IN BRIEF

THIS MONTH IN NATURE BIOTECHNOLOGY

Although recombinant adeno-associated virus (rAAV) vectors show promise as delivery vehicles for human gene therapy, their small packaging capacity has limited their usefulness in therapeutic strategies, especially for disorders associated with large genes, such as muscular dystrophy. In this issue (p. 544), Nakai et al. have addressed the problem with two complementary vectors created by splitting a single expression cassette and cloning the resulting sequences into two separate vectors. After injecting these two vectors into mouse portal veins, the sequences formed concatamers reconstituting the complete expression cassette. They assessed the hepatocyte transduction efficiency, as well as the total amount of transgene product, and found that the gene expression achieved by their two-vector method was an encouraging 60–70% of that obtained from mice that received a single vector containing the full expression cassette. JJ

Drawing a bead on bioassays

With the goals of increasing throughput and reducing reagent costs, scientists are developing methods for performing bioassays in microscopically small volumes. But since the sensitivity of an assay is typically directly related to its reaction volume, working with microliter-scale volumes make signal detection a real challenge. On page 548, Hänninen et al. describe a method to boost the sensitivity of microvolume-scale immunoassays by using two-photon microscopy to scan polystyrene beads that bind the molecule to be studied. This concentrates the signal and reduces background, providing high sensitivity and wide dynamic range in reaction volumes as small as 2 μ l. Since the method measures each microbead individually, it should be possible to design multiparameter tests with a mixture of beads with different bioactive coatings. ND

This Month in Nature Biotechnology written by Natalie DeWitt, Judy Jamison and Andrew Marshall.

Immunostimulatory DNA vaccines give tumor therapy a boost

DNA vaccines containing immunostimulatory DNA sequences (ISS) have been shown to induce antigen-specific cytotoxic T-lymphocyte (CTL) responses. In this issue, Cho et al. chemically coupled ISS sequences containing unmethylated cytosine/guanosine-rich motifs (CpG) to ovalbumin, a well-studied antigen. They found that vaccination with the ISS-ovalbumin conjugate induced a remarkable level of CTL activity against ovalbumin-expressing target cells injected into mice; surprisingly, the effect was independent of T-helper cells. The vaccine also had the unique property of stimulating antigen presentation by MHC class I. The vaccine protected mice against pre-established tumors expressing ovalbumin, suggesting that this approach could be used for stimulating CTL activity against tumor-associated antigens in cancer immunotherapy (see p. 509). ND

A key feature of psoriasis is uncontrolled epidermal cell growth, which leads to uncomfortable and disfiguring skin shedding. Numerous studies have suggested that this process may in some way be mediated by insulin-like growth factor 1 (IGF-1), prompting Wraight and colleagues to try inhibiting IGF-1 expression to alleviate these symptoms. In their study, they identify antisense oligonucleotides that reduce IGF-1 expression by an mRNA-dependent mechanism, and then show that several can reduce skin proliferation in cell culture. Finally, they use a nude mouse model with grafted human psoriatic lesions to show that antisense oligonucleotides, cutaneously administered, effectively reverse epidermal hyperproliferation and skin thickening in vivo, and thus are promising candidates for clinical therapies (p. 521). ND

Protein engineers have succeeded in building large libraries of potential biocatalysts, but the lack of efficient screening methods has posed a major barrier to finding useful enzymes from these libraries. In their paper on p. 544, Firestone et al. describe a method called QUEST, for detecting catalysis in vivo by coupling substrate turnover to transcription of a reporter gene. The substrate is designed to interfere with a two-hybrid reporter, but when the substrate is converted to product, the reporter is reactivated, turning on genes involved in the metabolism of arabinose. These events lead to acidification of the growth medium, which can be easily detected by a colorimetric assay. In the present study, they tailored the system to detect the fungal enzyme scytalone dehydratase, which is a pathological determinant of rice blast disease. However, QUEST could in theory be applied to detecting many types of enzymatic reaction, provided that interactions between the substrate and the two-hybrid reporter system can be properly jiggled. JJ

Feedback regulation in metabolic engineering

Metabolic engineering usually involves harnessing the cell's machinery to produce foreign metabolites. However, dysregulated expression of foreign products can lead to metabolic imbalances and disappointing yields. On page 533, Liao and Farmer use metabolic control engineering to model metabolic state and the effect of expression of particular genes in the production of lycopene, an important antioxidant found in food stuffs such as tomatoes. They altered the Ntr regulon, one of the global regulatory systems in *Escherichia coli*, to regulate expression of two key enzymes involved in lycopene production in response to carbon availability. Lycopene production was boosted threefold without compromising cell growth. JJ