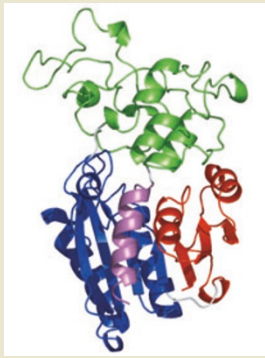
Protein permutations



Researchers have come up with a new twist on directed evolution that could potentially produce proteins with enhanced or altered activities. Dan Tawfik and colleagues use directed evolution to illustrate how 'permutation by duplication' may lead to the evolution of new protein topologies. Taking the DNA methyltransferase M.*Hae*III as an example, they use gene duplication and in-frame fusion, followed by partial degradation of the 5′ and 3′ ends of the first and second duplicated copies, to generate a library of duplicated and partially degraded forms. After testing these variants for enzyme activity, the N- and C-termini of the functional forms were found to cluster and coincide with natural methyltransferases, thereby indicating that the library overlaps substantially with folds generated during evolution. Although most forms exhibit a substantial decrease in enzyme activity, the authors suggest that subsequent point mutagenesis might compensate for changes in topology. As the authors use their method to discover a novel methyltransferase fold previously unidentified in nature, artificial evolution through permutation and subsequent point mutagenesis offers opportunities in protein design. (*Nat. Genet.* **38**, 168–174, 2006) *JWT*

Genomes from molds

Researchers in Europe, Japan and the United States have sequenced the genomes of three important *Aspergillus* fungi, a group of microorganisms that includes human pathogens as well as species traditionally used in food processing and industrial biotech. The largest of the three genomes reported corresponds to *Aspergillus oryzae*. Its sequence exhibits a bounty of genes associated with secondary metabolite production and transport, a quality that may explain *A. oryzae*'s versatility in contributing to the production of traditional Japanese fermented foods and beverages such as sake. The genome of *Aspergillus fumigatus*, a poorly understood fungus present in composting heaps and associated with high morbidity in immunocompromised patients, as well as with the triggering of severe asthma and sinusitis events in allergic individuals, contains genes encoding previously unknown allergens and a set of essential genes that may serve as potential drug targets. Finally, the sequence of model fungus *Aspergillus nidulans* provides an anchor with which evolutionary as well as other links among this group of organisms can be analyzed. Comparative analysis of all three sequences reported sheds new light onto the biology of this important and diverse group of microorganisms. (*Nature* **438**, 1105–1115, 2005; *Nature* **438**, 1151–1156, 2005; *Nature* **438**, 1157–1161, 2005) *GTO*

Antibiotic resistance lies fallow

Many clinically important antibiotics, and the genes that confer resistance to them, are derived from soil-dwelling actinomycetes. For the first time, Canadian researchers have undertaken a systematic survey of resistance in soil (the 'soil resistome') to identify the range of resistance mechanisms extant today. In 480 strains of culturable soil bacteria isolated from urban, agricultural and forest environments, they found resistance to all the antibiotics tested, including semisynthetic and purely synthetic ones. Every strain exhibited resistance to more than one antibiotic—from 2–15 of the 21 tested, with the majority in the 5–8 range. Analysis of resistance mechanisms revealed some similar to those found in clinical isolates, as with vancomycin resistance, which is caused by poor binding to a peptidoglycan in the clinic and in 80% of the resistant strains in this study. They also uncovered novel mechanisms, such as a hitherto unseen mutation in the quinolone resistance–determining region of DNA gyrase, conferring ciproflaxin resistance, in this case, to naive bacteria. These results, which may not reflect the extent of the diversity, as only spore-forming bacteria were studied, provides a glimpse at some of the mechanisms that might someday appear in the clinic, as well as fodder for developers of drugs to overcome resistance. (*Science* **311**, 374–377, 2006) *LD*

Life to the minimum

How many genes are required to sustain life? Although the ~580 kb genome of the smallest known self-replicating organism, *Mycoplasma genitalium* (which contains only 482 genes), was reported several years ago, a systematic experimental proof of the minimal number of genes essential for life has thus far been lacking. Venter and colleagues now revisit this question using transposon-mediated mutagenesis to attempt to disrupt each gene in the *M. genitalium* genome, one by one. In all, their screen reveals about 100 genes as nonessential. The vast majority (84%) of the nonessential genes were protein-coding genes; no structural RNA genes were disrupted. Several interesting mutants resulted, including one whose doubling time was 20% faster than wild type. Although a *M. genitalium* strain containing the minimal gene complement might survive in culture, some of the other 100 genes may well be necessary for growth in its natural environment. Ultimately, it is hoped that such studies can provide the foundation for 'minimal' bacterial cells that could be engineered with genetic circuits that produce compounds of pharmaceutical or nutritional interest. (*Proc. Natl. Acad. Sci. USA* **103**, 425–430, 2006) *TM*

DNA vaccine against hepatitis C

Infection with hepatitis C virus (HCV), a cause of life-threatening liver disease, has reached epidemic proportions, with >170 million people affected worldwide. There are no approved vaccines for HCV, and treatment options are limited. Viral heterogeneity represents a major hurdle to HCV vaccine development. Because the virus is highly mutable, vaccines based on stimulating the humoral immune response are considered unlikely to succeed, whereas those that act via cell-mediated immunity may be more promising. Nicosia and colleagues have developed a T cell–based DNA vaccine that protects chimpanzees against challenge with a heterologous viral strain. The vaccine sequence was taken from a nonstructural region of the HCV genotype 1b, *BK* strain, and delivered with an adenoviral prime and a plasmid boost. All five vaccinated animals saw a strong increase in HCV-specific $CD4^+$ and $CD8^+$ T cells. In four of the five, the T-cell response crossreacted with the sequence of the challenge virus, which differed by 13% from the vaccine sequence in the corresponding region. The challenge virus was given 49 weeks after vaccination; vaccinated animals showed much lower peak viral loads compared with controls and no signs of liver disease. (*Nat. Med.* **12**, 190–197, 2006) *KA*

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