Look who's docking

The mitogen-activated protein (MAP) kinases, such as ERK, JNK and p38, play a pivotal role in signal transduction. However, the mechanism by which they recognize their many substrates is unclear. Two recent studies illustrate the importance of docking sites, which tether the enzyme and substrate, allowing efficient substrate phosphorylation by the appropriate MAP kinase.

The *Caenorhabditis elegans* transcription factor LIN-1 is negatively regulated by ERK. A search for gain-of-function LIN-1 mutants revealed alterations in the conserved sequence FQFP, C-terminal to a region rich in ERK phosphorylation sites. Jacobs *et al.* demonstrate that this sequence is necessary for efficient phosphorylation of LIN-1 by ERK, and that it binds to ERK but not JNK[1]. A similar FXFP motif is found in several other ERK substrates, strongly suggesting that this is a bona fide ERK docking site. LIN-1 has a second docking site, the previously characterized ERK/JNK binding 'D-box'. The authors provide data showing that the two docking sites are functionally independent, and both are necessary for optimal phosphorylation by ERK. This modular system of docking sites, some with overlapping specificities, could explain why certain proteins are substrates for specific MAP kinases, whereas some can be phosphorylated by multiple enzymes.

In another recent study, Smith *et al.* examined the interaction of ERK with its substrate RSK (p90 ribosomal S6 kinase). They demonstrate that the sequence LAQRRVRKL at the C-terminus of RSK1 is crucial for its phosphorylation and activation by ERK[2]. Alignments of a number of RSK-related proteins suggest that variations in the number of basic residues in this sequence could determine specificity for ERK, p38 or both of these MAP kinases. However, this remains to be tested, and searches for other substrates containing this motif were not reported.

These studies might represent the tip of the iceberg, with many more docking sites being as yet undefined. Also, the binding regions on the MAP kinase are poorly understood, and how these interactions are regulated is unknown. Future structural studies will give us a much clearer picture of this area. Interestingly, peptides containing FXFP motifs can inhibit ERK *in vivo,* suggesting the possibility of designing docking site inhibitors for use as therapeutic agents.

1 Jacobs, D. *et al.* (1999) Multiple docking sites on substrate proteins form a modular system that mediates recognition by ERK MAP kinase, *Genes Dev.* 13, 163–175

2 Smith, J. A. *et al.* (1999) Identification of an extracellular signal-regulated kinase (ERK) docking site in ribosomal S6 kinase, a sequence critical for activation by Erk *in vivo*, *J. Biol. Chem.* 274, 2893–2898

Ménage à quatre: profilin regulates axon pathfinding *in vivo*

How an extracellular signal is decoded by the axonal growth cone and transformed to an intracellular signal leading to the appropriate turning or growth response is largely unknown. The highly motile membrane structures (filopodia and lamellipodia) at the tip of the growth cone are supported by a complex cytoskeletal architecture comprising actin and associated proteins. A powerful way to regulate assembly of actin polymers is through proteins, such as profilin, that can sequester monomeric G-actin. Dominant–negative profilin blocks neurite formation *in vitro*, and *Drosophila* has proven useful to identify and characterize proteins *in vivo* that are required for axon pathfinding.

Wills *et al.*[1] show that *stranded*, a previously identified zygotic-lethal mutation, corresponds to profilin/*chickadee*; motor axon extension and pathfinding are impaired in profilin mutants both *in vivo* and *in vitro*. Profilin binds to proteins of the Enabled (Ena) family, which are known to be phosphorylated by the tyrosine kinase Abl. Mutants of profilin and kinase-deficient Abl display an identical axon growth-arrest phenotype of the intersegmental motor neuron b (ISNb), suggesting that Abl and profilin act in the same process; this was further demonstrated by a dose-sensitive genetic analysis. Moreover, inappropriate crossings of axons at the central nervous system midline were observed[1]. Wills *et al.*[2] take things further and show that Abl acts antagonistically to Dlar, a receptor protein tyrosine phosphatase known to be involved in axon guidance; Abl and Ena are required for 'choice point' navigation, and biochemical evidence shows that they associate with and are substrates for the catalytic cytoplasmic domain of Dlar.

Abl, Ena and Dlar appear to participate in a phosphorylation-dependent switch that connects the cell surface to the actin-based machinery responsible for axon pathfinding at the tip of the growth cone. Lanier *et al.*[3] show a genetic interaction between mouse Ena (Mena) and profilin, as well as pathfinding defects. The question of how Ena and profilin ultimately regulate actin cytoskeletal dynamics is likely to be complex; it remains unclear how Abl, Ena and Dlar co-operate to regulate the activity of profilin.

1 Wills, Z., Marr, L., Zinn, K., Goodman, C. S. and Van Vactor, D. (1999) Profilin and the Abl tyrosine kinase are required for motor axon outgrowth in the *Drosophila* embryo, *Neuron* 22, 291–299

2 Wills, Z., Bateman, J., Korey, C. A., Comer, A. and Van Vactor, D. (1999) The tyrosine kinase Abl and its substrate enabled collaborate with the receptor phosphatase Dlar to control motor axon guidance, *Neuron* 22, 301–312

3 Lanier, X. *et al.* (1999) Mena is required for neurulation and commissure formation, *Neuron* 22, 313–325

This month's *headlines* were contributed by Søren Andersen, Volker Haucke, Arianne Heinrichs, Chung Lau, Sean Lawler and Kirsten Sadler.