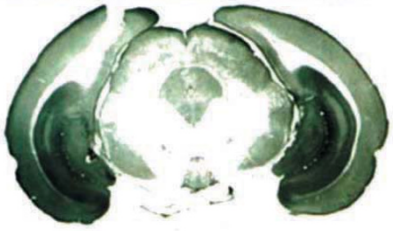
Gene-inducible behavior



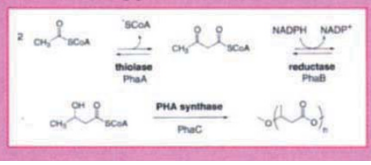
In order to study how molecules like nerve growth factor (NGF) specifically affect brain function and behavior, Federoff and colleagues have generated transgenic mice that only express NGF on activation with Cre recombinase (see p. 57). By injecting a herpes simplex viral vector carrying the Cre recombinase gene into the control center of spontaneous activity, the dorsal hippocampus, localized expression of NGF was induced. The "activated" transgenic mice showed increased activity compared with control mice.

Increased aptamer affinity and stability

An RNA ligand's utility as a therapeutic is dependent upon overcoming its inherent nuclease sensitivity. Ligands isolated using in vitro selection-amplification (SELEX) can be modified after SELEX by substituting 2′-amino (2′NH_2) or 2′-fluoro-(2′F) groups to confer nuclease resistance. This chemical modification can result in decreased binding affinity. Researchers at NeXstar Pharmaceuticals (Boulder, CO) have shown that high-affinity and nuclease-resistant ligands can be obtained by incorporating the modified triphosphates during SELEX. 2′F-substituted ligands for keratinocyte growth factor (KGF) were shown to have enhanced binding affinities compared with 2′NH_2-stabilized KGF ligands (see p. 68). The higher affinities of 2′F could be due to stronger intramolecular helices and therefore to higher rigidity of the ligand.

Engineered biopolymers

Biodegradable polymers are not yet a viable alternative to conventional plastics. They are more expensive to produce and average molecular weight and distribution of polyesters, which determine the properties of the final polymers, cannot be controlled. Now, Sim et al. have expressed polyhydroxyalkanoate synthase in *E. coli*, producing polyesters with lower molecular weights and broader size distribution (see pp. 17 and 63).

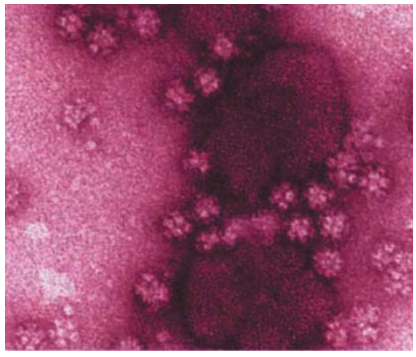


Single chain IL-12

Interleukin (IL)-12 has showed promise as an anticancer therapeutic, but its utility is somewhat compromised by the necessity of expressing both chains of the protein in stoichiometric amounts. This difficulty has been circumvented by designing a single-chain fusion protein in which the two subunits are linked by a polypeptide linker (see p. 35). The single chain IL-12 has similar bioactivities in vitro to the native form and in vivo resulted in regression of 80% of CMS-5 tumors in mice.

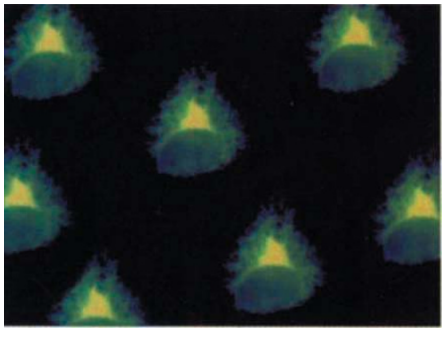
Minimal adenovirus vectors

The high-efficiency gene transfer of adenoviral vectors is often mitigated by an accompanying immune response to the virus. Chroboczek and colleagues have coexpressed the viral penton base and fiber, the proteins necessary for binding and uptake of the virus, to create dodecahedral vectors. These minimal vectors can transfect human cells and have the potential to be less antigenic than the commonly used viral vectors (see pp. 17 and 52).



Epitope mapping

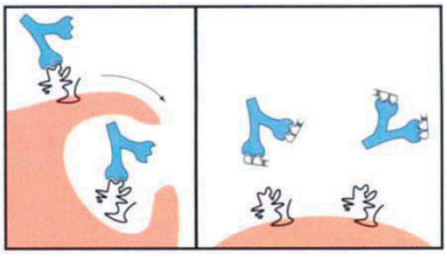
The mapping of antigenic epitopes often requires large libraries of variants. Kuwabara et al. have used a random epitope library created from a cDNA digest to isolate epitopes of the lectin galectin-3; a parallel random peptide library screening yielded none (see p. 74). The epitope method is more successful as it avoids low-frequency candidates and selective enrichment of peptides that do not form an epitope, yet have a high affinity with the selecting monoclonal antibody.



Antitumor immunotoxin secretion by T cells. A T-lymphocyte cell line transduced with an exotoxin-antiHIV k chain gene fusion results in antibody-toxin secreting lymphocytes that selectively destroy HIV-1 infected cells (see p. 46).

Optimized functional antibody screen

A cell-free in vitro expression system could be a rapid way of studying the effect of site-directed mutations in antibody genes on binding activities, but activity depends on the correct formation of a conserved intramolecular disulfide bond that does not readily form in vitro translation systems. Bond formation is catalyzed in vivo by a protein disulfide isomerase (PDI). Thus, a cell-free system has been optimized by translating single-chain (sc)Fv antibody genes in the presence of PDI, which enhanced the proportion of functionally active scFv fragments three fold (see p. 79). (Other molecular chaperones increased soluble protein, but did not affect the amount of functional scFv, although inclusion of chaperones did seem to enhance the effect of PDI.)



RNA decoys (shown in green) can be synthesized to protect cell surface acetylcholine receptors from myasthenic antibodies (see p. 41).

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