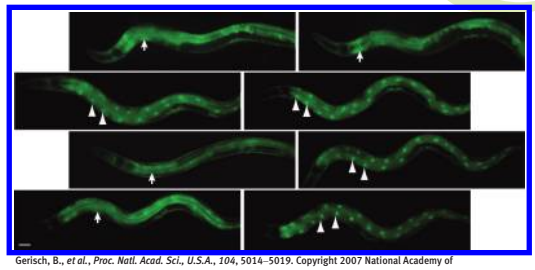
Spotlight

How Worms Age



Gerisch, B., *et al., Proc. Natl. Acad. Sci., U.S.A., 104*, 5014–5019. Copyright 2007 National Academy of Sciences, U.S.A.

We all have a vested interest in understanding the aging process. It is known that hormone-secreting endocrine systems in animals from worms to humans play crucial roles in growth and development processes and also in the response to changing environmental conditions. However, the role of hormones in regulating longevity is less well understood. Gerisch *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2007, *104*, 5014–5019) now provide compelling evidence that bile-acid-like steroids regulate the life span of the nematode *Caenorhabditis elegans*, a finding that may have implications in the mammalian aging process as well.

During *C. elegans* development, the nuclear receptor DAF-12 (which is homologous to the vitamin D and liver X receptors (LXR) in vertebrates) controls the worm's decision to either continue reproductive growth or pause at a long-lived larval stage called the dauer diapause. DAF-12 is activated by 3-keto bile acid-like steroids called dafachronic acids. It is thought that the interaction between DAF-12 and its ligand is necessary for normal life span, whereas activity of the receptor in the absence of ligand results in a longer life span. Indeed, the authors determined that the presence of Δ^4-dafachronic acid restored normal life spans to various long-lived *C. elegans* mutants in a DAF-12-dependent manner. In addition, consistent with the notion that increased longevity often correlates with stress resistance, hormone-deficient worms were more resistant to heat and oxidative stress. The authors suggest that increased longevity in the absence of ligand could reflect the animal's response to stress conditions, such as when steroid is not available. Notably, hormonal effects on the germ-line longevity pathway, in which life span is influenced by signals originating from the gonad and the absence of germ-line stem cells leads to longer life, were also examined. In contrast to the findings related to dauer signaling pathways, mutants without germ-line cells required Δ^4-dafachronic acid to restore longer life span. The apparent differing roles of the hormone in these pathways suggest that the signaling state of the animal has profound effects on the aging process. **Eva J. Gordon, Ph.D.**

MicroRNAs Go to the Oncologist

MicroRNAs (miRNAs) are small regulatory RNAs that affect the stability or translational efficiency of their target messenger RNAs (mRNAs) by forming base-pairing interactions. Using both computational and biochemical methods, researchers have uncovered hundreds of miRNAs in humans in the last several years. Because the interactions between the miRNAs and their targets involve just a short region of perfect base-pairing complementarity, the miRNAs have many predicted targets, and finding those that are causing a particular biological effect is challenging. Another recent quandary in the miRNA field is whether loss of binding sites on a particular mRNA can have significant consequences on the cell. Now, a study by Mayr *et al.* (*Science* 2007, *315*, 1576–1579) weaves together both target prediction and the effect of a miRNA on a particular gene with a disease link.

The gene, HMGA2, normally encodes a protein involved in chromatin maintenance. A number of tumor cells display a chromosomal translocation in HMGA2 that truncates the 3′ end of the protein-coding region and also eliminates the 3′ untranslated region (UTR). An earlier computational screen found that the 3′ UTR of human HMGA2 encodes seven elements with high complementarity to one particular miRNA, *let-7*. This led to an interesting question: is the oncogenic transformation from HMGA2 due to protein truncation or from misregulation of the mRNA when it lacks the *let-7* binding sites? To test the miRNA hypothesis, the authors made a

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Targeting the Other Cell Wall Enzyme

Targeting the enzymes that build the bacterial cell wall is a well-established and valid strategy for antibiotic design, but a disconcerting number of bacterial strains have grown resistant to the existing antibiotics of this class. The cell wall is constructed by transpeptidase enzymes, which are the target of the β-lactam group of antibiotics, and the GT_{51} family of peptidoglycan glycosyltransferase enzymes, for which no clinically approved inhibitors have yet been developed. GT_{51} generates peptidoglycan polymers from a lipid II-linked pentapeptide substrate, and molecules that inhibit this activity could be promising new antibiotics. Now, Lovering *et al.* (*Science* 2007, *315*, 1402–1405) present the crystal structure of penicillin-binding protein 2 (PBP2), a bifunctional enzyme containing separate transpeptidase and glycosyltransferase activities, providing structural insight into this relatively uncharted territory.

The structure of PBP2 was determined alone and in complex with moenomycin, the only well-characterized GT_{51} inhibitor. The GT_{51} domain is mostly α-helical and contains a globular "head" region and a smaller "jaw" region, each of which possesses a conserved glutamic acid residue. The structure is most similar to the bacteriophage λ lysozyme (which hydrolyzes peptidoglycan) yet strikingly dissimilar to the structures of other glycosyltransferases. The unliganded and moenomycin-bound structures offer provocative insights into both the mechanism of the polymerization reaction and the mechanism of inhibition by moenomycin. For example, it was previously hotly contested whether lipid II is the donor or acceptor in the polymerization reaction, but these structures provide compelling evidence that lipid II is the acceptor and the growing chain is the donor. In addition, scrutiny of the differences between the structure of moenomycin and the natural GT_{51} substrate revealed the interactions that likely play key roles in enzyme inhibition. This study lays the groundwork for future development of a new class of antibacterial drugs. **Eva J. Gordon, Ph.D.**

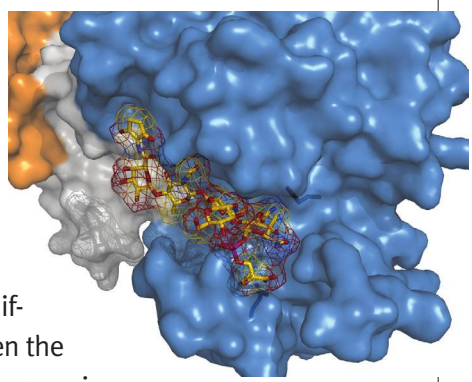

Image courtesy of A. L. Lovering and N. C. J. Strynadka.

MicroRNAs Go to the Oncologist,

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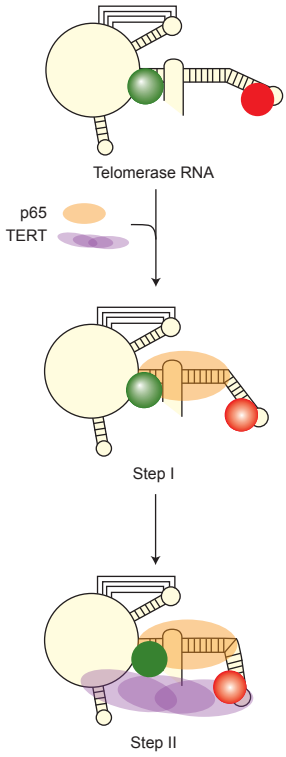
number of HMGA2 minigenes that deleted part of either the protein-coding region or the UTR region or simply mutated the *let-7* binding sites in the UTR. When these minigenes were introduced into mammalian cells, the HMGA2 protein levels were down-regulated in the presence of *let-7* miRNA, and the UTR sites were required for this effect. When the miRNA regulation was disrupted, it caused overexpression of the protein and higher colony formation, indicative of oncogenic transformation. The same cells injected into nude mice formed tumors more readily than those with proper *let-7* regulation. It is interesting that truncating the protein-coding region while maintaining the *let-7*-regulated UTR showed very low oncogenic potential. This study vindicates the computational efforts to find good targets for miRNAs, and it shows how one particular miRNA–mRNA interaction can have a profound effect on a cell's fate. Jason G. Underwood, Ph.D.

RNPs Made to Order

Ribonucleoproteins (RNPs) are at the heart of numerous cellular processes, including pre-messenger RNA splicing, translation, signal recognition, and telomere maintenance. For years, RNPs have been purified, cataloged, inventoried, and characterized, but as in the kitchen, just knowing the ingredients does not yield the complete finished product. How RNPs assemble from their protein and RNA components is a pivotal question, and methodologies for studying this difficult problem have often proved dubious. In a recent study, Stone *et al.* (*Nature* 2007, *446*, 458–461) use a clever biophysical technique to take on an important eukaryotic RNP enzyme in a purified system.



The subject of interest, telomerase, is an enzyme involved in maintaining the repetitive sequence elements found at the termini of chromosomes. With its RNA template and a reverse transcriptase protein, TERT, this RNP enzyme catalyzes nucleotide addition onto the telomere. As with most RNPs, the telomerase case is not so simple. Additional factors are bound to the telomerase RNA, and it is thought that many of these act to chaperone the RNA–protein interactions that are critical for telomerase function. To study the mysterious road to assembly, Stone *et al.* made use of the well-characterized *Tetrahymena* telomerase RNA and two of its binding partners, TERT and p65. The RNA was specifically labeled with two separate fluorophores, and the distance between the two was monitored by FRET. The proteins could then be added singly or in combination, and the relative conformation of the RNA helices monitored by FRET. Monitoring the folding orientation of the RNA in real time revealed a hierarchical assembly mechanism. The p65 protein appears to bind first and induce an RNA structure that in turn promotes the binding of the catalytic TERT. After RNAs labeled at various positions were tested, an essential bulge structure in one of the stems of telomerase RNA was shown to act as a hinge upon protein binding. After the authors tested RNAs labeled at various positions, it was shown that an essential bulge structure in one of the stems of telomerase RNA acts as a hinge upon protein binding. This study demonstrates the power of the FRET system in watching how complexes assemble and hints that viewing even more complicated RNP assembly might be around the corner. Jason G. Underwood, Ph.D.

An Elusive Enzyme Exposed

Telomerase is a ribonucleoprotein enzyme that uses a built-in RNA template to guide DNA synthesis at the ends of eukaryotic chromosomes. The human enzyme has been linked to cancer, aging, and a number of genetic diseases over the last 20 years. Because this specialized reverse transcriptase functions at just 46 chromosomal ends in a typical human cell, the number of copies of telomerase is vanishingly low. This has made purification and a complete characterization of the human enzyme particularly challenging. Dozens of proteins were thought to be telomerase-associated, but stoichiometry and precise composition remained a mystery. Now, Cohen *et al.* (*Science* 2007, *315*, 1850–1853) take on the human telomerase with an impressive new approach by purifying the enzyme complex from an immortal human cell line.

Using gradient sedimentation, the authors estimated the telomerase size at 650 kDa in five cancer cell lines and in embryonic kidney cells. The latter was chosen as the starting material for a highly specific three-step purification. One step even exploited the catalytic property of telomerase to ensure that only the active enzyme was purified. A partially purified telomerase fraction was bound to an immobilized DNA corresponding to the mammalian telomeric repeat. This interaction is highly stable, with a dissociation rate of 12 h at RT. After unbound factors were washed away, the enzyme was activated with deoxynucleoside triphosphates. After a telomeric repeat was added by telomerase, it then fell off the telomere. This complex has a dissociation time of just a few minutes. The final product was highly pure and, surprisingly, only two proteins were found with the telomerase RNA: the expected reverse transcriptase, hTERT, and another known subunit, dyskerin. The researchers attribute the size of the complex to two copies of each of the three molecules and estimate the telomerase count in this cell line to be ~20–50 molecules per cell. This study opens up the potential for studies on catalytically active telomerase in a more pure system. It also begs the question of what the other telomerase-associated proteins are doing in the cell if they are not part of the active holoenzyme. **Jason G. Underwood, Ph.D.**

acs chemical biology

Targeting the Mutants

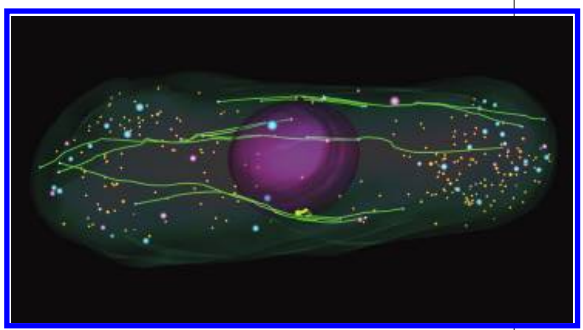
The ideal cancer treatment would kill all cancer cells and leave healthy cells untouched. Molecular targeted therapy, in which a drug is specifically designed against a molecule known to contribute to the progression of the cancer, holds much promise toward this goal. However, targeted therapy requires that the molecules involved be identified and characterized sufficiently for the design of effective drugs. To this end, Yun *et al.* (*Cancer Cell* 2007, *11*, 217–227) report structural and kinetic insight into the interactions between wild-type epidermal growth factor receptor (EGFR) and EGFR mutants implicated in non-small-cell lung cancer with several EGFR inhibitors.

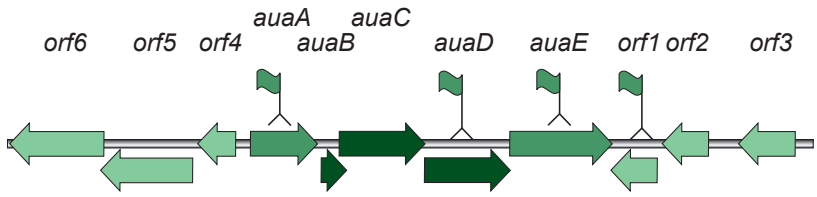
X-ray crystallography and various kinetic assays provided intriguing information about the relationships between EGFR, EGFR mutants, and EGFR inhibitors that also sheds light on observations in the clinic. Wild-type EGFR and two EGFR mutants, L858R and G719S (which are both found in non-small-cell lung cancer patients), were examined with several EGFR inhibitors, including gefitinib (marketed as Iressa), AEE788, and the staurosporine analogue AFN941. The authors first determined that the mutant kinases were 10–50× more active than the wild-type kinase. The structural basis for this was apparent upon examination of the crystal structure, because the mutations destabilized the inactive conformation of the enzyme. In addition, examination of the inhibitor complexes revealed similar binding interactions between wild-type and mutant proteins, except in the case of AFN941, which had a marked rotation in the binding cleft of the G719S mutant. However, kinetic analysis revealed that gefitinib and AEE788 bind more tightly to the mutant enzymes than to wild-type protein. These striking observations provide insightful details into the molecular basis for correlations between the responsiveness of cancer patients harboring specific mutations and particular drugs. Moreover, this information will facilitate the design of future generations of molecular targeted agents. **Eva J. Gordon, Ph.D.**

Microtubules in 3D

Without microtubules, cells would have a tough time maintaining their shape or moving around. Although countless molecular and dynamic characterizations of these fascinating structures have been conducted, a detailed understanding of microtubule length, polarity, distribution, and the nature of microtubule association with other cellular structures has been limited by the scope of the methods (such as light, fluorescence, or electron microscopy) used to investigate them. Höög *et al.* (*Dev. Cell* 2007, *12*, 349–361) now use electron tomography to characterize microtubule architecture, uncovering structural details not possible to discern with traditional methods.

Electron tomography provides detailed 3D images of macromolecular structures. Using this technique, the authors reconstructed a complete fission yeast cell, revealing several remarkable features of microtubules. For example, they determined that the cell contained 16 microtubules arranged in 3 bundles, corresponding to a total length of 34.5 μm and concentration of 2.78 μM of polymerized tubulin in the cell. In addition, the resolution was sufficient to determine that most microtubules were open at one end and capped at the other, convincing evidence of their polarity. New insights into the connections among microtubules with each other and with other structures, such as the nuclear envelope, mitochondria, and vesicles, were also revealed. For instance, the authors found that microtubules were connected to each other and to the nuclear envelope *via* electron dense filamentous bridges. In addition, microtubules were often found to be bent around or toward mitochondria. In contrast, little evidence existed of any association of microtubules with vesicles. Future applications of electron tomography to the study of whole cells should provide many additional exciting revelations about the architecture of the cell. **Eva J. Gordon, Ph.D.**





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Myxobacteria Mix It Up

Myxobacteria are a soil-dwelling Gram-negative bacteria that often produce a distinctive mix of biologically active secondary metabolites. The aurachins are a unique family of quinoline alkaloid compounds that have structural similarities to vitamin K and are known inhibitors of electron transport in the respiratory chain. They also share structural features with isoprenylated quinoline antibiotics and 4-hydroxy-2-alkylquinolines and thus may possess potential antibiotic activity or cell signaling properties as well. Despite the rich biological potential of the aurachins, little was known about their biosynthesis. Sandmann *et al.* (*Angew. Chem., Int. Ed.* 2007, *46*, 2712–2716) now report the characterization of the aurachin biosynthetic pathway, including the surprising involvement of a type II polyketide synthase (PKS).

The authors employed HPLC/MS to screen a 4096-member transposon mutant library in their hunt for genes involved in aurachin biosynthesis. A gene cluster with five open-reading frames was discovered, and genes with high similarity to a prenyltransferase, a type II PKS system, and a benzoate-CoA ligase were identified. Notably, this is the first known example of a type II PKS system in a Gram-negative bacterium. These enzyme complexes typically contain an acyl carrier protein, a ketosynthase responsible for iterative condensation of malonyl-CoA units, and a chain length factor responsible for decarboxylation of the starter unit and determination of chain length. The presence of these genes in the aurachin gene cluster, along with previous evidence suggesting that the starting unit of the aurachins is anthranilate, enabled the authors to propose a biosynthetic pathway for aurachin D. Anthranilate is converted to anthraniloyl-CoA by the benzoate-CoA ligase, transferred to the type II PKS enzymes for extension with two malonyl-CoA units, and finally delivered to the prenyltransferase for addition of the isoprenoid side chain. It is interesting that on the basis of the sequence analysis of several type II and III PKS systems from bacteria and plants, the authors speculate that the aurachin PKS is undergoing an evolutionary transition from type II into type III PKS and/or FABs, or *vice versa*. **Eva J. Gordon, Ph.D.**

No Protection Needed

The synthesis of structurally complex molecules typically employs the temporary installment of protecting groups to mask functional groups in the compound that would otherwise fall prey to the reaction conditions. In fact, the use of protecting groups has become so common that suggesting otherwise seems somewhat radical in nature. In practice, however, protecting group manipulations are often easier said than done, and they add at least two steps to the synthesis. Baran *et al.* (*Nature* 2007, *446*, 404–408) develop a strategy for vastly improving the efficiency of the synthesis of complex molecules, in large part by eliminating the use of protecting groups.

The authors present several guidelines based on known principles in organic chemistry for improving the efficiency of organic syntheses. For example, focusing on maximizing the number of carbon–carbon bond-forming reactions, linearly escalating the overall oxidation state of the intermediates through the synthesis, using cascade reactions where possible, and exploiting the innate reactivity of existing functional groups can collectively result in a dramatically more

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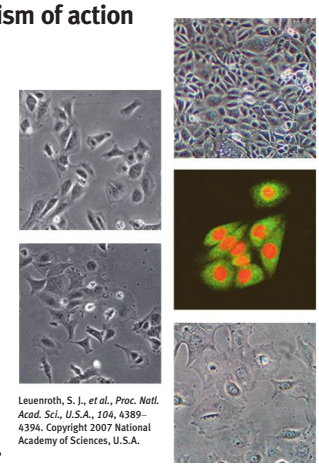
Exploring the "Thunder God Vine"

Triptolide is a natural product isolated from the Thunder God Vine, or *Tripterygium wilfordii* Hook F, a vine that has been used in traditional Chinese medicine for centuries. A diterpene with anticancer and anti-inflammatory properties, triptolide's mechanism of action is not well understood, as is the case for many substances used in Chinese medicine. Examination of how traditional medicines such as those in the Thunder God Vine work would enable more effective treatment strategies with fewer side effects. Now, Leuenroth *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2007, *104*, 4389–4394) discover a molecular target of triptolide and report its therapeutic potential in the treatment of polycystic kidney disease.



Leuenroth, S. J., *et al.*, *Proc. Natl. Acad. Sci., U.S.A.*, *104*, 4389–4394. Copyright 2007 National Academy of Sciences, U.S.A.

Using ^{3}H-labeled triptolide and extensive chromatographic purification, the authors isolated the calcium channel polycystin-2 (PC2) as a potential triptolide target. Regulation of calcium levels by PC2 is important during kidney organogenesis, and the genetic disorder autosomal dominant polycystic kidney disease (ADPKD), characterized by formation of fluid-filled cysts, is the result of misregulation of this process. Using various cell lines with altered PC2 expression, the authors demonstrate that exposure of cells to triptolide results in calcium release that is dependent on the presence of PC2. The calcium signaling turned on by triptolide is important because it leads to cell growth arrest, which ultimately prevents growth of the cysts. It is encouraging that treatment with triptolide reduced cyst formation in a mouse model of ADPKD, without adverse effects on kidney development. These results suggest PC2 as a promising target for ADPKD and triptolide as a promising candidate for ADPKD treatment. The studies presented here exemplify the value in exploring the mechanisms behind the effects of traditional medicines. **Eva J. Gordon, Ph.D.**

No Protection Needed, *continued from page 208*

efficient synthesis. The total syntheses of four natural products, hapalindole, fischerindole, welwitindolinone, and ambiguine, were executed according to these principles, and the benefits of the approach were impressive, to say the least. Enantioselective construction of hapalindole U was accomplished in just 8 steps without the use of a single protecting group, whereas previous attempts required 20 steps and multiple protecting groups just to arrive at the racemic compound. Hapalindole U was then efficiently and cleverly converted to structurally related ambiguine H by exploiting the natural reactivity of the functional groups that would normally be protected in such a transformation. In the syntheses of fischerindole and welwitindolinone, elimination of protecting groups and careful reaction optimization also led to considerably improved yields of enantiomerically pure material. Given the challenges associated with the synthesis of complex biologically active compounds, application of these principles should increase our access to such molecules for biological and medicinal purposes. **Eva J. Gordon, Ph.D.**