EGFR SIGNALING

Acetylation regulation

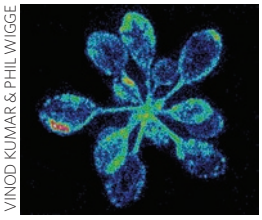
Sci. Signal., published online 22 December 2009, doi:10.1126/scisignal.2000576

Epidermal growth factor receptor (EGFR) activation by ligand binding initiates signaling by a large network of receptor-associated proteins to downstream pathways. Though some components that interact with inactivated EGFR have been identified, a complete picture of how the receptor is maintained in a state that is competent for immediate activation is unclear. Lissanu Deribe *et al.* used a modified split ubiquitin-based membrane two-hybrid assay to identify proteins that interact with EGFR. The authors focused on an interaction with the cytoplasmic lysine deacetylase HDAC6. Interaction with HDAC6 could stabilize and increase the abundance of EGFR, and this was dependent on its deacetylase activity. Knockdown of HDAC6 accelerated the delivery of EGFR to lysosomes and therefore its degradation after ligand stimulation. The authors also found that HDAC6 deacetylates α-tubulin in stimulated cells—a process known to slow transport through the secretory pathway—and identified a feedback mechanism where EGFR inactivates HDAC6 through phosphorylation, increasing α-tubulin acetylation. These results lead to a model where HDAC6 binding to EGFR allows for immediate downregulation though acetylation of microtubules after ligand stimulation. *MB*

PLANT GENE EXPRESSION

A histone thermocouple

Cell **140**, 136–147 (2010)



VINOD KUMAR & PHIL WIGGE

Plants are exquisitely sensitive to changes in environmental conditions, such as light and temperature. For instance, small increases in ambient temperature are known to accelerate growth and trigger early flowering in plants. Yet the molecular basis for temperature sensation in eukaryotes is not understood. Kumar and Wigge now establish that nucleosomes containing histone H2A.Z are conserved master sensors of the transcriptional response to changes in ambient temperature. Noting that *Arabidopsis thaliana HSP70* is upregulated uniformly with small temperature increments, the authors constructed an HSP70-luciferase reporter system and used it to perform a screen of *Arabidopsis* mutants to identify genes involved in temperature sensation. The screen revealed that the alternative histone H2.AZ is involved in the global ambient temperature response. Chromatin immunoprecipitation studies showed that H2A.Z-containing nucleosomes are enriched at transcriptional start sites (TSS) of temperature-responsive genes under low temperatures and depleted at higher temperatures. Further biochemical analyses suggested that H2A.Z-containing nucleosomes wrap DNA more tightly than H2A-containing nucleosomes and restrict RNA polymerase access to the TSS of these genes. This mode of temperature sensing appears to be conserved, as the H2A.Z homolog in budding yeast, also modulates temperature-dependent gene expression. Taken together, these results support a model in which H2A.Z nucleosomes act as key molecular temperature sensors and control gene expression at the transcriptional level. *TLS*

PROTEIN LABELING

Reversible cysteine labeling

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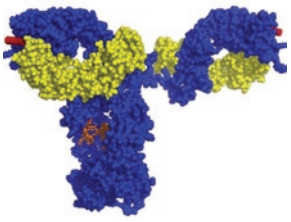
The nucleophilicity and low natural abundance of cysteine residues in proteins has made them attractive sites for chemoselective protein modification reactions. For example, proteins can be decorated with reporters through the conjugate addition of cysteine thiols to N-substituted maleimides. Though these reactions are selective and efficient, they are generally irreversible and permit the introduction of only a single label. Smith *et al.* now demonstrate that protein labeling with N-substituted bromomaleimide derivatives overcomes many of these limitations. Building on an earlier study exploring the chemical reactivity of bromomaleimides, the authors now show that *N*-methylbromomaleimide reacts efficiently with an SH2 domain model protein containing a single cysteine. The resulting maleimide adduct could be reversed to the starting protein by treatment with a phosphine. Alternatively, it could be converted to a more complex bioconjugate by addition of a second thiol reagent. Dibromomaleimide reacts similarly and can be used to produce either a mono- or dithioether product that can be reversed by treatment with excess thiol such as glutathione. Dibromomaleimide and its N-modified derivatives were also shown to be able to reversibly insert into the disulfide bond of the cyclic peptide somatostatin. Thus it has been shown that bromomaleimide derivatives offer opportunities for multiple bioconjugation reactions that can be reversed under conditions likely to be encountered in the intracellular medium. They may therefore offer a substantial improvement over current maleimide-based protein labeling methods. *TLS*

ANTIBODY RECOGNITION

HIV-1 Trp-ed up

Proc. Natl. Acad. Sci. USA, published online 4 January 2010, doi:10.1073/pnas.0909680107



CALTECH/JOSHUA KLEIN

The HIV-1 glycoprotein gp41 is associated with the viral membrane and involved in fusion to cellular membranes. Antibodies such as 4E10, derived from the host's adaptive immune system, have been discovered that neutralize a broad range of HIV-1 isolates by binding to gp41. Because 4E10 can bind lipids, and antibody recognition is enhanced in a membrane context, it is thought that 4E10 binds to viral membrane lipids and that this is important for viral neutralization. To determine the role of lipids, Scherer *et al.* focused on the H3 loop of the 4E10 antibody heavy chain, which binds only a few gp41 residues. The authors mutated the two most lipophilic residues, tryptophans, in this region with either alanine or aspartic acid, together or separately. Mutant antibodies could still bind gp41 peptides, but antibodies showed decreased reactivity to viral membrane mimetic liposomes and were less potent at viral neutralization. The alanine mutants performed slightly better in these assays and also in an epitope affinity assay, but both single mutations and the combined mutations, which appeared to act cooperatively, could only neutralize a highly sensitive HIV-1 strain. These results suggest that the tryptophan residues within the antibody H3 loop enable its interactions with viral lipids and that the membrane environment configures the lipophilic residues of the H3 loop for optimal recognition. *MB*

Written by Mirella Bucci, Catherine Goodman, Joanne Kotz & Terry L. Sheppard