Journal Club

Anatomy of a molecular switch: structural basis of Ephb2 activation

Now that, in theory, the various genome projects have given us a list of all the parts used by various organisms to go about their daily lives, the next challenge is to understand how those parts work, interact and are connected. One of the most important aspects of understanding any complex system is to learn how it is controlled and where the switches are. A common switching mechanism used by cells is to attach negatively charged phosphate groups to proteins using protein kinases. This simple modification can cause the proteins to change their structural conformation, thereby allowing them to react with or bind other proteins that contain phosphate-binding domains, such as the SH2 domain, and initiate a signal cascade. The results of signal transmission, that is, turning on or off the switch, can have profound effects on cells. Indeed, many malignancies and developmental abnormalities are caused by malfunctioning molecular switches.

Wybenga-Groot *et al.* [1] describe the detailed workings of such a molecular switch. They have solved the structure of the Ephb2 receptor kinase domain and the juxtamembrane region in the autoinhibited state. Their results show that, in its unphosphorylated form, the juxtamembrane region adopts a helical structure that impinges on the ordering of the activation segment and distorts the conformation of the small lobe of the kinase domain, thereby disrupting the active site and preventing autophosphorylation. The authors also suggest that the structure shows how phosphorylation of the juxtamembrane tyrosines would remove this autoinhibition by permitting a structural realignment of the kinase domain, which would result in it becoming active. The data presented also indicate that phosphorylation of the juxtamembrane region would cause its release from the active site. The environment around each of the so-called two switch regions is hydrophobic in nature, but exposed to solvent. The addition of negatively charged phosphate groups to region one would cause electrostatic repulsion. Region two contains five negatively charged residues, so that phosphorylation of the region would result in additional electrostatic repulsion and cause the release of this residue from its binding pocket. Mutation of the two tyrosines to phenylalanine in the juxtamembrane region was shown to drastically reduce the kinase activity of Ephb2, lending strong support to the proposed mechanism. In addition, the now free phosphorylated juxtamembrane region of the receptor would be able to adopt a new conformation and bind to SH2 domain-containing molecules to assemble a signaling complex.

It has long been known that cells respond to external stimuli through the binding of ligands to receptors, which in their turn transmit the signal inside the cell to initiate an appropriate response. The ability to oscillate between catalytically active and inhibited states in a regulated manner is central to the ability of protein kinases to act as versatile molecular switches. Kit and Flt3 receptors have been implicated in the regulation of tyrosine kinase activity. There are oncogenic variants known in mice and human cell lines that have amino acid substitutions or deletions in the juxtamembrane region that result in constitutive activation of the kinase domain. This leaves the switch permanently on, with disastrous consequences. This suggests that other molecules might use the mechanism proposed for the Ephb2 receptor. By opening the trap door, priming the trap with the phosphate bait, these molecular switches are turned on causing further molecules to be trapped and the signal to be passed down the line. By understanding the way in which the switches work and where they are, we might be able to turn off a broken switch.

1 Wybenga-Groot, L.E. *et al.* (2001) Structural basis for autoinhibition of the Ephb2 receptor tyrosine kinase by the unphosphorylated juxtamembrane region. *Cell* 106, 745–757

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Mapping the combinatorial regulation by transcription factors

Microarray experiments have become central to transcription studies. Several computational methods (e.g. based on principal components analysis) are currently used to analyse such experiments on a genome-wide scale. Moreover, comprehensive databases of gene sequences and transcription factors are now available and thus it is promising to combine both sources of information. So far, only a few studies in this context address the fact that, in eukaryotes, one and the same promoter can be regulated by the coordinated action of multiple transcription factors. This brings about a combinatorial control, so that each gene can respond to a variety of environmental conditions without the need for a huge number of different transcription factors.

Pilpel *et al.* [1] identified synergistic effects of transcription factors in *Saccharomyces cerevisiae*. They propose an approach consisting of the following steps: (1) build a database of known and putative promoter motifs (i.e. portions of promoter sequence to which transcription factors can bind); (2) for each motif pair, identify all the genes that involve this pair in their promoters; (3) calculate the 'expression coherence score,' that is, a measure of the overall similarity of the expression profiles of all the genes containing that motif pair, based on a Euclidean distance; (4) identify significantly synergistic combinations; and (5) build motif synergy maps and compare the effect of individual motifs and combinations of motifs on gene expression. This was performed with yeast in several different conditions, including different stages of the cell cycle, sporulation, diauxic shift, heat and cold shock, as well as treatment with pheromone and DNA-damaging substances.

Several experimentally established motif associations were confirmed in the analysis. For example, the binding sites for Mcm1 and SFF are synergistic in certain phases of the cell cycle. Moreover, Pilpel *et al.*

