PLC-ε, through membrane targeting by Ras.

Song, C. *et al. J. Biol. Chem*. **276**, 2752–2757 (2001)

A novel bifunctional phospholipase C that is regulated by Gα12 and stimulates the Ras/MAP kinase pathway.

Lopez, I. *et al*. *J. Biol. Chem.* **276**, 2758–2765 (2001)

Phospholipase Cε: a novel Ras effector.

Kelley, G. G. *et al*. *EMBO J.* **20**, 743–754 (2001)

These papers report a new mammalian phospholipase C that is activated by Ras (as well as the α-subunit of a heterotrimeric G protein) but can also activate Ras through its guanine nucleotide exchange factor domain.

DNA RECOMBINATION

Branch migration and Holliday junction resolution catalyzed by activities from mammalian cells.

Constantinou, A., Davies, A. A. & West, S. C. *Cell* **104**, 259–268 (2001)

A key intermediate of homologous recombination and double-stranded-break repair is the Holliday junction. This dynamic structure can move (branch migrate) to generate stretches of heteroduplex DNA, and it is resolved by a junction-specific endonuclease. These reactions are well characterized in bacteria, and West and colleagues now describe analogous activities in mammalian cell-free extracts. This report highlights the conservation of this pathway from prokaryotes to mammals.

DNA REPAIR

XRCC1 stimulates human polynucleotide kinase activity at damaged DNA termini and accelerates DNA single-strand break repair.

Whitehouse, C. J. *et al*. *Cell* **104**, 107–117 (2001)

XRCC1 is involved in repairing single-stranded DNA breaks, but little is known about its biochemical function. Now, using XRCC1 as bait in a yeast two-hybrid screen, the authors have identified a new partner for it — human polynucleotide kinase (PNK). XRCC1 stimulates the DNA kinase and phosphatase activities of PNK at damaged DNA termini, and this accelerates the repair reaction. It is, claim the authors, "a novel pathway for mammalian single-strand break repair".