Methods

Preparation of plasmids and enzyme. Plasmid pRH3 is a 6.16 kilobase derivative of plasmid pBR322, which contains two *Eco*KI sites at base pairs 3,458 and 5,831. Plasmid pBRSK15 is a derivative of pBR322, which has only one *Eco*KI site at base pair 1,668 (G. Davies, pers. comm.). Plasmids containing non-methylated *Eco*KI sites were prepared from *E. coli* NM679 (ref. 26) and purified using standard techniques[27]. *Eco*KI was purified as described[5]. Either plasmid alone or plasmid plus *Eco*KI, at a stoichiometric ratio of 1:2, were incubated in 33 mM Tris-acetate, pH 7.9, 10 mM magnesium acetate, 66 mM potassium acetate and 0.5 mM dithiothreitol at 37 °C for 10 min. All samples were supplemented with 50 μM *S*-adenosylmethionine. Samples were then diluted in the same buffer to a final plasmid concentration of 1 nM, and 50 μl droplets applied to freshly-cleaved mica. Samples were bound to mica in the presence of 10 mM magnesium acetate.

Atomic force microscopy imaging. Imaging was performed using a BioScope Atomic Force Microscope (Digital Instruments) equipped with a fluid tip holder. Samples were imaged using tapping mode with a root mean square amplitude of ~0.7 V and drive frequency of ~8.6 kHz. Commercially available, 100 μm long, oxide-sharpened Si_3N_4 cantilevers with a force constant of ~0.38 N m^{-1} were used (Nanoprobes, Digital Instruments).

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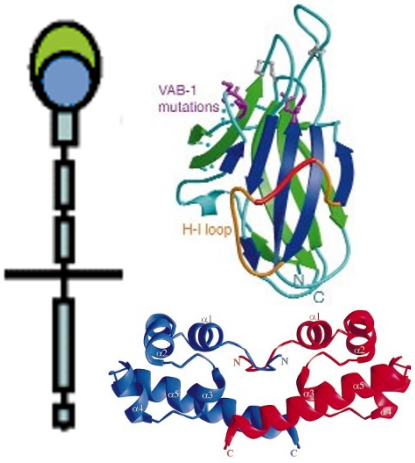
Ephrin receptors divided



Eph receptor tyrosine kinases are involved in many cellular processes, including axon guidance, cell migration and angiogenesis. Like many other receptor tyrosine kinases, the Eph receptors are activated by oligomerization. However, the ephrin protein ligands are unable to induce receptor oligomerization directly. Thus, the question is: how does binding of ephrin lead to activation of the Eph receptor? To understand the mechanism in detail, it will be necessary to determine the structures of Eph receptor family members, in both the presence and absence of the ligand. But, such a task may be difficult: Eph receptors are large, multi-domain, membrane-anchored proteins. The diagram illustrates a typical Eph receptor, which consists of (from top to bottom) a globular region, a cysteine-rich region, two fibronectin type III repeats, a membrane-spanning region, a tyrosine kinase domain and a SAM (sterile α motif) domain. Two groups of researchers have recently determined crystal structures of two isolated Eph receptor domains — the SAM domain (page 44 of this issue) and the ligand-binding domain (Himanen, J.-P., Hendemeyer, M. & Nikolov, D.B. *Nature* **396**, 486–491; 1998).

The globular ligand-binding domain (top structure) has a jellyroll β-sandwich topology. Mutational analysis has demonstrated that the H-I loop (red) participates in ephrin binding, and this loop is proximal to residues (magenta) that, when mutated, lead to signaling defects in the *C. elegans* VAB-1 Eph receptor. However, without a ligand-bound structure, it is difficult to predict how altering the structure of this region could activate the receptor. Even less is known about how the SAM domain may participate in activation. SAM domains (bottom structure) form dimers, both in the crystal and in solution. This dimerization could be important for clustering Eph receptors during activation, or the association could keep receptors in a repressed state. Alternatively, interaction with other SAM domain-containing proteins could play a role. The challenge in this system will be to determine how information about ephrin binding is transmitted through the membrane to the tyrosine kinase and SAM domains and to understand the roles played by each domain. *Tracy Smith*