# Evolving sturdier gene therapy vectors

For gene therapy applications, retroviral vectors must be purified and concentrated by ultracentrifugation, a process that tends to strip off surface proteins needed for cell infectivity. In this issue, Powell et al. use DNA shuffling to evolve variants of the type C Moloney murine leukemia virus (MLV) retrovirus—the parent for most retroviral gene transfer vectors—with enhanced resistance to envelope breakdown during ultracentrifugation. They shuffled the envelope genes from six murine retroviruses, put them back into a retrovirus backbone, and reassembled viral particles by transfecting cells with the recombinant viral library. The retroviruses were subjected to several rounds of ultracentrifugation and selection, and a number of variants were identified with heightened resistance. Stress resistance of this kind is difficult to engineer by rational design, so the success of DNA shuffling to improve this property suggests that the technology could be harnessed to overcome previously intractable limitations of viral gene therapy vectors (see pp. 1244 and 1279). *JJ*
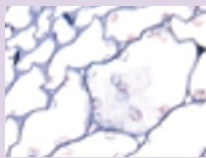
Fungal pathogens cause devastating crop losses worldwide, but unfortunately, new cultivars and fungicides have been only partially successful in controlling these losses, and transgenic plants expressing antifungal proteins have proved disappointing in the field. In this issue, Gao et al. show that in transgenic potato plants, expression of the alfalfa antifungal peptide "defensin"confers strong, sustained resistance to damage from the fungal pathogen *Verticillium dahliae*. After isolating the peptide from seeds of *Medicago sativa*, they cloned the gene, expressed it in potato, and showed that the transgenic plants accumulated high levels of the alfalfa defensin. The potatoes performed well in the greenhouse and, more importantly, as well as commercial fumigants in field trials, suggesting that defensin genes have important commercial potential for effective fungal control in economically important crops (see p. 1307). *JJ*

Brighter aptamers for biosensing

Nucleic acid aptamers can be tailored to bind a wide spectrum of molecules, and therefore are natural candidates for biosensors. Until now, however, aptamer biosensing technologies have relied on indirect methods for readout of binding. In this issue, Jhaveri et al. develop an aptamer that increases fluorescence upon target binding, providing a direct readout. They design a pool of RNA aptamers with randomly incorporated fluorescent uridines, select the ones that bind to adenosine,their target molecule, and then screen for those that displayed significant increase in fluorescence upon adenosine binding as a result of a conformational change. Jhaveri et al. identify several, and go on to test their specificity and the structural requirements for their signaling function. For high-throughput sensor arrays, such molecules could potentially be incorporated into a variety of formats, such as fiber optic cables and etched microarrays (see p. 1293). *ND*

Gene amplification for plants

Gene amplification and the *cis*-acting DNA elements responsible are well characterized in mammalian cells and provide an effective means of increasing protein production in cell culture. Now Borisjuk et al. have identified the first such sequence in plants and have shown that it increases the copy number and expression of adjacent heterologous genes. To identify the amplification-promoting sequence (*aps*), they zeroed in on a region of plant chromosomes that seemed prone to spontaneous amplification, and found a domain with homology to the A+T-rich tracks and autonomously replicating sequence consensus sequences found in mammalian amplification-stimulating DNA elements. When placed next to the genes for an herbicide resistance gene and GFP, the sequence significantly, though variably, increased the genes' copy numbers and expression, and in the case of the herbicide resistance, enhanced the phenotype as well. The effects were stably inherited, suggesting applications in agriculture and pharmaceutical production in crops (see p. 1303). *ND*

Technical Reports

On page 1314, Zhang et al. describe a way to efficiently clone large target sequences in a single step. First an appropriate bacterial host containing a highly efficient phage recombination system is transformed with the target DNA along with a PCR-generated fragment that contains a replication origin, a selectable antibiotic resistance marker, and short homologous ends to the target DNA. The phage homologous recombination machinery then transfers the target DNA into the linear cloning vector. *MS*

The effective regulation of therapeutic transgenes will in some cases require delivery of their complete genomic loci, to include regulatory sequences such as promoters and introns. To this end, vectors that can accommodate large inserts and shuttle between bacterial and mammalian cells have been developed. On page 1311, Wade-Martins et al. show that such a system can be used to correct a disease phenotype in human cells by delivering a 115 kb genomic transgene on an episomal plasmid. The episome expressed the transgene and remained stable in the cell line for six months. *ND*

Review

Over the past decade, several biological display technologies have emerged for generating large, diverse libraries of molecules through rounds of mutation and selection that mimic the process of natural evolution. Display libraries consist of modularly coded molecules, each of which contains a displayed entity of interest, a common linker, and a corresponding identifying code. They have transformed our ability to rapidly identify proteins, and in particular antibodies/antibody fragments, that bind to orphan targets of interest, and have subsequently found application in basic research and drug/agrochemical discovery. On page 1251, Min Li summarizes the concepts unifying the various display approaches and the types of coding formats/applications described to date, in the context of the technology's suitability for adaptation to studies of protein–protein/target interactions on a global (proteomic) scale. *AM*