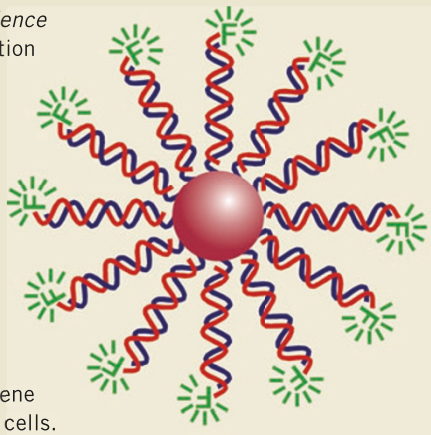
## Silence is golden



A recent paper in *Science* describes a new addition to the toolbox of techniques for knocking down gene expression in cultured cells. Mirkin and colleagues study the utility of gold nanoparticles decorated with antisense oligos for silencing a reporter gene in mouse endothelial cells. Two versions of the construct were tested: particle A carried 45–50 oligos and particle B carried 110–120 oligos. Despite their overall negative charge, both particles were efficiently taken up by a range of cell types (endothelial, macrophage, cervical carcinoma, fibroblast and kidney cells). As expected, particle B had a higher affinity for the target mRNA than particle A and was more effective at reducing gene expression. Gold-bound oligos were more resistant to nuclease digestion than free oligos. The approach also proved advantageous compared with use of the commercial transfection agents, lipofectamine and cytofectin, showing greater knockdown and lower toxicity. (*Science* **312**, 1027–1030, 2006) *KA*

## Clipping away at protease substrates

Proteases are abundant and are important for maintaining human health. Yet, substrates for only 10% of the roughly 1,000 human proteases are known. Now, a technique described by Boulware and Daugherty that uses fluorescent-activated cell sorting to screen large peptides libraries may help speed this process along. In their technique, which they call CLiPS (cellular libraries of peptide substrates), 5- to 6-amino-acid-long peptides, linked to a ligand for a fluorescent probe (streptavidin-phycoerythrin), are displayed on the surface of *Escherichia coli*. Incubating the library with proteases releases only those peptides recognized by the enzyme, removing them from the cell surface, along with the fluorescent probe-binding region. After several rounds of sorting for nonfluorescent cells, the researchers pulled out sets of substrates for two proteases, the well-characterized caspase-3 and the less well-characterized enteropeptidase. Using CLiPS, they could rank individual peptides by their kinetics, and found several that cleaved more rapidly than the canonical target sequence. Compared with phage display, CLiPS screens a greater number of substrates displayed on the cell surface, allows real-time quantitative measurements and does not require the synthesis of individual fluorogenic substrates. (*Proc. Nat. Acad. Sci. USA* **103**, 7583–7588, 2006) *LD*

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## CAGE rattles off transcription starts

Partly because of the 3′ bias of many complementary DNA (cDNA) libraries, many transcription start sites (TSS) in the human genome remain unidentified. Carninci *et al.* have now carried out a genome-wide 5′-end analysis of the mouse and human transcriptome using cap analysis of gene expression (CAGE) to define TSS and analyze their diversity and evolutionary conservation. After deriving CAGE tags (short oligonucleotides derived from the 5′-end of capped mRNAs) from 145 mouse and 41 human cDNA libraries, the authors map the tags onto the mouse and human genomes, identifying several hundred thousand putative TSS in each species. The promoters separate into two classes: highly conserved TATA-box containing promoters with precise TSS; and less conserved CpG-rich promoters with less defined TSS. The authors also identify TSS within exons of predominantly highly expressed and tissue-specific transcripts (that might be involved in RNA processing) and within 3′ untranslated regions (that may encode noncoding RNAs involved in RNA regulation). (*Nat. Genet.* **38**, 626–635, 2006) *JWT*

## Reconstituted validamycin A

With the sequencing and functional analysis of a 45-kb stretch of DNA from *Streptomyces hygroscopicus*, researchers have successfully reconstituted the biosynthetic pathway of validamycin A, an antifungal agent used to treat sheath blight disease in rice that also has insecticidal properties. Bai *et al.*'s analysis uncovered 16 structural and 2 regulatory genes, 5 genes involved in transport and 4 genes with no obvious identity to known genes. Using gene knockout and homology information, the team homed in on 8 of the sequence's 27 genes as essential for validamycin synthesis and subsequently introduced them as a construct into the heterologous host *Streptomyces lividans*, which successfully produced the antibiotic. Now that the essential gene sequence is available, it may be possible to engineer rice and other vulnerable crops to express validamycin A to protect them against the sheath blight fungus (*Pellicularia sasakii*). The work may also make more efficient the production of several drug precursors, such as valienamine, a validamycin A degradation product that is important in the production of voglibose, a diabetes drug. (*Chem. Biol.* **13**, 387–397, 2006) *TM*

## Tumor treatment triumvirate

A combination of cancer treatments that simultaneously target antibody-mediated tumor-cell apoptosis and T-cell activation may both synergistically kill tumors and reduce toxic effects. The strategy, termed trimAb (triple monoclonal antibody) therapy, involves treating mice with a cocktail of three monoclonal antibodies (mAbs). Whereas the first of these stimulates TRAIL (tumor necrosis factor–related apoptosis-inducing ligand) receptors, which are expressed by several classes of tumor, the other two mAbs, agonists to CD40 and CD137, attack from a different front. They augment the pro-apoptotic effect by activating interferon-γ–producing $CD8^+$ T cells that may also prime the immune system for defense against recurrence of that tumor type. In studies involving mice, an 80% efficiency of tumor rejection after provision of the three mAbs together compares favorably with less than 30% tumor rejection using single- or double-antibody combinations. Depending on how well trimAb therapy performs in human trials, combinatorial approaches may emerge as valuable strategies towards tumor eradication and protection against recurrence. (*Nat. Med.*, published online 7 May 2006, DOI: 10.1038/nm1405) *PH*