SYSTEMS BIOLOGY

Spotting interactions

Online links

EGF: http://us.expasy.org/cgi-bin/niceprot.pl?P01133
SH2: http://www.ebi.ac.uk/interpro/DisplayIproEntry?ac=IPR000980
ERBB1: http://us.expasy.org/uniprot/P00533
ERBB2: http://us.expasy.org/uniprot/P04626
ERBB3: http://us.expasy.org/uniprot/P21860

When epidermal growth factor (EGF) and its relatives bind the ERBB family of receptors, they trigger a network of signalling pathways that culminate in responses ranging from cell division to death, and adhesion to migration. Using a genome-wide approach, Jones *et al.* now reveal a quantitative network that provides new insights into the recruitment of proteins to the activated ERBB receptors.

Ligand binding to the extracellular domain of these receptors promotes receptor dimerization and activation of the intracellular tyrosine-kinase domain. Activated receptors phosphorylate each other on a number of tyrosine residues, which function as docking sites for the Src-homology-2 (SH2) or phosphotyrosine binding (PTB) domains of enzymes or adaptor proteins. Extensive analysis has provided useful information on the binding partners of the receptors, but could a biophysical analysis of protein recruitment, carried out on a genome-wide scale, provide insights into the nature of ERBB signalling at a system level?

The authors initiated their approach by cloning, expressing and purifying every SH2 and PTB domain that is encoded in the human genome. They then synthesized 17–19-residue, phosphotyrosine-containing peptides that represented the physiological sites of tyrosine phosphorylation in the four ERBB receptors (ERBB1–4). The interactions of 159 domains with 61 peptides were tested using microarrays that were probed with 8 concentrations of each peptide to measure the strength of each possible interaction.

The 77,592 independent biochemical measurements that were collected helped the authors to construct a graphical representation of phosphotyrosine-mediated recruitment that illustrates the biophysical interactions between signalling proteins and known sites of tyrosine phosphorylation on the ERBB receptors. This has provided, for the first time, a system-level view of the interactions that are mediated by these receptors. Comparing these data, previously reported interactions between SH2- and PTB-containing proteins and ERBB1–3 receptors, and data from Scansite (a program that uses consensus-binding information) revealed that the microarrays detected 43 of the 65 known interactions. However, they also uncovered many strong interactions that had not been previously reported. Several proteins bound to ERBB1–3, including the ones that initiate canonical signalling pathways. But the authors also found that proteins that had not previously been considered in the context of ERBB signalling could bind with high affinity to all four receptors.

By slicing through the network for ERBB1–3 at different affinity thresholds, this study showed intriguing differences between the receptors. The ERBB3 network changed very little as the threshold was lowered, whereas ERBB1 (also known as EGFR) and ERBB2 (also known as HER2/neu) became much more promiscuous, which indicates that cells should be less sensitive to changes in the levels of ERBB3 (also known as HER3) relative to ERBB1 and ERBB2. But what can each receptor do that the others cannot? When the tightest interactions were considered, the only protein that bound to ERBB1 was the SH2-domain-containing phosphatase SHP2. Further analysis showed that, under stringent conditions, the ERBB2–ERBB3 complex is broadest in scope, followed by ERBB1–ERBB3, ERBB1–ERBB2 and finally ERBB1–ERBB1.

This study provides a quantitative protein-interaction network that reveals new interactions between the ERBB receptors and SH2 and PTB domains, and shows surprising differences between these receptor tyrosine kinases. Because these receptors are overexpressed in many cancers, the oncological implications of these findings are equally intriguing.

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ORIGINAL RESEARCH PAPER
Jones, R. B. *et al.* A quantitative protein interaction network for the ErbB receptors using protein microarrays. *Nature* 6 Nov 2005 (doi:10.1038/nature04177)