SIGNAL TRANSDUCTION

Split personality

Steroid hormone receptors, best known as transcriptional activators, are showing a new side to their character. A study by Dean Edwards and colleagues in *Molecular Cell* adds to growing evidence that steroid hormone receptors can modulate cytoplasmic signalling pathways by identifying a polyproline motif in the amino-terminal domain of the progesterone receptor (PR) that is able to activate Src kinases through a Src-homology-3 (SH3) domain interaction.

The authors carried out a yeast two-hybrid screen using the amino terminus of PR as bait, and pulled out a clone corresponding to the carboxy-terminal-most SH3 domain of *c*-Cbl-associated protein (CAP). By expressing the SH3 domain of CAP and a panel of other signalling molecules as glutathione-*S*-transferase (GST) fusion proteins, the authors showed that PR bound directly — through SH3-domain interactions — to the Src family members *c*-Src and Hck, as well as CAP. Conversely, they identified the extended left-handed polyproline (PPII) helix within the amino-terminal domain (PPPPLP-PR) of PR as being necessary and sufficient for SH3-domain binding.

Of the other nuclear receptors tested — oestrogen, glucocorticoid, androgen and thyroid hormone — none was able to bind to SH3 domains *in vitro*. This is hardly surprising, as they all lack an obvious polyproline recognition sequence.

To test the hormone dependency of the PR–SH3 interaction in cells, Edwards *et al.* added R5020 — synthetic progestin — to normal breast epithelial cells expressing recombinant PR, and saw that it increased the interaction with GST–*c*-Src-SH3. R5020 also enhanced the interaction of PR and *c*-Src in a co-immunoprecipitation assay in breast cancer cells that express both proteins endogenously.

So what effect does PR binding have on Src kinases? To investigate this, the authors expressed Hck with *c*-Src kinase (CSK) to phosphorylate tyrosine 527 of Hck, a residue involved in the autoinhibition of catalytic activity through intramolecular associations. Adding purified PR that was bound to R5020 stimulated Hck kinase activity in a concentration-dependent manner by displacing intramolecular interactions between the SH3 domain and a polyproline-like helix — the second important autoinhibitory mechanism.

The authors then showed that progestin transiently stimulated *c*-Src activity in mammalian cells, which also led to a transient increase in the activity of mitogen-activated protein kinase (MAPK) — a downstream effector. Mutations in the second zinc finger of the DNA-binding domain of PR that destroy its transcriptional ability had no effect on the ability of progestin to stimulate *c*-Src activity, but mutating three key prolines to alanines in the PPII motif of PR (PR_{mPro}) abolished both the interaction with the SH3 domain of Src and Src's ability to be activated by progestin.

So, what is the biological significance of PR–SH3 domain interactions? In normal mammary epithelium and breast cancer cells, progestin mainly exerts a growth inhibitory effect. In the present study, continuous treatment with R5020 for five days led to growth arrest in normal mammary cells expressing PR, whereas cells expressing PR_{mPro} experienced a delayed onset of growth arrest. Similarly, expressing PR_{mPro} in *Xenopus* oocytes had no effect on the rate of progesterone-induced maturation, in contrast to normal PR, which accelerated the rate of maturation.

So it seems that PR acts as a dual-function protein, first by directly interacting with target DNA in the nucleus and thereby modulating gene transcription, and second by interacting with SH3 domains to modulate cytoplasmic cell signalling pathways.

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References and links

ORIGINAL RESEARCH PAPER
Boonyaratanakornkit, V. *et al*. Progesterone receptor contains a proline-rich motif that directly interacts with SH3 domains and activates *c*-Src family tyrosine kinases. *Mol. Cell* **8**, 269–280 (2001)

CELL SIGNALLING

Moving forward in reverse

Signalling by Eph receptors and ephrins is a two-way process — each can act both as a receptor, which transduces signals, but also as a ligand that can send such signals. More is known about the 'forward' signalling pathway — where Eph receptors, as their name suggests, act as receptors — than the events that occur in the reverse direction. Reporting in *Nature*, however, Cowan and Henkemeyer now describe some of the proteins involved in reverse signalling following the engagement of B-ephrins.

The authors started with a two-hybrid screen to identify proteins that interact with the cytoplasmic domain of ephrin-B1 in a phosphotyrosine-dependent manner. All the resulting 19 clones encoded an adaptor protein called Grb4 (also known as Nck-2 and Nckβ), which contains one Src-homology-2 (SH2) domain, essential for the interaction with ephrin-B1, and three SH3 domains. The authors used glutathione-*S*-transferase pull-down experiments and co-immunoprecipitation to verify this interaction in mammalian cells, and then visualized the recruitment of Grb4 to ephrin-B1 in live cells.

What is the effect of this interaction? Cowan and Henkemeyer examined the cytoskeleton after ephrin-B1 reverse signalling, and found a marked loss of polymerized actin. Not only that, but cells rounded up and shrank, as though adhesive contacts had been lost, and the focal adhesion marker paxillin was seen to re-distribute from the plasma membrane to the cytoplasm. Finally, catalytic activity of focal adhesion kinase (FAK) was found to increase.

To find out how reverse signalling leads to such effects, the authors used the three Grb4 SH3 domains as bait in another two-hybrid screen. They pulled out five proteins — dynamin, heterogeneous nuclear ribonucleoprotein K (hnRNPK), Abl-interacting protein-1 (Abi-1), *c*-Cbl-associated protein (CAP) and axin — and showed that one of them, CAP, redistributes with Grb4 to activated spots of ephrin-B1. The authors therefore propose a model, shown below, in which clustered tyrosine-phosphorylated ephrin-B1 forms a high-affinity binding site for the Grb4 SH2 domain, and that the SH3 domain of Grb4 recruits various proteins to this site.

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References and links

ORIGINAL RESEARCH PAPER Cowan, C. A. & Henkemeyer, M. The SH2/SH3 adaptor Grb4 transduces B-ephrin reverse signals. *Nature* **413**, 174–179 (2001)