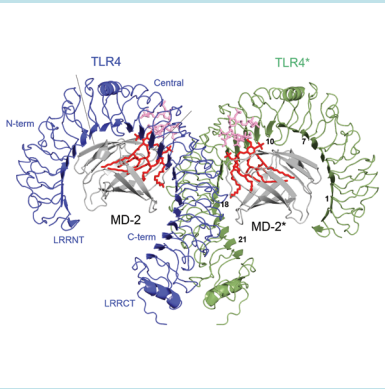
Spotlight

Displaced Activation



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Luckily for us, during the early stages of infection our immune system is primed to detect tiny amounts of a component of the outer membrane of certain bacteria called lipopolysaccharide (LPS). In turn, an immune response is launched to help protect us from further infection. LPS is recognized by two receptors on the surface of certain white blood cells, Toll-like receptor (TLR) 4 and myeloid differentiation factor 2 (MD-2), but the ligand specificity of the complex is not well defined. Park *et al.* (*Nature* advance online publication March 1, 2009; DOI: 10.1038/nature07830) now present the crystal structure of the TLR4–MD-2–LPS complex, revealing some remarkable mechanisms employed by the receptor to accommodate and differentiate distinct LPS molecules from diverse microorganisms.

The TLR4–MD-2–LPS complex is a symmetrical dimer, with the two TLR4 molecules adopting their characteristic horseshoe-like shape and the two MD-2 molecules in a β-cup fold structure, inside which sits a large hydrophobic pocket for LPS binding. Hydrophobic interactions between the lipid chains of LPS and hydrophobic residues in the receptors, as well as hydrophilic interactions between the hydroxyl and anionic groups of LPS and hydrophilic residues in the receptors, play key roles in the dimerization. The structural data also offer a compelling explanation for why certain antagonists with only four lipid chains do not activate the receptor but an LPS agonist from *E. coli* that has six lipid chains does. Apparently, the additional space required for the *E. coli* LPS lipid binding is generated by displacing the glucosamine backbone upward, which strategically locates the phosphate groups such that they can interact with a cluster of positively charged residues on the TLR4 molecules. This interaction in turn promotes dimerization and activation. These insights into LPS recognition might contribute to efforts to combat septic shock syndrome and other immunological diseases caused by a mis-regulated immune response. **Eva J. Gordon, Ph.D.**

Anticancer Vaccines Just Got Sweeter

A promising strategy in the fight against cancer is the development of vaccines designed to elicit an immune response against specific tumor-associated antigens. Many tumor cells display aberrant levels and patterns of carbohydrates on their surface, suggesting that vaccines displaying such carbohydrate antigens may be effective stimulators of the immune system. In addition, a family of glycoproteins known as mucins are also overexpressed on cancer cell surfaces, indicating that incorporation of such mucin-like features into vaccines may also enhance a tumor-specific immune response. Zhu *et al.* (*J. Am. Chem. Soc.* 2009, *131*, 4151−4158) now present the design and synthesis of an impressive new class of antitumor vaccines that encompasses both carbohydrate- and peptide-based elements.

Building off previous work in which vaccines displaying multiple carbohydrate-based tumor antigens were created, the new design also incorporates mucin-like peptides into the backbone of the vaccine. In the hopes of generating a vaccine targeting ovarian cancer, globotriaosyl ceramide (Gb_3) was selected as the carbohydrate antigen and MUC5AC was selected as the mucin antigen, as both of these structures are overexpressed on ovarian tumor cell surfaces. Key to the success of the synthetic approach was the preparation of a Gb_3-MUC5AC thioester cassette. This cassette was an efficient building block for the construction of the final vaccine, composed of multiple repeats of the carbohydrate and mucin antigens. This inspiring construction of a synthetic vaccine, which is currently undergoing immunological evaluation, is compelling evidence of the power of synthetic chemistry for the generation of unique therapeutic agents. **Eva J. Gordon, Ph.D.**

"Outside-the-Box" Nicotine Addiction

A clear picture of the molecular basis for nicotine addiction would be a valuable step toward preventing the more than four million smoking-related deaths that occur annually across the globe. Nicotine binds to the acetylcholine (ACh) family of receptors, which are located both in muscle and in the brain. In the brain, the α4β2 ACh receptors have been linked to nicotine addition, but the distinct interactions responsible for such physiological consequences have re-

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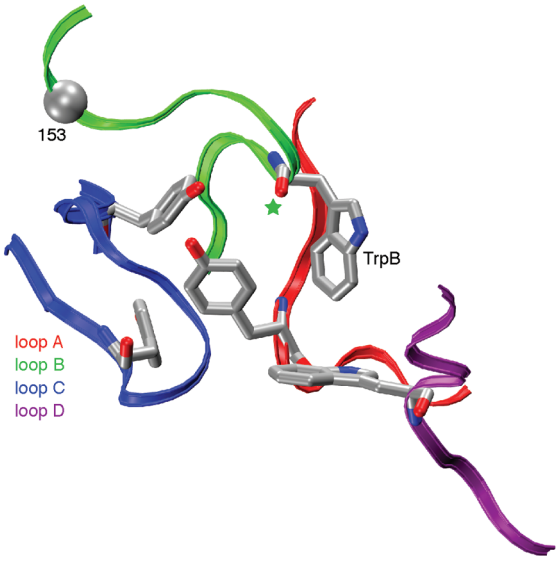
mained elusive. Xiu *et al.* (*Nature* 2009, *458*, 534–537) now demonstrate the remarkable role played by a cation-π interaction between nicotine and α4β2 receptors.



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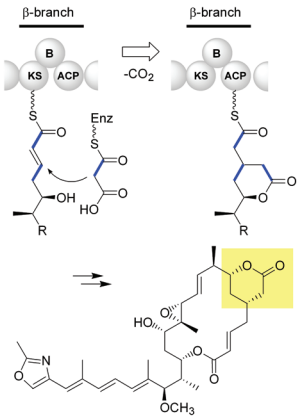
A common method for evaluating cation-π interactions is through the incorporation of fluorinated amino acid analogs into the receptor. Indeed, a decrease in cation-π energy for both acetylcholine and nicotine was observed when the tryptophan residue capable of the cation-π interaction in α4β2 was replaced with fluorinated analogs. Notably, a similar decrease was not observed in the analogous experiment using the muscle ACh receptor. Furthermore, a hydrogen bond between nicotine and the same tryptophan residue was found to be enhanced in α4β2 relative to the muscle receptor. Five residues comprise an aromatic box in which the cation-π and hydrogen bond interactions occur, and these residues are identical in both the brain and muscle receptors. However, additional mutagenesis experiments indicated that a single amino acid difference lysine in the brain receptor *versus* glycine in the muscle receptor–positioned "outside the box" but near the tryptophan alters the shape of the binding site such that nicotine interacts much more strongly with the brain receptor. These insights into the molecular basis of nicotine interactions in the brain will facilitate drug design efforts for nicotine addiction, as well as numerous other diseases for which ACh receptors are established targets. **Eva J. Gordon, Ph.D.**

Expanding the Polyketide Playbook

The polyketide rhizoxin, produced by bacteria that live inside the rice pathogenic fungus *Rhizopus microsporus,* is an antimitotic agent with a somewhat unusual structure. Polyketide biosynthesis typically begins with the generation of a linear carbon backbone that is decorated with branching units by various enzymes, resulting in side chains containing a wide range of functionality. Unlike the more common branching mechanism in which a carbon side chain is added α to a carbonyl group of the polyketide backbone, rhizoxin has a δ-lactone-containing carbon branch at a β position. Through close examination and manipulation of the rhizoxin biosynthetic gene cluster, Hertweck *et al.* (*Angew. Chem., Int. Ed.*, published online Mar 5, 2009; DOI: 10.1002/anie200900277) decipher the novel branching mechanism used in the biosynthesis of this structurally unique polyketide.



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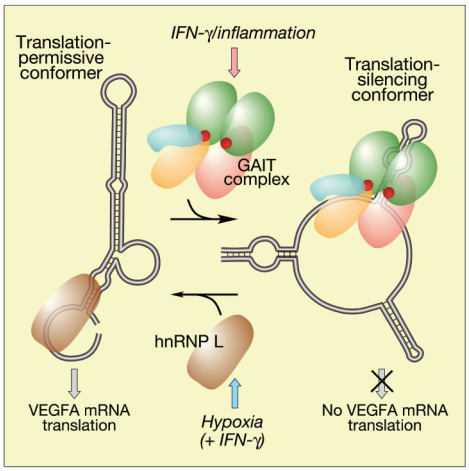
Polyketides that do contain alkylation at the β position all appear to use a similar set of enzymes to achieve the necessary carbon–carbon coupling, similar to those used in terpene biosynthesis. Intriguingly, the rhizoxin gene cluster did not contain any of those enzymes. Structural analysis of the intermediates generated after disruption of the rhizoxin polyketide synthase in the thioesterase and the β-branching domain enabled elucidation of the final steps in the biosynthetic pathway. It was determined that the δ-lactone branch is introduced through an unprecedented Michael-type addition of a malonyl unit to a Michael acceptor, likely followed by decarboxylation and nucleophilic attack by a hydroxyl group to yield the δ-lactone ring. Identification and manipulation of the genes involved in this unusual pathway establish the existence of another branching mechanism for polyketide biosynthesis. This expansion of the reaction playbook for polyketides adds yet another layer of complexity and versatility to the fascinating biosynthetic machinery responsible for generation of these important biomolecules. **Eva J. Gordon, Ph.D.**

Humans Flip a Riboswitch Too

Sensing the environment and adjusting gene expression to external cues are critical tasks for cells ranging from the tiniest bacterium

to the largest mammal. In the past 10 years, a large body of work indicates that bacteria and fungi can sense their environments with the help of riboswitches. These RNA modules respond to a metabolite and change their conformational status such that a nearby gene involved in the synthesis of that metabolite is adjusted up or down in transcription or translation efficiency. But could this type of mechanism be active in complex eukaryotes such as mammals? Now, a study by Ray *et al.* (*Nature* 2009, *457*, 915−919) answers this question with a resounding yes and a new mechanism for how RNA structure can modulate mammalian gene expression.



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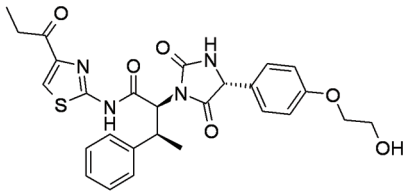
The study focused on the 3′ untranslated region (UTR) of an important human growth factor gene, VEGF. Under normal oxygen conditions, the protein displays weak translation, a property that was previously shown to be controlled by 3′ UTR sequences. Expression of VEGF is upregulated during hypoxic stress, such as when the cell is trapped within the confines of a tumor. By dissecting the RNA elements in the 3′ UTR, the authors found an interesting conformational switch. Instead of a small molecule ligand like the riboswitches, the VEGF RNA uses changes in protein concentration to switch between two states. Under hypoxic conditions, hnRNP L bound to an RNA element to promote a compact structural motif that favors translation of the VEGF RNA. Under normal oxygen conditions, hnRNP L was downregulated by protein degradation and the RNA formed a different conformation displaying low translational efficiency. Showing mutually exclusive binding with hnRNP L was the GAIT complex, an interferon-γ-regulated protein complex previously shown to downregulate VEGF translation. Thus, this 3′ UTR acts as a type of human riboswitch that integrates information from upstream signals, tweaks the level of key proteins, and unlocks RNA structures that turn gene expression on or off. Uncovering the first riboswitch in bacteria proved to be just scratching the surface, so mammalian cells may be the next frontier for this type of riboregulation. **Jason G. Underwood, Ph.D.**

Old Target, New Inhibitor

Despite tremendous research efforts toward the development of new cancer drugs, the prevalence of the disease coupled with the tendency for many cancers to acquire drug resistance underscores the urgent need for new anticancer agents, especially those that are effective against otherwise drug-resistant cancers. Many of the proteins in the mitogen-activated protein kinase (MAPK) signal transduction pathway are well-validated cancer targets, and several therapeutic agents targeting such proteins are in clinical trials, with varying degrees of success. Now, Daouti *et al.* (*Cancer Res.* 2009, *69*, 1924−1932) report the discovery of a RO497350, a novel small molecule inhibitor of the MAPK protein kinase MEK, and potential new anticancer drug.



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RO497350 was discovered through high-throughput screening efforts, immediately distinguishing itself from other MEK inhibitors by its chemical structure. In *in vitro* assays, it was shown to bind with high affinity to unphosphorylated MEK and to potently inhibit MEK kinase activity. In several cancer cell lines, the compound was a potent inhibitor of cell proliferation and was demonstrated to arrest cells in the G_1 phase of the cell cycle. Notably, RO497350 inhibited phosphorylation of ERK, the only known substrate of MEK, and also the phosphorylation of MEK, an activity unique to RO497350 and its analogs. Investigation into its mechanism of action confirmed its distinct properties; unlike other MEK inhibitors, RO497350 appears to prevent a feedback increase of MEK that is thought to contribute to development of drug resistance. Importantly, RO497350 also exhibited significant antitumor activity and was well tolerated in mouse cancer models. These studies offer compelling evidence for the promising applications of RO497350, both as a single agent and in combination with other anticancer drugs targeting cancers with MAPK pathway aberrations. **Eva J. Gordon, Ph.D.**