Spotlight

The Periwinkle Prescription

Image courtesy of Weerawat Runguphan.

Medicinal plants deftly choreograph the assembly of molecules, particularly complex natural products such as alkaloids, but with only a few exceptions halogenation remains outside their synthetic repertoire. Now, in a clever study, Runguphan *et al.* (*Nature* 2010, *468*, 461−464) have introduced chlorination enzymes from soil bacteria into a plant species leading to the production of a host of chlorinated and brominated natural products.

The researchers worked with *Catharanthus roseus*, the Madagascar periwinkle, a plant that produces more than 100 different indole alkaloids from the amino acid tryptophan. Previous research had shown that the plants could produce halogenated alkaloids when fed halogenated molecules. Therefore, the researchers suspected that if they could engineer these plants to include enzymes that tack chlorines onto tryptophan, they could produce halogenated alkaloids.

Runguphan *et al.* chose PyrH and RebH, soil bacteria enzymes that chlorinate the indole ring of tryptophan at the 5- and 7-positions, respectively. Vectors containing one of these enzyme genes along with the gene for a required reductase, RebF, were used to produce transgenic "hairy root" cultures of *C. roseus*. One of the downstream enzymes in the plant, strictosidine synthase, could not accept 5-chlorotryptamine. So the researchers added a previously reported engineered gene to the PyrH-RebF system. The researchers monitored alkaloid production using LC−MS and validated their results using authentic standards of the alkaloids.

This transgenic system successfully produced up to 26 μg of chlorinated alkaloid per gram of fresh plant. In the RebH-RebF system, the resulting chlorinated alkaloids had carbon structures that are not typically observed in alkaloids from wild-type *C. roseus*, suggesting that a different set of metabolic enzymes in the plant showed specificity for 7-chlorinated substrates. In low chlorine conditions supplemented with KBr, the RebH-RebF system also produced brominated alkaloids.

Halogens tend to boost the bioactivity of natural products. *C. roseus* already produces an important anticancer drug, vinblastine, and the availability of halogenated alkaloids through plants could provide a variety of new drug candidates. In addition, because halogens are easily modified, these structures could provide a gateway for the synthesis of other complex molecules. **Sarah A. Webb, Ph.D.**

Conformational Coaxing by Cytohesins

Transmembrane receptors use a variety of tactics for passing information from the outside of the cell to the cytoplasm. Many receptor tyrosine kinases sense a molecule outside of the cell and, in response, dimerize and autophosphorylate their own cytoplasmic domains. Phosphorylation leads to new proteins associating with the intracellular domain, with the net effect being up- or downregulation of subsequent signaling pathways. The epidermal growth factor receptor family, otherwise known as the ErbB family, are receptor kinases that respond to growth factors and affect many processes including differentiation and cell proliferation. As such, improper ErbB function has been implicated in numerous diseases and cancers, including the well-known Her2 receptor, which is amplified in some breast cancers. Now, a new player in the ErbB mechanism enters the field to add a new degree of complexity. Bill *et al.* (*Cell* 2010, *143*, 201−211) demonstrated that cytohesins, a family of guanine nucleotide exchange factor proteins that reside in the cytoplasm, are involved in enhancing a growth factor's signal, but from the inside of the cell.

Through a variety of inhibition and overexpression experiments in human cancer lines cells, the authors showed that cytohesins are

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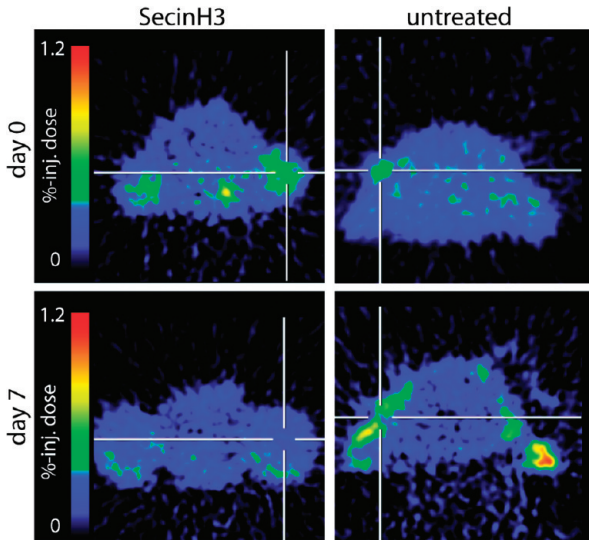

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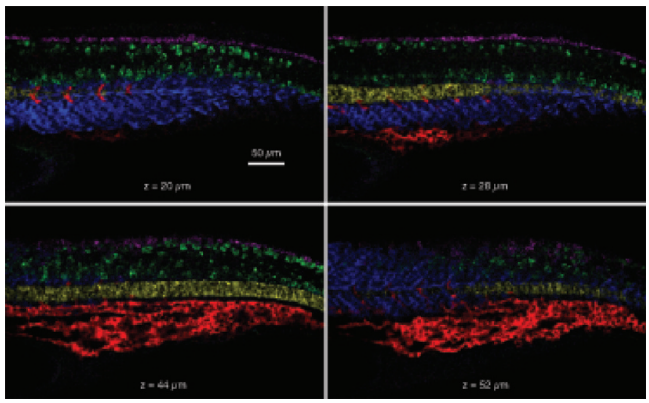
important activators of signaling through all of the ErbB receptors. The action of these cytoplasmic partners appears to be in binding to and directly enhancing the phosphorylation of the ErbB cytoplasmic domain. Cytohesins do not seem to physically enhance the dimerization event that occurs upon extracellular ligand binding but instead promote a conformational change in the already dimerized intracellular ErbB domains. Finally, the connection between cytohesins and ErbB signaling was investigated by correlating cytohesin levels with the phosphorylation levels of proteins known to be activated during epidermal growth factor receptor (EGFR) signaling. Indeed, human lung cancers showed a high correlation between expression levels of the cytohesins and the activation of both EGFR itself and signaling proteins downstream of EGFR. In fact, treating a human lung cancer cell line with SecinH3, a chemical inhibitor of cytohesins, actually inhibited growth of a tumor when the cells were placed into a mouse. These results demonstrate a new way that receptor signals are tuned in the cytoplasm and indicate a new possible target for drug intervention when ErbB signaling goes awry. **Jason G. Underwood, Ph.D.**

Multiple FISH in Fish

Each cell in a multicellular organism contains the same genetic material. Regulatory control of these identical genomes is the basis for cellular differentiation. Over the past few decades, fluorescent *in situ* hybridization has become a common tool used for the swift and precise visualization of nucleic acid molecules in a morphological context. *In situ* hybridization methods have revolutionized the mapping of mRNA expression patterns within intact biological samples; however, simultaneous mapping of multiple target mRNAs has proved challenging within intact vertebrate embryos, a significant limitation in attempting to study interacting regulatory elements in systems most relevant to human development and disease. Due to variability between biological specimens, accurate mapping of adjacent and overlapping regulatory loci requires multiplexed experiments in which multiple target mRNAs are imaged simultaneously within a single sample. Choi *et al.* (*Nat. Biotechnol.* 2010, *28*, 1208−1212) report the development of a robust, multiplexed fluorescent *in situ* hybridization approach based on orthogonal amplification using hybridization chain reactions. They demonstrate simultaneous mapping of five target mRNAs in a single intact zebrafish embryo.



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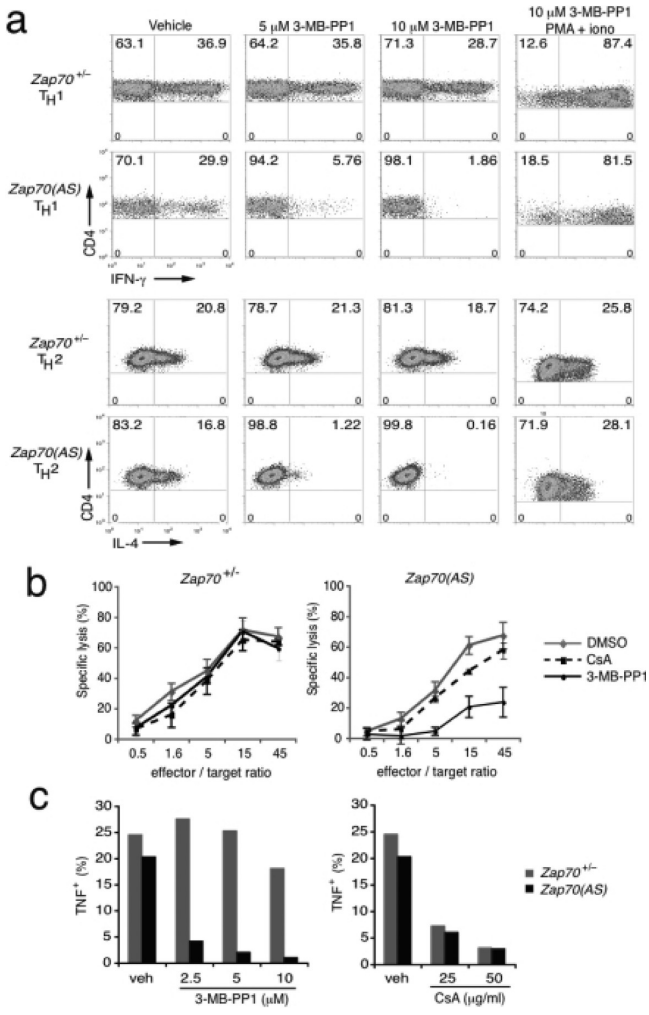
This method depends on two species of fluorescently labeled metastable RNA hairpins with regions of complementarity to each other. A third RNA probe base pairs to the mRNA target of interest, but a portion of this probe also binds to one species of the metastable RNA hairpins. The binding causes the hairpin to open and initiates a sequence of hybridization reactions among the metastable hairpins that leads to the assembly of fluorescently labeled "amplification polymers" tethered to the target mRNA. This amplification leads to a high signal-to-background ratio that allows for the accurate mapping of mRNA expression patterns within intact zebrafish embryos. Amplification occurs via a triggered self-assembly process at the site of mRNA targets, enabling deep sample penetration of small probes and amplification components. The amplification polymers remain tethered to the target mRNAs, yielding sharp signal localization. For multiplexed experiments, probes for different target mRNAs activate orthogonal hybridization chain reactions (labeled with spectrally distinct fluorophores) that operate independently within an embryo. As a result, mapping the expression patterns of five target mRNAs within a single sample requires no more time than mapping the expression pattern of a single target mRNA. **Jitesh A. Soares, Ph.D.**

Teasing Out Kinase Function in T Cells

Various types of T cells engage in a numerous activities that enable the immune system to function properly, including mounting a response to a previously encountered pathogen (memory T cells), producing cytokines to assist other immune cells in their functions (effector T cells), and suppressing T cell activity when it is no longer needed (regulatory T cells). These activities are triggered by activation of the T cell antigen receptor (TCR), which in turn initiates a collection of signaling cascades that are mediated in part by phosphorylation of the tyrosine kinase Zap70. The precise functions of Zap70 have been challenging to elucidate, in part due to a lack of small molecule inhibitors capable of selective Zap70 inhibition. Now, using a cleverly designed mutant Zap70 that is selectively inhibited by an analog of the small molecule kinase inhibitor PP1 called 3-MB-PP1, Au-Yeung *et al.* (*Nat. Immunol.* 2010, *11*, 1085−1092) explore the role of Zap70 in memory, effector, and regulatory T cells.

Using 3-MB-PP1 and cells from genetically engineered mice expressing the mutant Zap70, it was first demonstrated that T cell proliferation as well as early events in T cell activation, such as maintenance of increased calcium levels and activation of the mitogen-activated protein kinase pathway, rely on the catalytic activity of Zap70. In addition, Zap70 kinase activity was required for cytokine production by previously activated effector and memory T cells. Unexpectedly however, Zap70 kinase activity was not required for the suppressive activity of regulatory T cells. Further investigation revealed that Zap70 does participate in T cell suppression, but as a phosphorylated scaffolding protein that contributes to an integrin-dependent cell adhesion process that mediates T cell suppression. The elucidation of these specific functions of Zap70 points to potential advantages of Zap70 as a therapeutic target and importantly, this strategy can be broadly applied to tease out the functions of additional kinases in various other cellular processes. **Eva J. Gordon, Ph.D.**



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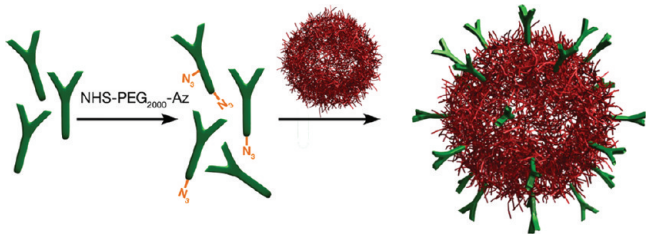
Clicking on Cancer Cells

The journey made by a drug as it travels through the body is wrought with distractions (such as other cells and tissues with compelling binding surfaces) and destructive forces (like hydrolytic enzymes and harsh pH environments). Drug delivery vehicles that can protect therapeutic agents from these surroundings while escorting them to their intended targets have the ability to greatly improve the effectiveness of the drug and minimize its adverse effects. To this end, Kamphuis *et al.* (*J. Am. Chem. Soc.* 2010, *132*, 15881−15883) now report a general method for the creation of polymer capsules that function as both drug delivery vehicles and cell targeting agents.



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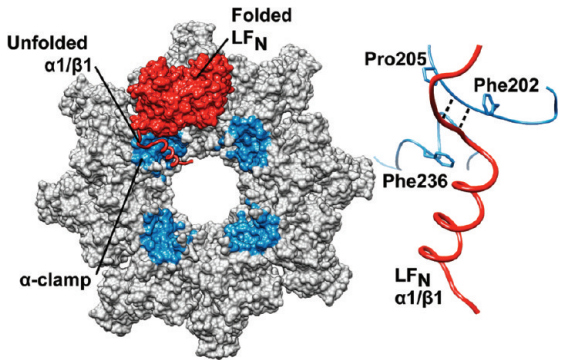
The method exploits the versatility and efficiency of click chemistry, in which a cycloaddition between an azide and an alkyne is facilitated by a copper(I) catalyst. Poly(*N*-vinylpyrrolidone) polymer capsules displaying a small amount of alkyne groups were coupled via click chemistry to azide-functionalized antibodies directed against the A33 antigen, a protein expressed on the surface of most colorectal cancer cells. The coupling reaction was assessed us-

ing flow cytometry, which demonstrated that the antibody was indeed incorporated on the capsule surface. Antibody activity and adsorption experiments further showed that the antibody was capable of binding A33 and that tens of thousands of antibodies were present on each polymer capsule. Cell binding assays and flow cytometry experiments next demonstrated that the antibody-functionalized capsules selectively and specifically bind only cells expressing A33, even when such cells constituted a very small percentage of the total cell population. This innovative approach for the nanoengineering of drug delivery vehicles capable of targeted cell delivery could be applied toward a variety of drug delivery and cell targeting strategies. **Eva J. Gordon, Ph.D.**

The Tale of Anthrax Unfolds

Thanks to vaccines and various eradication programs, anthrax is a relatively rare disease. However, there remains much to learn about the mechanism of attack used by this still dangerous infectious agent. Anthrax toxin invades its host through the use of a transmembrane protein-delivery system, which injects a cytotoxic enzyme called lethal factor (LF) into target cells. However, the molecular determinants that guide this process are not well-defined. Now, Feld *et al.* (*Nat. Struct. Mol. Biol.* 2010, *17*, 1383−1390) report the crystal structure of LF's amino-terminal domain in complex with its translocation machinery, a transmembrane translocase called protective antigen (PA).



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This structure of a PA octamer in complex with four LF PA-binding domains reveals the central core of a lethal toxin (LT) complex, which generally contains multiple copies of LF bound to a PA oligomer. Notably, Feld *et al.* observe that the LF domain partially unfolds upon binding the PA oligomer. The nature of the interaction between LF and PA was also extensively characterized using a variety of methods, including protein mutagenesis, cytotoxicity assays, assembly assays, and electrophysiology. Examination of the X-ray structure and biochemical and cellular data suggested that the polypeptide unfolding proceeds through nonspecific binding interactions in multiple locations, which is thought to stabilize the unfolding intermediates and facilitate the docking of the polypeptide chain into the cleft situated between two adjacent PA subunits, called the α clamp. The unfolded polypeptide chain is then directed toward the center of the translocation channel to a second catalytic active site, called the ϕ clamp. The nonspecific polypeptide binding ability of the α clamp is likely a key contributor to the proper assembly of the translocation channel, the unfolding of LF, and the translocation process. These insights into the anthrax modus operandi elucidate fascinating details about protein translocation mechanisms that further our understanding of both normal and pathogenic processes. **Eva J. Gordon, Ph.D.**