EGF signalling — it's all in SHC1's timing

When activated, the Tyr kinase EGFR (epidermal growth factor receptor) phosphorylates the scaffold protein SHC1. This enables SHC1 to recruit SH2 (SRC homology 2) domain-containing proteins, such as the adaptor GRB2 (growth factor receptor-bound 2), to trigger the activation of downstream pathways. Pawson and colleagues now uncover the true complexity of this system, revealing that SHC1 is phosphorylated on both Tyr and Ser/Thr residues at specific time points after EGF stimulation; this allows it to interact with three discrete clusters of proteins that change the output of EGF signalling over time.

To investigate events downstream of EGFR-mediated SHC1 phosphorylation, the authors generated cells stably expressing a tagged version of the 52 kDa isoform of SHC1. They mapped SHC1 phosphorylation sites and the time after EGF stimulation at which phosphorylation peaks at each site. SHC1 was phosphorylated on Tyr239, Tyr240 and Tyr313 (1–2 minutes after EGF stimulation, in line with these sites being direct EGFR substrates), Ser29 (3 minutes), Thr214 (5 minutes) and Ser335 (20 minutes). They also sought to identify the kinases that phosphorylate SHC1 on Ser/Thr residues. EGFR-mediated SHC1 phosphorylation activates the RAS–ERK and PI3K–AKT pathways. Using different kinase inhibitors, the authors found that SHC1 is phosphorylated by AKT on Ser29 and by ERK on Thr214. Thus, the earliest SHC1 phosphorylation events trigger the activity of the kinases that phosphorylate SHC1 later. The kinase responsible for Ser335 phosphorylation remains to be determined.

Next, the authors assessed how SHC1 interacts with the 41 downstream binding partners that they identified, over time. They classified proteins that associated with SHC1 1–2 minutes, 2–5 minutes and 15–20 minutes after EGF stimulation, as cluster 1, cluster 2 and cluster 3 proteins, respectively. Cluster 1

included effectors involved in stimulating mitogenic signalling, cluster 2 included the Tyr phosphatase PTPN12 and cluster 3 included proteins involved in cytoskeletal reorganization as well as phosphatases and GTPase-activating proteins that downregulate RAS–MAPK signalling. The timing of these SHC1–protein interactions suggests that SHC1 initially stimulates mitogenic signalling before switching its signalling output to the control of cytoskeletal architecture and signal reversal.

But, do all of the downstream effects of SHC1 depend on its interaction with GRB2? The interaction of SHC1 with cluster 1 proteins, but not cluster 2 and cluster 3 proteins, was abolished in GRB2-depleted cells. Further experiments showed that Ser29 phosphorylation is important for the GRB2-independent interaction of SHC1 with the cluster 2 protein PTPN12, and mutation of Ser29 enhanced the binding of cluster 1 proteins to SHC1.

Thus, the authors propose that the recruitment of PTPN12 (a known human tumour suppressor), which could dephosphorylate Tyr residues in SHC1 to reduce GRB2 binding, might switch this scaffold from a protein that stimulates mitogenic pathways via cluster 1 proteins to a protein that attenuates signalling and promotes cytoskeletal reorganization via cluster 3 proteins. Interestingly, they found that the cluster 3 protein Sgk269 also contributes to this 'switch' by acting as an adaptor to recruit other cluster 3 proteins to SHC1. Therefore, SHC1 is not simply a static scaffold, but rather a dynamic device that modifies EGFR signalling output over time.

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ORIGINAL RESEARCH PAPER Zheng, Y. *et al.* Temporal regulation of EGF signaling networks by the scaffold protein Shc1. *Nature* **499**, 166–171 (2013)

FURTHER READING Avraham, R. & Yarden, Y. Feedback regulation of EGFR signalling: decision making by early and delayed loops. *Nature Rev. Mol. Cell Biol.* **12**, 104–117 (2011)