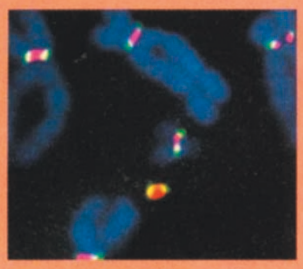
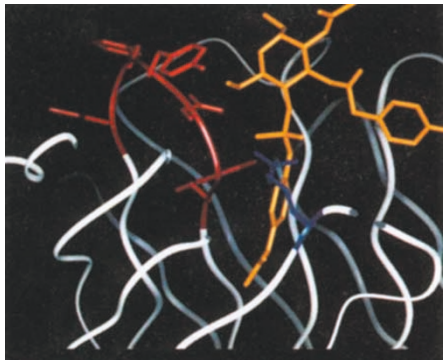
Artificial chromosomes get real

Although viral and nonviral gene therapy vectors are making their way out of the laboratory and into the clinic, stably inherited episomal vectors capable of introducing large segments of DNA and regulated gene expression over many cell divisions have not yet been developed. By introducing into cultured human cells yeast artificial chromosomes, which contained repetitive alphoid DNA arrays from human chromosome 21 and had been retrofitted with human telomeres and a drug-resistance marker, Masumoto and colleagues succeed in isolating microchromosomes that do not acquire host DNA (see pp. 415 and 431). These artificial human chromosomes will not only allow the dissection of chromosomal element function, but may address a major hurdle in the development of this type of gene therapy vector.





By using phage display for the in vitro maturation of an antibody fragment that binds to a transition state analog for ester hydrolysis, Fuji and colleagues (see pp. 423, 463) have been able to alter the catalytic activity of a primordial catalytic antibody.

No bioactivity, more cytotoxicity

Brain tumor glioma cells express a receptor for interleukin 13 (IL-13R) that, because it does not contain an α-chain that is shared with IL-4R, is tumor specific and thus an attractive therapeutic target. Taking a rational engineering approach, Debinski et al. have altered the receptor-interacting region of IL-13 so that it is no longer bioactive in that it does not interact with IL-4R present on normal cells. By fusing the mutated interleukin to a plant cytotoxin they have created a molecule with tumor cell specific cytoxicity that is more toxic than the wild-type fusion protein both in culture and mouse models (see p. 449). They show that virally infected tumor cells xenografted onto mice can be killed by administration of an inactive prodrug that is only activated within the tumor cells.

Research briefs written by Philip Bernstein.

Boosting antigen mimicry

Mimics of tumor-associated antigens, such as carcinoembryonic antigen (CEA), can overcome immune tolerance toward this type of self-antigen. Anti-idiotypic antibodies to CEA—which mimic CEA—can induce anti-CEA antibodies, but the level of immunity is insufficient to establish a clinical response. By affinity crosslinking a peptide from the C3d protein of the complement cascade to the anti-idiotypic CEA antibody, Lou and Kohler have recruited components of innate immunity to induce a strong protective immune response in a mouse model (see p. 458).

By searching for proteins that bind to potato starch, Kossman and colleagues have identified a protein that regulates its phosphorylation (see p. 473). Using antisense, they inhibit the expression of the protein without effecting the plant's growth or the starch content of the tubers. The resultant tubers, however, are resistant to cold-induced sweetening, a problem in potato storage where starch is converted to soluble sugar.

Multigenic metabolic engineering

For the production of certain proteins—particularly those whose activity is dependent upon complex post translational modifications—only a mammalian expression system will do. Using a coordinated approach for the expression of a cyclin-dependent kinase inhibitor and Bcl-x_L, an antiapoptotic protein, Bailey and colleagues are able to maintain cells in a viable state for an extended period of time (see pp. 416 and 468). Upon introduction of a gene of interest, they are able to overcome the natural tendency of these cells to undergo apoptosis and produce 30 fold more protein than would be synthesized using a single gene approach.

Targeted HSV prodrug therapy

Herpes simplex virus (HSV) is dependent upon its ribonucleotide reductase gene, *Hsrr*, for replication. Because several types of human tumor cell have a high level of expression of this enzyme, an HSV strain with a mutant *Hsrr* gene should be able to replicate only in these cells. By replacing the *Hsrr* gene of HSV with a gene that activates chemotherapeutic prodrugs, Chase et al. have engineered a virus with antitumor potential (see p. 444).

Whooping immunogenicity by attenuation

By engineering pathogens so that they no longer express disease-causing genes, effective live vaccines can be produced, although they are often less immunogenic than the wild type. On the basis that pertussis toxin is the key virulence factor of *Bordetella pertussis*, the pathogenic agent of whooping cough, Mielcarek et al. have created an attenuated vaccine (see p. 454). Not only is the attenuated pathogen as effective in inducing protection as wild-type *B. pertussis*, but also it results in increased immunogenicity to the filamentous hemagglutinin (FHA). By fusing a heterologous antigen from *Schistosoma mansoni* to the FHA, this re-engineered strain elicited simultaneous protection against two pathogens in a mouse model system.

Cytotoxic transport

A persistent problem for gene therapy has been the inability to get consistent widespread dissemination of the protein of interest. The herpes simplex virus-1 (HSV-1) protein VP22, is able to spread between cells, even when covalently linked to heterologous peptides. Peter O'Hare and colleagues have exploited the properties of VP22 to produce an effective bystander effect for gene therapy (see pp. 418 and 440). They show that a fusion protein consisting of p53—a protein that can induce apoptosis—covalently attached to HSV-1 VP22 has a widespread cytotoxic on a p53 negative human osteosarcoma cell culture model, even when it is initially delivered to small number of cells.