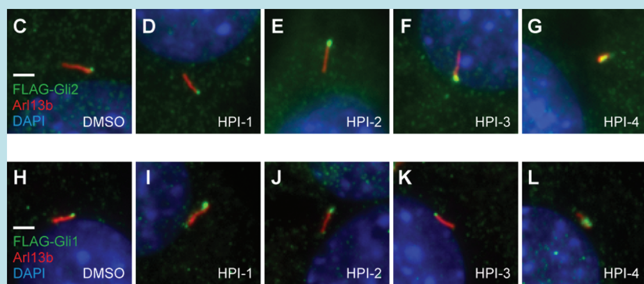
# Spotlight

## Tackling Hedgehog




Hyman, J. M., *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, DOI:10.1073/pnas.0907134106. Copyright 2009 National Academy of Sciences, U.S.A.

The Hedgehog (Hh) signaling pathway is a key regulator of cell growth, differentiation, and proliferation, especially in developing embryos. Initiated by the Hh family of secreted proteins, this pathway relies on numerous uniquely named proteins to control expression of various target genes. These include Patched1, which represses the G protein-coupled receptor-like protein Smoothened, which regulates the Gli family of transcription factors, which is negatively regulated by the nucleocytoplasmic protein Suppressor of Fused. Misregulation of this pathway has been implicated in various types of cancer, and while inhibitors of Smoothened have demonstrated promising anticancer activity, compounds that target the pathway downstream of this receptor may offer a more effective treatment strategy. Now, Hyman *et al.* (*Proc. Natl. Acad. Sci. U.S.A.*, published online August 5, 2009; DOI: 10.1073/pnas.0907134106) report the identification of four Hh pathway inhibitors that act downstream of Smoothened.

Over 120,000 compounds were screened for inhibition of Hh target gene expression induced by a Smoothened agonist. Four inhibitors, HPI1–4, were identified, and investigation into their mechanisms of action suggested that they act at the level of the Gli transcription factors. Probing further, it was determined that each inhibitor functions by a distinct mechanism. While HPI1 and HPI4 promote the proteolytic processing of Gli2 into a transcriptional repressor in Hh-stimulated cells, HPI2 and HPI3 appear to block the conversion of full-length Gli2 into a transcriptional activator. HPI4 also seems to act by disrupting the formation of primary cilia, where many proteins that participate in the Hh pathway are localized. In addition to their value as tools for dissecting the Hh pathway, these small molecules are intriguing leads for novel anticancer agents. **Eva J. Gordon, Ph.D.**

## Acting on Actin

Actin, one of the most abundant proteins in eukaryotic cells, is a fundamental component of how cells control their shape, internal structure, and motility. The actin-related protein (Arp)2/3 complex directs actin polymerization, but its precise role among the various other mechanisms for actin polymerization has not been elucidated. Nolen *et al.* (*Nature*, published online August 2, 2009; DOI: 10: 1038/nature08231) search for small molecules tools that could help elucidate Arp2/3 complex function, and identify two classes of novel Arp2/3 complex inhibitors.

Over 400,000 small molecules were screened using *in vitro* fluorescence assays in the search for inhibitors of actin polymerization triggered by the Arp2/3 complex. Two compounds were identified, CK-636 and CK-548, that reduced or prevented the formation of branched actin filaments compared to control samples. These compounds also reversibly decreased the formation of actin filament comet tails induced by bacterial infection in a human cancer cell line and prevented the formation of podosomes (actin-rich adhesive structures found in white blood cells), demonstrating their activity in live cells. Two additional inhibitors, one an analogue of CK-636 and the other a derivative of CK-548, were subsequently discovered, offering insight into the structural features of the compounds that are important for inhibition. To explore the mechanism of action of the compounds, crystal structures of Arp2/3 with CK-548 and CK-636 were solved. CK636 binds in a pocket between Arp2 and Arp3 and, based on the location of this pocket, likely locks Arp2/3 complex in an inactive conformation. The crystal structure data does not illuminate the mechanism of CK-548 quite as clearly, but it does offer some clues. For example, binding of the compound induces a large conformational change in Arp3, and this may disrupt one of the steps on the way to branch formation. These inhibitors will facilitate future studies of Arp2/3 complex function in live cells. **Eva J. Gordon, Ph.D.**

## The Dynamics of Post-transcription

Gene expression is an extraordinarily complex process that is regulated at multiple levels, including post-transcriptional events such as splicing, trafficking, stability, and translation of mRNA. Posttran-

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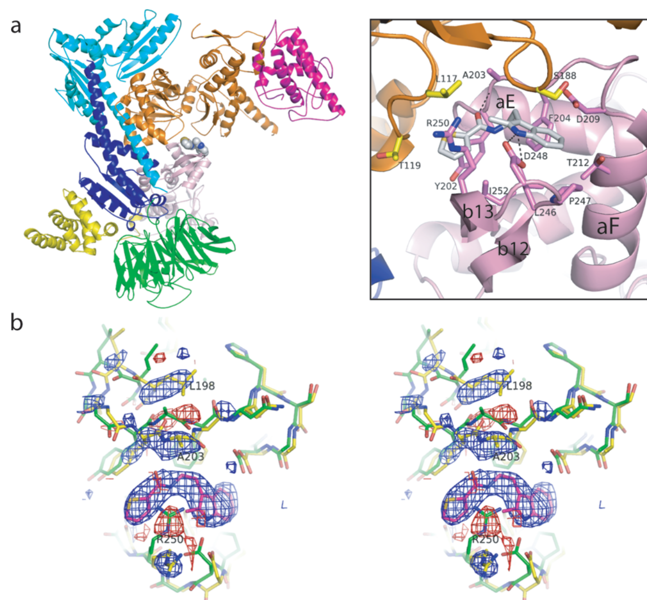


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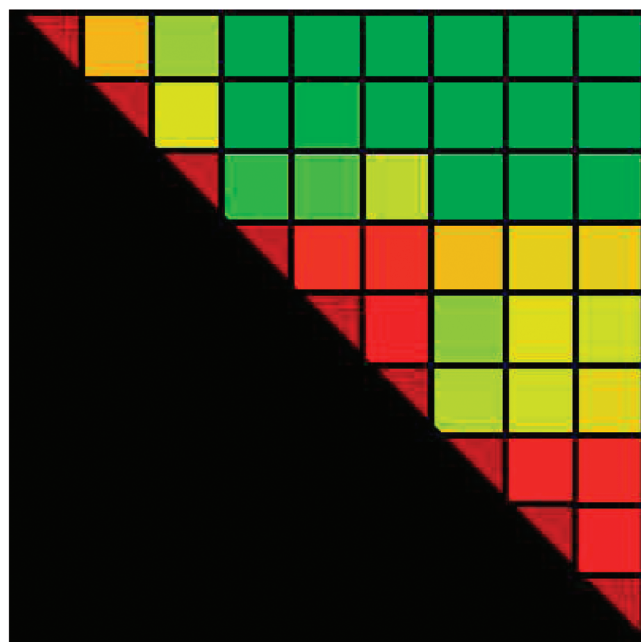
scriptional regulation is coordinated by ribonucleoprotein (RNP) complexes, which promote interactions between mRNA and post-transcriptional regulatory factors such as RNA-binding proteins (RBPs) and noncoding RNAs. The dynamics of RNP interactions have not been investigated on a global scale yet are undoubtedly critical in the control of post-transcriptional events and in the effects of such events on gene expression. Mukherjee *et al.* (*Mol. Sys. Biol.*, published online July 28, 2009; DOI: 10:1038/msb.2009.44) now present a strategy for exploring the dynamics of RNP interactions over the course of a critically important and fascinating process, the activation of T-cells.

As mRNA stability and splicing events are altered substantially during T-cell activation, post-transcriptional regulation is likely an important aspect of the regulation of immune function. Statistical modeling was combined with the “RIP chip” method, which employs ribonucleoprotein immunoprecipitation and microarray analysis to identify RNP complexes, to analyze the interactions of two RBPs, HuR and PABP, with mRNA during the mitogen-induced activation of T-cells. HuR was found to interact with various populations of mRNAs, dominated by those that encode proteins involved in the Wnt pathway, which is linked to numerous growth, development, and pathogenic processes, and in T-cell signaling. Notably, these RNP interactions were found to undergo substantial remodeling during T-cell activation. In addition, the HuR mRNA targets overlapped with the targets of other post-transcriptional regulatory factors, hinting at the existence of an intricate network of post-transcriptional regulatory events. Finally, the dynamics data were used to identify candidate small molecules that modulate post-



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transcriptional events. Indeed, the cyclooxygenase inhibitor resveratrol (present in red wine and famous for its antiaging effects) was found to alter the subcellular localization of HuR during T-cell activation. This points to a strategy for finding small molecules that can modulate the activity of the post-transcriptional machinery. **Eva J. Gordon, Ph.D.**

## Some Answers About Androgen

It is estimated that 1 in 6 men will be diagnosed with prostate cancer at some point during their lifetime. The pathogenesis of this very prevalent disease is intimately linked to the androgen receptor (AR), a member of the nuclear hormone receptor superfamily, which is typically activated by androgen ligands. However, advanced stages of prostate cancer are characterized by the switch from an androgen-dependent state to an androgen-independent state, yet evidence suggests that AR signaling pathways are still critical to the progression of androgen-independent prostate cancer (AIPC). Using gene expression profiling, Wang *et al.* (*Cell* 2009, *138*, 245–256) explore the role of the AR in AIPC.

The profiling experiments indicated that certain cell-cycle regulatory genes, specifically those directly involved in mitosis (M-phase), were upregulated in AIPC cells and that the AR selectively upregulates those genes to promote cancer cell growth. Identification of one of the upregulated genes, ubiquitin-conjugating enzyme E2C (UBE2C), was particularly intriguing due to its recent implication in inactivating the M-phase checkpoint. Indeed, in AIPC cells, two spe-

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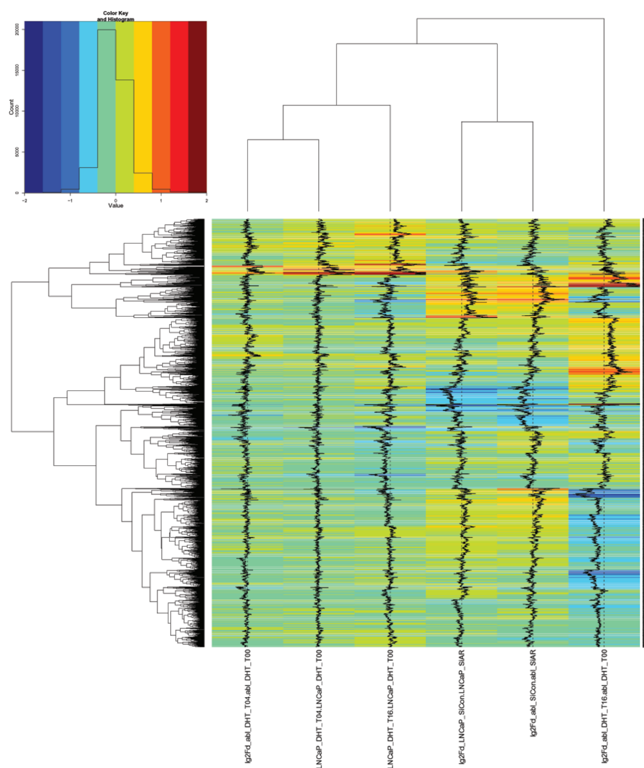
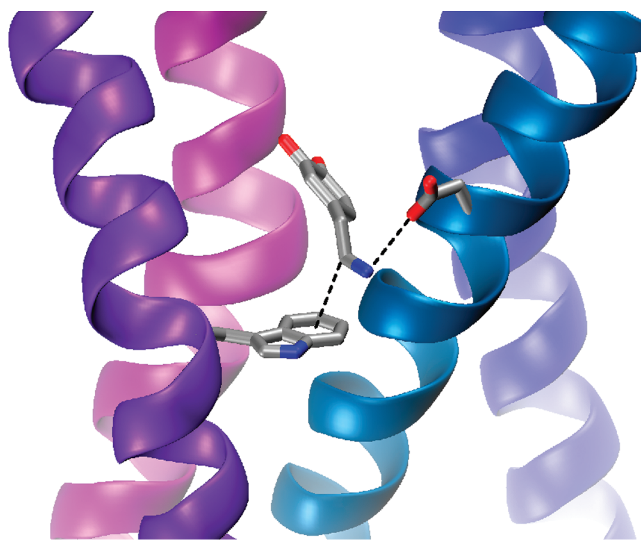


Reprinted from *Cell, 138*, Wang, Q., *et al.*, Androgen Receptor Regulates a Distinct Transcription Program in Androgen-Independent Prostate Cancer, 245–256, Copyright 2009, with permission from Elsevier.

cific UBE2C AR enhancer binding sites exhibited increased histone methylation and transcription factor binding, which in turn promoted increased AR binding, ultimately resulting in increased UBE2C gene expression. Thus, it appears that AIPC cells have cleverly managed to mastermind their own, epigenetically regulated method for proliferation even in the absence of androgen. These insights into how AIPC cells thrive is an exciting step forward toward the development of new strategies to combat prostate cancer. **Eva J. Gordon, Ph.D.**

## Probing GPCRs with Unnatural Amino Acids

G-protein coupled receptors (GPCRs) dominate transmembrane signaling in humans and represent a sizable proportion of drug targets for the pharmaceutical industry. Based on X-ray crystallography studies, a cascade of structural changes activates GPCRs, but the chemical mechanisms that underlie those chemical switches remain unclear. Unnatural amino acid mutagenesis and expression of those proteins within *Xenopus* oocytes provide a valuable tool for studying these proteins by modifying specific protein side chains and comparing their function with wild-type receptors. Because of the small amounts of modified protein produced in these experiments, researchers have typically designed such systems to study proteins with a measurable electrophysiological response, such as ion channels. However, Torrice *et al.* (*Proc. Natl. Acad. Sci., U.S.A.* 2009, *106*, 11919–11924) have now cleverly extended this method to GPCRs, developing a general system that they demonstrate for studying cation-π interactions.



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Because GPCRs are not ion channels, the researchers looked for the most direct secondary readout system. They monitored an ion channel directly downstream, the G protein-coupled, inward rectifying $K^+$ channel (GIRK). By optimizing the concentrations and timing of mRNA sequences injected into the oocyte in experiments with the M2 muscarinic acetylcholine receptor, they obtained reproducible quantitative results based on the GIRK response.

Substituting fluorine atoms for hydrogens on an aromatic ring deactivates that donor moiety in cation-π interactions. In previous studies of Cys-loop receptors, the researchers had established a mathematical relationship in cation-π interactions: increasing fluorine substitution on the aromatic ring also increases the dose of the small molecule needed to activate the receptor. The researchers incorporated a series of fluorinated tryptophan residues and phenylalanine residues at conserved locations in the M2 receptor and the D2 dopamine receptor. The W6.48 side chain of the D2 receptor showed a clear cation-π interaction with the dopamine ligand, but the comparable side chain in the M2 receptor did not. This interaction in the D2 receptor requires a rotation of the tryptophan side chain from a perpendicular orientation to a parallel one relative to the membrane, which supports the rotamer-switch model for activation of the receptor. **Sarah A. Webb, Ph.D.**