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SPOTLIGHT

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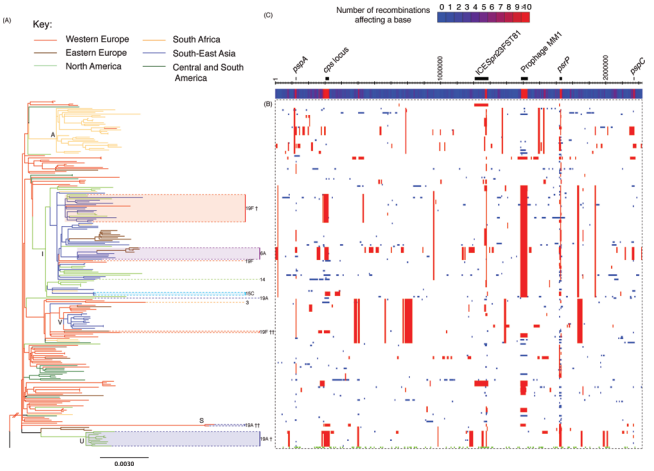
The Evolution of Resistance

The alarmingly rapid emergence of microbial pathogens that have acquired drug and vaccine resistance has fuelled exploration into strategies for the discovery of effective new treatments, as well as investigations into the mechanisms that underlie the acquisition of resistance. Indeed, delineation of the genetic changes that accompany drug and vaccine resistance may enable the development of new strategies to target drug-resistant strains. To this end, Croucher *et al.* (*Science* 2011, *331*, 430−434) examined the genome sequences of 240 isolates of *Streptococcus pneumoniae*, a bacteria capable of causing numerous, potentially life-threatening infections including pneumonia and meningitis, and offer insight into how the genomic plasticity of this shrewd pathogen facilitates its rapid adaptation to clinical interventions.

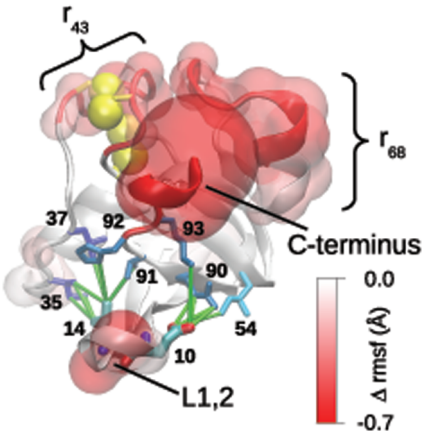


From Croucher, N. J., *et al.*, *Science*, 2011, *331*, 430. Reprinted with permission from AAAS.

Lineages derived from the first recognized multidrug-resistant *S. pneumoniae* clone, called Pneumococcal Molecular Epidemiology Network clone 1, have been found throughout the world. Sequencing of 240 members of this global collection enabled construction and detailed analysis of the phylogeny. Over 57,000 single-nucleotide polymorphisms, including 702 recombination events, were identified. Close examination revealed that the sequence variations likely arose primarily through incorporation of imported DNA, not the accumulation of base substitutions, and the sequence changes often affected genes that encoded major antigens. Further analysis suggested that the human immune system drives increased recombination at specific loci, and that *S. pneumoniae* has distinct responses to different anthropogenic pressures. For example, changes in serotype (subgroups defined by cell surface antigen expression) that follow vaccine introduction appear to result from the depletion of the resident population and expansion of preexisting capsular variants, as opposed to a change in capsule type. In contrast, evolution of resistance to various antibiotics, including fluoroquinolones, rifampicin, and macrolides, occurred on numerous occasions and involved supplementation or replacement of the affected gene. These insights into the remarkable adaptability of this organism will inform efforts to control future, drug-resistant *S. pneumoniae* strains and guide investigations of other drug-resistant pathogens. **Eva J. Gordon, Ph.D.**

Networking within a Protein

Proteins are dynamic macromolecules that carry out many important functions within a cell. Proper execution of these functions is typically dependent on fluctuations in protein folding between conformations that support catalysis and those that stabilize complex formation. Factors such as phosphorylation, cofactor or ligand binding, etc. can provide the switch from an inactive to an active protein conformation. However, not much is known about the internal networks of amino acid residues in proteins and the role they play in controlling protein function. To learn more about the role of internal networks on modulating protein function, Nechushtai *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2011, *108*, 2240−2245) studied the influence of a loop region distant from the redox active iron−sulfur cluster on the overall structure and function of plant ferredoxin.



Nechushtai, R., *et al.*, *Proc. Natl. Acad. Sci., U.S.A.*, *108*, 2240−2245. Copyright 2011 National Academy of Sciences, U.S.A.

Iron−sulfur proteins perform an essential function in several key processes such as photosynthesis, nitrogen fixation and respiration. Ferredoxins contain iron−sulfur cluster(s) with very low redox potentials (up to −450 mV), which mediate some of the most reductive reactions in the cell. The plant-type ferredoxin (Fdx) has a single surface-exposed [2Fe-2S] iron−sulfur cluster, which serves as an electron donor in several essential metabolic pathways. This single domain protein contains a flexible loop approximately 20 Å away from the iron−sulfur cluster. The truncation of this loop to a rigid β-turn resulted in minimal alteration to the global structure of the protein as determined by X-ray crystallography. Additionally, the thermal stability of the mutated Fdx remained unaffected as observed by thermal-dependent circular dichroism. However, protein-film voltammetric experiments showed that despite minimal disruptions in the structure, the redox potential of the mutated Fdx had changed by ∼57 mV relative to the wild-type protein. All-atom structure-based

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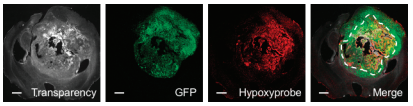
simulation revealed dynamic coupling between the distantly situated flexible loop and iron−sulfur cluster regions. This remarkable observation suggests intrinsic communication channels that connect active site centers to distal sites in single-domain proteins through a network of short-ranged interactions. **Jitesh A. Soares, Ph.D.**

Glioblastoma-Derived Endothelial Cells May Thwart Antiangiogenesis Therapies

The aggressive form of brain cancer, glioblastoma multiforme, has confounded cancer researchers with its resistance to many therapies. Particularly puzzling is glioblastoma's limited response to drugs that target vascular endothelial growth factor (VEGF): these tumors undergo angiogenesis, forming characteristic tufts of endothelial cells and blood vessels that feed these fast-growing tumors. But research in other cancers such as lymphoma, myeloma, and breast cancer has recently shown that endothelial cells can differentiate from tumor cells, not just from normal cells. Now Soda *et al.* (*Proc. Natl. Acad. Sci. U.S.A.*, published online January 24, 2011, DOI: 10.1073/pnas.1016030108) demonstrate that glioblastomas also produce tumor-derived endothelial cells (TDECs), which may explain this resistance to anti-VEGF drugs.



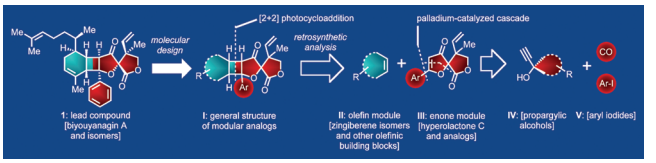
Soda, Y., *et al. Proc. Natl. Acad. Sci., U.S.A.*, DOI: 10.1073/pnas.1016030108. Copyright 2011 National Academy of Sciences, U.S.A.

The researchers started by studying a mouse model of glioblastoma that expresses GFP in the tumor cells. They found a subset of cells that expressed GFP as well as several angiogenic growth factors. These tumor derived cells form functional blood vessels, and such cells arise even when a tumor is transplanted into a mouse. Most of these TDECs were found deep within the tumors, suggesting a role of hypoxia and the activation of HIF-1 in the transdifferentiation of TDECs from the tumor. Not only did the researchers confirm that TDECs are formed in response to HIF-1α, they also found that the process was independent of VEGF. These cells proved to be resistant to VEGF receptor inhibitors, suggesting that these cells may play a role in the VEGF resistance in glioblastomas. Soda *et al.* confirmed that these cells are present in human glioblastoma tumors.

In melanoma and colon cancer, fluid connecting channels can be formed that do not contain endothelial cells, but these cells formed by glioblastomas express known endothelial cell markers. Because this process is independent of VEGF, it could be a mechanism by which tumors acquire resistance to drugs that target VEGF. As a result, treatments may require new combinations of cancer therapies. **Sarah A. Webb, Ph.D.**

Designed, Naturally

The discovery and characterization of novel natural products and the design of innovative synthetic compound libraries are key strategies for supplying our drug arsenal with new and improved medicines for all kinds of diseases. The inherent structural complexity and biological activity of many natural products often inspire the design of synthetic analogs with improved selectivity and pharmacokinetic properties, thus bridging these important drug discovery approaches. Nicolaou *et al.* (*Proc. Natl. Acad. Sci. U.S.A.*, published online January 18, 2011, DOI: 10.1073/pnas.1015258108) elegantly illustrate this tactic with the design, synthesis and biological evaluation of a compound library inspired by biyouyanagins, natural products found in the plant *Hypericum chinense*.

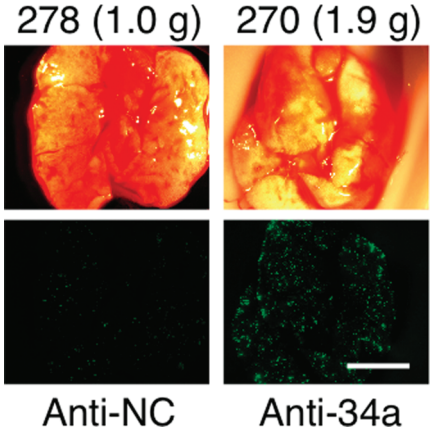


Nicolaou, K. C., *et al., Proc. Natl. Acad. Sci., U.S.A.*, DOI: 10.1073/pnas.1015258108. Copyright 2011 National Academy of Sciences, U.S.A.

The plant metabolite biyouyanagin A is known to inhibit HIV replication in lymphocytes and prevent cytokine production, pointing to potential applications of molecules of this class as antiviral and anti-inflammatory agents. To further exploit these intriguing biological properties, a compound library centered around the biyouyanagin structure was synthesized. The two key steps in the synthetic route to biyouyanagins included a palladium-catalyzed cascade reaction combining acetylenic alcohols, aryl iodides and carbon monoxide, and a [2 + 2] photocycloaddition reaction. Over fifty biyouyanagin analogs were generated, and the compounds were tested in several antiviral and cytokine production assays. Notably, four compounds, including biyouyanagin A, were selective inhibitors of lymphocytic choriomeningitis virus, a human pathogen and potential bioterrorism agent. In addition, specific library members effectively blocked HIV replication in lymphocytes, and a distinct subset prevented cytokine release in macrophages. These compounds are exciting leads for further antiviral and anti-inflammatory drug discovery efforts, and are also powerful molecular tools for probing viral and inflammatory processes. **Eva J. Gordon, Ph.D.**

MicroRNAs, Macro Potential

MicroRNAs (miRNAs) are short strands of RNA that function as post-transcriptional regulators by binding to mRNA (mRNA) molecules, typically resulting in decreased protein production. Cancer stem cells, which recent studies have shown can self-renew, are particularly talented at initiating and promoting tumor growth, and have an enhanced ability to resist chemotherapy, express different levels of certain miRNAs than cancer cells lacking such stem cell-like properties. In an effort to exploit this point of differentiation, Liu *et al.* (*Nat. Med.* 2011, *17*, 211−215) demonstrate that the miRNA miR-34a is a key negative regulator of prostate cancer stem cells, and provide compelling evidence of its potential as an anticancer agent.



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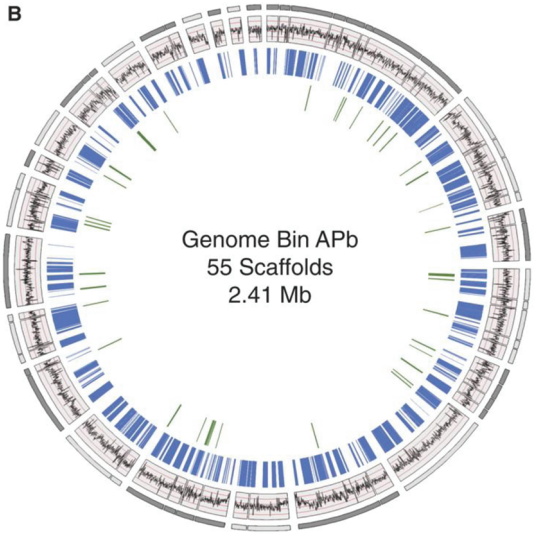
Using quantitative real-time polymerase chain reaction (qRT-PCR), it was determined that miR-34a was expressed at significantly lower levels in $CD44^+$ prostate cancer cells, which have stem cell-like properties, than in $CD44^-$ prostate cancer cells, which do not. It was next demonstrated that when cells transfected with miR-34a were implanted in mouse prostates, tumor growth was substantially inhibited compared with cells transfected with control miRNA. The tumor-suppressive effect of miR-34a was even greater when it was ectopically expressed in the $CD44^+$ prostate cancer cells, suggesting that miR-34a negatively regulates the tumor-initiating activity of cancer stem cells as opposed to other types of cancer cells. To demonstrate the therapeutic potential of miR-34a, mice with prostate tumors treated either intratumorally or systemically with the miRNA experienced dramatically reduced tumor growth or metastasis and exhibited extended survival. Notably, protein expression analysis in $CD44^+$ prostate cancer cells and prostate tumor tissue revealed a strong inverse correlation between miR-34a and CD44 levels. Furthermore, results from a luciferase reporter assay suggested that miR-34a binds to two complementary sites in the 3′ untranslated region of the CD44 mRNA. Finally, knockdown of CD44 in prostate cancer cells phenocopied expression of miR-34a, further supporting the notion that miR-34a directly targets CD44. Together, the data offer compelling evidence of the exciting therapeutic potential of miR-34a in prostate cancer. **Eva J. Gordon, Ph.D.**

Biofuels: Learning How from a Cow

For decades, the political and environmental issues surrounding fossil fuels have generated significant interest in alternative energy sources. Among these sources, biofuels have gained momentum recently. Though fuels containing plant-based ethanol or diesel are found at fuel stations worldwide, governments and private industry are betting on the next generation of biofuels to help solve the energy crisis. Among the biggest hurdles is understanding how to build a better fermenter that converts plant or algae biomass into useful fuels. Now, a new study (Hess *et al. Science* 2011, *331*, 463−467) takes aim at this goal by taking a biological snapshot of how a highly efficient living fermenter, the cow gut, breaks down tough plant cellulose.



From Hess, M., *et al.*, *Science*, 2011, *331*, 463. Reprinted with permission from AAAS.

The researchers inserted a permeable nylon bag containing switchgrass into the cow foregut by way of a fistula, a port that is surgically installed to access the cow's rumen. After 72 h, the bag containing the partially digested grass was recovered along with the diversity of rumen microbes that were busy digesting the plant matter. They then used high throughput DNA sequencing of the complex microbe mixture to determine what species are at work in the rumen and identify candidate genes involved in carbohydrate metabolism. This metagenomics approach yielded 268 billion base pairs of DNA sequence which was further assembled into millions of predicted open reading frames. They then selected genes containing any sequence similarity to known protein domains involved in carbohydrate binding or catabolism. Interestingly, only a small subset of those candidate open reading frames showed high homology to known full length proteins in curated databases. This is probably due to the fact that most of these microbes have eluded the DNA databases due to their inability to be cultured by standard laboratory means. The researchers went on to choose a random set of these candidate carbohydrate-metabolizing proteins for biochemical characterization. From a set of 90 test proteins ranging in sequence identity to known proteins, an impressive 51 showed enzymatic activity on one of 10 carbohydrate test substrates. Finally, though the DNA sequencing data was a mixed bag of small fragments from many different organisms, the researchers were able to assemble 15 draft genomes for new microbes belonging to four different phylogenetic orders and validate some genomes with single-cell genome sequencing. This study demonstrates not only the power of DNA sequencing to uncover new tools for biofuel production, but also the power of metagenomics for unveiling the genetic code of interesting organisms previously inaccessible to the scientist's eye. **Jason G. Underwood, Ph.D.**