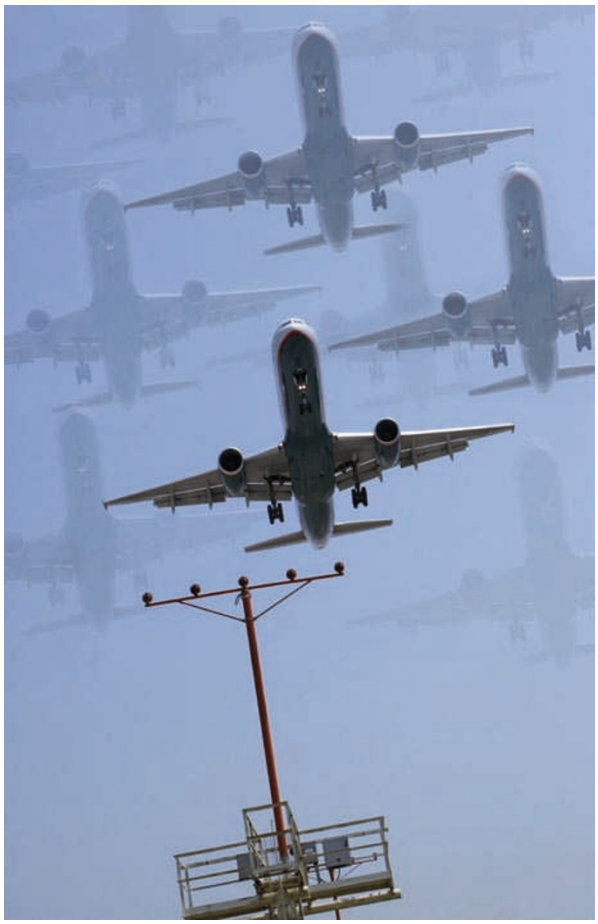
*Nature Reviews Molecular Cell Biology* | AOP, published online 13 January 2010; doi:10.1038/nrm2839

ENDOCYTOSIS

# Acetylation controls EGF traffic

> HDAC6 regulates EGFR endocytosis and degradation

When epidermal growth factor (EGF) binds its receptor, EGFR, intracellular signal transduction results in the regulation of cellular functions such as growth, proliferation, differentiation and survival. Signalling is terminated by the internalization and trafficking of EGF–EGFR through endocytic compartments to the lysosome for proteolytic degradation. Although EGF signalling through EGFR is well characterized, little is known about the association of other proteins with EGFR before EGF stimulation.



BRAND X

Now, Deribe *et al.* identify the EGF-unoccupied EGFR interactome and reveal a role for histone deacetylase 6 (HDAC6) in EGFR trafficking.

A modified split ubiquitin-based membrane yeast two-hybrid assay was used to analyse membrane protein interactions with the human EGF-unoccupied EGFR. In this assay EGFR is fused to the carboxy-terminal part of ubiquitin and a transcription factor. When EGFR interacts with proteins fused to the amino-terminal part of ubiquitin, the ubiquitin halves are brought together and the transcription factor is released, activating the transcription of reporter genes. The authors replaced EGFR's signal sequence (required for protein targeting) with that of the yeast α-mating pheromone precursor to circumvent the problems of self-activation and improper incorporation of mammalian single-pass transmembrane proteins (such as EGFR) in yeast.

This screen identified 87 candidate proteins that interact with EGF-unoccupied EGFR. These proteins include the cytoplasmic HDAC6, which co-immunoprecipitated with endogenous EGFR to a similar extent before and after EGF stimulation. Overexpression of HDAC6 increased the amount of EGFR in several cell lines, and knockdown of HDAC6 caused an early onset of EGFR degradation following EGF stimulation. Together with an observed colocalization of HDAC6 with EGF–EGFR in endosomes, these data suggest that HDAC6 might influence the trafficking and sorting of EGFR along the endocytic pathway. Indeed, following HDAC6 knockdown, the colocalization of EGF–EGFR with early endosomal antigen 1 (EEA1) and the rate of travel of EGF-containing endosomes towards the perinuclear area (where receptor degradation occurs) were increased.

To investigate whether EGF-induced protein acetylation regulates the kinetics of EGFR trafficking, the authors looked for acetylated proteins in complex with EGFR and in the endocytic apparatus. Mass spectrometry analysis revealed that, after EGF stimulation, α-tubulin is acetylated on Lys40, which enhances interactions between microtubules and motor proteins to accelerate motor protein-driven endocytic cargo transport along microtubules. Furthermore, EGFR inactivates HDAC6 by phosphorylating it on Tyr570. EGFR therefore increases the acetylation of α-tubulin by inactivating HDAC6, thereby functioning in a complex negative feedback system that regulates EGFR trafficking along microtubules.

So, this study identifies the human EGF-unoccupied EGFR protein interactome and shows that HDAC6 regulates EGFR endocytosis and degradation. This implicates post-translational modification by acetylation as a regulatory mechanism in receptor trafficking, and provides a modified membrane yeast two-hybrid assay that can be applied to the study of other receptor tyrosine kinases.

*Debbie Walker*

**ORIGINAL RESEARCH PAPER** Deribe, Y. L. *et al.* Regulation of epidermal growth factor receptor trafficking by lysine deacetylase HDAC6. *Sci. Signal.* **2**, ra84 (2009)
**FURTHER READING** Sorkin, A. and Zastrow, M. Endocytosis and signalling: intertwining molecular networks. *Nature Rev. Mol. Cell Biol.* **10**, 609–622 (2009)