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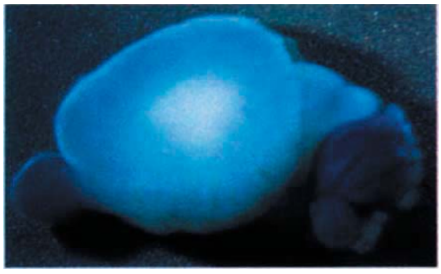
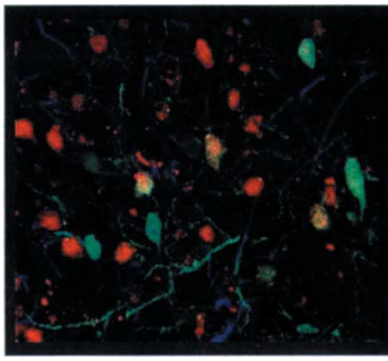


Artificial chromosomes—from various origins, including bacteria, yeast and mammals—have enabled the introduction of large DNA segments into transgenic animals for the analysis of gene function. Despite the relative ease with which bacterial artificial chromosomes (BACs) can be handled, the introduction, deletion, or substitution of marker genes has, until now, not been possible. By transiently inducing *recA*-mediated recombination, Yang et al. (see pp. 839 and 859) have stably modified a 131 kb BAC with a reporter gene, such that the proper spatial expression induced by the regulatory elements of a murine zinc finger gene are observed in the brain of a transgenic mouse.



Lentiviruses—such as HIV—can stably infect nondividing cells, a characteristic that would be ideal for some gene therapy protocols. As these viruses are highly pathogenic, however, their clinical use is tempered by the possibility of generating a replication-competent virus. A major step in reducing this risk has been achieved by Zufferey et al. (see p. 871) who have removed four of the so-called accessory genes (suspected virulence factors), along with the *env* gene, to develop an attenuated vector that is able to deliver transgenes into adult neurons as efficiently as the parental vector.

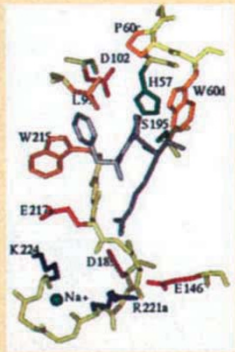
Ribozymes: Half empty or half full?

Characterized by the ability to specifically cleave or repair RNA in vitro, ribozymes (and other nucleic acid based molecules) are being examined as therapeutic agents. However, it has been difficult to assess the potential clinical utility of trans-splicing ribozymes—and thus develop strategies to improve their efficiency—in absence of the ability to measure their activity in vivo. Jones and Sullenger (see p. 902) have devised a competitive reverse transcriptase/PCR protocol to do just that. By adjusting the ribozyme/substrate ratio, they are able to achieve an encouraging 50% trans-splicing efficiency.

Seeing blue-green by mass spectrometry

Water blooms, which are caused by the rapid growth of cyanobacteria—blue-green algae—can be toxic to both animals and humans because of the production of certain secondary metabolites. The identification of toxic strains has traditionally relied upon laborious processes of metabolite isolation. By using MALDI-TOF mass spectrometry, Erhard et al. (p. 906) have been able to identify intact microorganisms on the basis of their secondary metabolite production profile. Furthermore, this approach has been used to identify a new cyclic peptide.

Research Briefs written by Philip Bernstein.



Comparisons of the ability of related enzymes to bind substrate can often be made by site-directed mutagenesis of the substrate-binding domain. However, when the alteration of these sites would prevent substrate binding or enzymatic activity, or the enzyme is of single allosteric form, this approach may not be feasible. An alternative—using small substrate libraries—has been adopted by Vindigni et al. (see p. 891) to identify the structural differences between the slow (anticoagulant) and fast (procoagulant) forms of thrombin.

Mimicking cell-cycle arrest

Progression through the cell cycle involves a number of critical decision points, such as when the cell replicates its genome in the transition from G1 to S phase. This pathway is controlled by the tumor suppresser protein pRb, which downregulates downstream transcription factors. Misregulation can result in tumorigenesis. A small peptide based on the structure of one downstream transcription factor has been used to antagonize pRb activity, causing apoptosis (see p. 896). The demonstrated specificity of this mimetic makes it a promising lead therapeutic candidate.

Chilling out

An untimely frost can devastate crop production. Antifreeze proteins (AFPs) isolated from cold water fish (which bind to and inhibit ice crystal formation) have been introduced into plants with the hope that the transgenic plant would resist freezing. The spruce budworm, a forest pest insect, survives severe winters in part as a result of the production of a thermal hysteresis protein. The cloned and heterologously expressed protein has cryoprotective activity far in excess of any previously identified fish AFP (see pp. 844 and 887).

Boosting an antimalarial DNA vaccine

In two papers (see pp. 842, 876, and 882), Zanetti and coworkers describe antimalarial strategies that may have implications for vaccine development in general. These authors have inserted the malarial antigen $(NANP)_3$ into a region of an immunoglobulin gene (Ig)—called the complementarity-determining region (CDR)—that tolerates insertions, thus allowing the presentation of heterologous sequences. They then used this construct as a DNA vaccine to inoculate mice by the unusual transplenic route. The recombinant gene was apparently integrated into B cells, leading to an anti-idiotypic immune response. This group has also shown that more than one CDR within an Ig can express heterologous proteins. They have successfully introduced both a B-cell and a T-helper cell epitope of the malaria parasite into two different sites within a single Ig. When delivered intrasplenically, the expression of both epitopes resulted in enhanced production of anti-$(NANP)_3$ antibodies.

A unification of gene therapy vectors

Retroviruses and adenoviruses each have attributes that make them attractive human gene therapy vectors. By designing a gene-delivery system in which adenoviral vectors are used to deliver retroviral vector and packaging proteins to cells in vivo, Feng et al (see pp. 840 and 866) achieve efficient delivery characteristic of adenoviruses along with the stable gene expression of retroviral vectors. The initial adenoviral infection results in a cell that transiently produces recombinant retrovirus particles, which subsequently induce the stable transduction of neighboring cells.