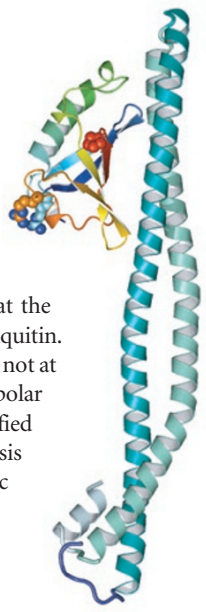
PI3K polar patch



Phosphoinositide 3-kinases (PI3Ks) regulate many cellular processes and are linked to several cancers. The PI3K catalytic subunit, p110α, dimerizes with the p85 regulatory subunit, resulting in enzyme inhibition. Some oncogenic hot spots in p110α map to the adaptor-binding domain (ABD), which binds one of the three SH2 domains (the coiled-coil iSH2 domain) in p85. These hot spots have now been examined by Backer, Williams and colleagues. The authors crystallized the ABD–iSH2 complex, finding that the previously unsolved ABD structure resembles ubiquitin. Oddly enough, the oncogenic ABD mutations are not at the iSH2 interface, mapping instead to a distinct polar patch that may interact with an as yet unidentified factor. Modeling of the holoenzyme on the basis of available structures suggests how oncogenic mutations in the p110α helical domain might function. This was further pursued by testing whether the helical domain interacts with the N-terminal SH2 domain (nSH2) of p85. The oncogenic helical domain mutant E545K is active even in the presence of p85. To test whether this mutation disrupts a charge-charge interaction with the regulatory subunit, the authors introduced charge-compensatory mutations at the predicted interaction site in p85. These designed mutations in p85 restore its ability to inhibit the p110α E545K mutant. As the nSH2 inhibitory surface overlaps with the known p85 phosphopeptide-binding site, the authors suggest a mechanism for kinase activation where the phosphopeptide competes with the catalytic subunit for regulatory subunit binding. Their data complete the structural picture of a p110 catalytic subunit, identify additional interactions with p85 and suggest testable mechanisms for PI3K activation; together, these findings may prove useful for drug design. (*Science* **317**, 239–242, 2007) *SL*

Coordinating from a distance

Internal protein motions seem to be closely linked to functions such as enzyme catalysis. To investigate this interconnection, it is important to be able to characterize the relevant time-dependent protein motions from the time-averaged three-dimensional structure. Using NMR relaxation dispersion measurements, biochemical experiments and mutagenesis, Loria and coworkers have studied the protein motions of RNase. RNase is an enzyme whose rate-limiting step is a conformational change coupled to the conversion of substrate to product. If hydrogen bonds are involved in the conformational change, then replacement with deuterium should slow the rate of motion, and solvent isotopes should therefore affect enzyme kinetics. The authors find that protein motions and catalytic activity in RNase A are coupled and show identical solvent isotope effects. They zero in on a histidine residue (His48) as a key regulator of this coupling. Despite the fact that it is 18 Å away from the site of RNA bond cleavage, mutation of this residue to alanine results in a loss of protein motion and a decrease in k_{cat}, indicating a change in the rate-limiting conformational motion. Thus, a single residue far removed from the active site has a central role in modulating and coordinating the catalytically productive motions of RNase. (*Proc. Natl. Acad. Sci. USA* **104**, 11981–11986, 2007) *BK*

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Putting a brake on TopIB

DNA topoisomerases relieve DNA supercoiling, an activity essential for cell survival. Type IB topoisomerase (TopIB) forms a transient covalent bond with DNA, creating a single-stranded nick that allows DNA to swivel; this relieves the torsional stress caused by either positive or negative supercoiling. TopIB is the target for camptothecins such as topotecan, a chemotherapeutic agent approved by the US Food and Drug Administration. Camptothecins trap TopIB on DNA, and such drug-enzyme adducts are thought to pose a physical barrier for the replication machinery, accounting for camptothecins' cytotoxicity. However, a recent study from Koster and colleagues reveals that there might be more to it than that. Using a single-molecule approach, the authors observed that topotecan slows down DNA uncoiling by human TopIB, suggesting that topotecan hinders the rotation of DNA bound to TopIB. This effect is more pronounced on positive supercoils, but the structural basis of such asymmetry is not understood. Positive supercoils are formed *in vivo* ahead of advancing transcription bubbles and replication forks. Treatment of yeast cells with camptothecin resulted in the accumulation of positive supercoils, an effect that was dependent on TopIB. The authors propose an alternative mechanism for the antitumor properties of camptothecins: the slowing down of TopIB's uncoiling activity leads to the accumulation of positive supercoils in the chromosome, which may stall the progression of the replication fork, resulting in fork collapse and lethal DNA lesions. Understanding these drugs' mode of action in detail is crucial for the development of new chemotherapeutics. (*Nature* **448**, 213–217, 2007) *IC*

Nanoswitch signaling

The MAP kinase (MAPK) pathway transmits signals from receptors at the plasma membrane into the cell to regulate cell differentiation and proliferation. Key to this pathway is the activation of Ras by GTP binding, which occurs after receptor phosphorylation. Recent studies have shown that Ras activation results in the formation of signaling nanoclusters, discrete regions in the plasma membrane that concentrate Raf, MEK and ERK, all downstream components of the MAPK cascade. Nanocluster formation has been proposed to improve signaling efficiency, but how it does so is unclear. Using mathematical modeling and *in vivo* studies, Hancock and colleagues have examined how the nanoclusters mediate efficient signal transduction. They start by demonstrating that Raf-mediated MEK activation occurs only at Ras-GTP nanoclusters. Interestingly, they find that ERK activation, mediated by MEK, occurs in a binary fashion, with any amount of activated Raf triggering maximal ERK phosphorylation. In contrast, they find a linear correlation between the amount of extracellular ligand applied and the degree of ERK activation. These findings suggest that Ras nanoclusters act like nanoswitches, producing a maximal output in response to activation. However, because nanoclusters form only when Ras-GTP is present, the clusters are still able to produce an analog response that is directly proportional to the amount of signaling ligand and that can be regulated by the amount and lifetime of Ras-GTP. Because many other signaling pathways use lipid rafts and nanoclusters for organization, this analog-digital-analog readout may be shared by other signal transduction systems. (*Nature Cell Biol.*, advance online publication 8 July 2007, doi:10.1038/ncb1615) *MM*