Hot lips and phosphorylation of protein kinases

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ONE of the successes of the study of signal transduction has been the elucidation of several 'MAP kinase' pathways. These pathways involve the sequential activation of protein kinases, and they control diverse cellular responses in eukaryotes including proliferation and differentiation[1].

Another milestone has now been reached with the publication by Zhang *et al.*, on page 704 of this issue[2], of the crystal structure of the mammalian MAP kinase ERK2. This is only the third protein kinase structure to have been solved, the first being the catalytic subunit of cyclic AMP-dependent kinase (cAPK)[3,4], and the second cyclin-dependent kinase 2 (CDK2)[5]. Overall, the organization of the three kinases is similar — all have a bilobate structure in which ATP is bound in the cleft between the two lobes and the carboxy-terminal lobe binds the substrate. But the newly determined structure is in some ways very different from those of cAPK and CDK2, providing insight into substrate specificity and the way in which the activity of MAP kinases is regulated by phosphorylation.

DFGFAKRVKGRT.....WTLCGTPEYLAPE	cAPK
DFGLARIADPEHDHTGFLTEYVATRWYRAPE	ERK2 (ref. 6)
DFGLARAFGVPVRTY...THEVVTLWYRAPE	CDK2
DFGFAKQLRAENGLL...MTPCYTANFVAPE	RSK (ref. 12)

Activating phosphorylation sites in protein kinases (single-letter amino-acid code). The activating phosphorylation sites are highlighted.

MAP kinases were first discovered in mammalian cells as cytoplasmic protein kinases whose activity is rapidly stimulated by growth factors. Their distinguishing characteristic is that they are activated by phosphorylation on threonine and tyrosine residues within the motif Thr-Glu-Tyr[6], a process which is carried out by a dual specificity protein kinase, MAP kinase kinase[7] (known, in mammalian cells, as MEK).

A mechanism of MEK activation is through phosphorylation by the serine/threonine protein kinase Raf; Raf itself is activated following the stimulation of receptor tyrosine kinases, by a mechanism which involves the formation of Ras/GTP and binding of that complex to the Raf kinase[8]. Similar signalling pathways from receptor tyrosine kinases have been shown to operate in the nematode *Caenorhabditis elegans* and the fruitfly *Drosophila melanogaster*.

One end result of the activation of MAP kinases is their ability to phosphorylate and thereby activate a number of transcription factors, a process which may hinge on their ability to enter the nucleus. A function of MAP kinases may therefore be to provide a link between transmembrane signalling and the nucleus. The biochemical logic of having a MAP kinase regulated through threonine and tyrosine phosphorylation by a dual specificity MAP kinase kinase, which is itself activated by serine/threonine phosphorylation by MAP kinase kinase kinases, is not only used by multicellular organisms but also by single-cell eukaryotes. In the yeasts, for example, MAP kinase pathways are required for the maintenance of cell shape, osmotic integrity and the responses to mating pheromones. Each of these pathways has its own MAP kinase(s), with distinct MAP kinase kinases and upstream kinases[9].

Since the discovery that MAP kinases are regulated by threonine and tyrosine phosphorylation, a central question has been how these phosphorylation events turn on the catalytic activity. The crystal structure of ERK2 is a great help in understanding this mechanism, for it suggests why dual phosphorylation is required to activate ERK2. The threonine (Thr 183) and tyrosine (Tyr 185) residues, phosphorylation of which by MEK activates ERK2, are contained in a loop (L_{12}) which connects the protein kinase domains VII and VIII; these domains include residues Asp-Phe-Gly and Pro-Glu essential to the catalytic activity of all protein kinases[10]. The catalytic activities of cAPK and CDK2 also require phosphorylation of a threonine residue contained within this loop, but Zhang *et al.*[2] show that the structure of the L_{12} loop in ERK2 is quite different. In ERK2, L_{12} is longer (by six amino acids compared with cAPK, three compared to CDK2) and forms what the authors term the 'lip'. The Thr 183 is exposed on the surface, and is therefore likely to be accessible to MEK, but Tyr 185 is buried in a hydrophobic pocket. The location of Tyr 185 is perhaps the most striking feature of the structure and would not have been predicted by model building. To phosphorylate Tyr 185, MEK must presumably alter the conformation of the 'lip'; co-crystals of ERK2 and MEK are eagerly awaited.

The positioning of Tyr 185 in the inactive, nonphosphorylated form of ERK2 suggests that the 'lip' may occlude the substrate-binding site. On phosphorylation, Tyr 185 probably changes the position of its side- and main-chains so that substrate can now bind. If phosphorylation of Tyr 185 regulates substrate binding, what is the role of phosphorylation of Thr 183? Unlike the inactive form of CDK2 (ref. 5), the structure of the bound ATP is not distorted in inactive ERK2. But comparison of the structure with the structure of active cAPK shows that the catalytic residues of inactive ERK2 are not aligned correctly for phosphotransfer, the ATP-binding cleft being more open than in active cAPK. Phosphorylation of Thr 183 is predicted to lead to rotation of the amino- and carboxy-terminal domains, so that the catalytic residues become correctly aligned. If the crystal structure of active ERK2 confirms these predictions, we will have a most elegant explanation of why the activation of MAP kinases requires a dual phosphorylation event.

Phosphorylation within the L_{12} loop is emerging as a regulatory mechanism common to many kinases. Activation of cyclin-dependent kinases requires phosphorylation by a cyclin-activating kinase at a threonine in this region (Thr 167 in *Schizosaccharomyces pombe* CDC2, Thr 161 in human CDC2 and Thr 160 in human CDK2), as well as interaction with a cyclin. Within the mammalian MAP kinase cascade, both the upstream MEK (ref. 11) and downstream RSK (ref. 12) are activated by phosphorylation in L_{12} (see figure). So the L_{12} loop and its 'lips' are likely to be happy hunting grounds for those interested in the regulation of protein kinase activity. □

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