Adenovirus vector gutted

Because it infects virtually all cell types, can carry large DNA insertions, and is easy to produce in quantity, adenovirus has been viewed as a promising gene therapy vector. One of the system's major flaws, however, is the vigorous immune response generated against adenovirus-infected cells. In an attempt to address this problem, researchers at Duke University Medical Center have used an adenovirus vector that lacks both the E1 regulatory gene and the viral polymerase gene. E1-deleted viruses have been studied previously by gene therapists, but the Duke team reasoned that eliminating the polymerase would prevent viral DNA replication, which is required for the expression of all of the virus's immunogenic late gene products. "We actually knock out the ability of the vector to express, at a minimum, 10 other viral proteins," explains Andrea Amalfitano, a professor in the department of Genetics at Duke and senior author on the study. In immunocompetent mice, the double-deletion virus can stably infect liver cells and express a reporter gene for at least two months, while a virus lacking only E1 is rapidly cleared by the immune system. While emphasizing that the results are still preliminary, Amalfitano describes the new vector as being "as good as anything I've seen out there [for gene therapy]." The findings are reported in *Human Gene Therapy* (**10**, 355–364, 1999).

IL-2 boosts cancer vaccine

Researchers developing a cancer vaccine based on dendritic cells have substantially improved the vaccine's efficacy using the cytokine interleukin-2 (IL-2), suggesting that future cancer therapies might rely on exploiting the natural regulatory pathways of the immune system. The team, whose results appear in *PNAS* (**96**, 2268–2273, 1999), had previously developed a technique in which host dendritic cells are exposed to tumor cell lysates, then re-introduced into the host. Because dendritic cells present the antigens to T cells to initiate an immune response against the tumor, the scientists reasoned that IL-2—a T cell growth promoter that has already been approved for clinical use—might help the vaccine produce a more robust response. Tests of the new approach in mice showed that the combined vaccine can render the animals immune to lethal tumor challenge and cause the regression of micrometastases in mice with established tumors. According to James Mulé, a professor in the Department of Surgery at the University of Michigan and the senior author on the new study, a phase I trial on the dendritic cell vaccine has shown that "small numbers of dendritic cells can prime patients to react strongly to that antigen when presented by dendritic cells," and that the cells have low toxicity. Phase II trials on the combined vaccine are slated to begin in the next few months.

Research News Briefs written by Alan Dove and Andrew Marshall

Haploinsufficiency drug screen

Gene haploinsufficiency is generally bad news for a diploid organism because loss of that gene means that the organism is unable to survive. Now, in a multi-center effort called the *Saccharomyces* Genome Deletion Project, researchers have exploited the trait to identify drug targets (*Nature Genet.* **21**, 278—283, 1999). In their approach, each deletion strain lacks one copy of a given gene and carries in its place a unique DNA "bar code" that allows scientists to identify it on a DNA microarray. When the strains are cultured with the different deletions together, those in which the single-copy deletion causes a growth disadvantage gradually disappear and these strains identify genes that exhibit haploinsufficiency. Similarly, a drug that inhibits a particular gene product will place strains with only one copy of that protein's gene at a competitive disadvantage, resulting in their death. By comparing the drug-induced haploinsufficiency data with the original data, the scientists are able to find the drug's protein targets. The initial proof-of-concept experiment used 233 deletion strains, but the Genome Deletion Project will ultimately yield a much larger set of mutants. Guri Giaver, a researcher in the department of biochemistry at the Stanford University School of Medicine and first author on the new study, says "there is no foreseeable technical reason to think. . .that the system cannot scale to accommodate the complete genome set of 6,000 strains."

Mix and match *Streptomyces*

Scientists have succeeded in turning *Streptomyces* into a system akin to combinatorial chemistry for producing molecular diversity. By mixing and matching enzyme domains in two *Streptomyces* polyketide synthases (PKSs)—6-deoxyerythronolide B synthase (DEBS) and rapamycin PKS—researchers at KOSAN Biosciences (Hayward, CA) have generated a library of >50 macrolides that would be impractical to produce by chemical methods. The modular nature of the DEBS enzyme allowed the KOSAN team to substitute corresponding domains from the rapamycin PKS encoding alternative substrate specificities. According to Robert McDaniel, lead author on the paper (*PNAS* **96**, 1846–1851, 1999), engineered DEBS containing single, double, or triple catalytic domain substitutions catalyzed the production of erythromycin macrolactones with corresponding single, double, or triple modifications. "The relatively high success rate of the approach suggests that combinatorial mutations are well tolerated by the PKS," he says. "To my knowledge, this is the first demonstration that you can combine mutations in a polyketide synthase in a combinatorial fashion." Kosan is currently collaborating with Johnson & Johnson (New Brunswick, NJ) to apply the technology to novel antibacterial discovery as well as exploring opportunities in anticancer drugs, immunosuppressants, and agricultural products.

Drug targeting looks inward

When researchers try to design drugs for specific cellular signaling pathways, they usually target extracellular receptors, which often have well defined and separate binding and signaling components. New work reported in *Science* (**283**, 1332–1335, 1999) suggests that it may soon be open season on intracellular proteins as well. Scientists studying G proteins, key intermediates in many cellular signaling pathways, have found that the binding and signal transduction functions of a G protein subunit are separate, and have defined the amino acid sequences responsible for each. Ravi Iyengar, professor of pharmacology at the Mt. Sinai School of Medicine (New York, NY) and senior author on the new study, suggests that analogs of the signal transduction domain could be used as activators of the pathway, whereas analogs of the binding domain might act as inhibitors. Although drugs that bind to receptors may be more specific than those targeting intracellular targets, Iyengar points out that "when you want a drug that is an activator, it will always be advantage to target it intracellularly, because the major desensitization events occur at the level of the receptor."