HIGHLIGHTS

CYTOSKELETON

Eph receptors tip the balance

As mediators of axonal guidance, Eph receptors have long been thought to influence the cytoskeleton. So far, however, a direct connection between these plasma-membrane-bound receptor tyrosine kinases and the regulation of actin dynamics has been unsubstantiated. Now, Michael Greenberg and colleagues describe in *Cell* the identification of a guanine nucleotide exchange factor (GEF) that interacts directly with the EphA4 receptor and activates Rho-family GTPases.

The GEF — termed ephexin for Eph-interacting exchange protein — was identified by yeast two-hybrid screening using the intracellular domain of EphA4 as bait, and contains a tandem Dbl-homology–pleckstrin homology (DH–PH) motif that is conserved in all Dbl GEFs. After confirming the interaction between EphA4 and ephexin in mammalian cells, the authors showed that the interaction occurs independently of EphA4 autophosphorylation. By generating EphA4 deletion mutants, they then demonstrated the requirement for the carboxy-terminal lobe of the kinase domain for ephexin binding. Reciprocally, the DH–PH module of ephexin was found to be enough for EphA4 binding.

Three different assays were used to show that ephexin activates the Rho GTPases RhoA, Cdc42 and Rac1. All assays revealed a clear pecking order in the extent of the response of these GTPases — the largest increase in activity was observed in RhoA, whereas Rac1 showed only a modest increase in exchange activity. Wild-type ephexin was then shown to activate Pak, a downstream effector of Rac1 and Cdc42, as detected by a phospho-specific anti-Pak antibody.

The authors then used the anti-phosphoPak antibody to demonstrate that activating EphA receptors using the EphA ligand, ephrin-A1, inhibited Pak phosphorylation in embryonic cortical neurons. Furthermore, ephrin-A1 induced growth-cone collapse in retinal ganglion cells. As this would require changes in actin dynamics, it was not surprising that further investigation revealed that co-expression of both EphA4 and ephexin in embryonic fibroblasts considerably increased the proportion of cells with actin stress fibres in comparison to cells expressing EphA4 alone, whereas the percentage of cells extending actin processes such as lamellipodia and filopodia was markedly decreased.

Although it is not clear how activation of EphA receptors alters ephexin activity, the implication is that activating EphA4 potentiates the RhoA-activating ability of ephexin, while inhibiting its activity towards Rac1 and Cdc42. The resultant shift in the balance between the activities of the three GTPases is thought to induce localized cell contractility and cell retraction, ultimately leading to growth-cone collapse.

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References and links

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FURTHER INFORMATION Eph receptors

ENCYCLOPEDIA OF LIFE SCIENCES Ephrins | Cytoskeleton | Axon guidance