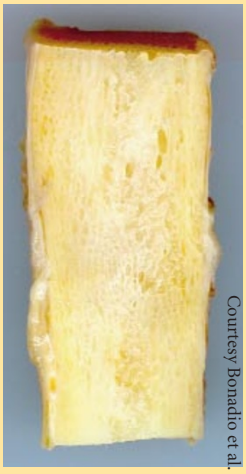
Boning up on gene therapy

Researchers at the University of Michigan Medical School (Ann Arbor, MI) have reported a new technique for localized delivery of gene products that accelerates healing of a bone fracture. The work, reported in *Nature Medicine* (**5**, 753–759, 1999), uses a system in which plasmid DNA encoding human parathyroid hormone is trapped within a polymer gene-activated matrix (GAM) carrier. The polymer, which is biodegradable, can be implanted near a broken bone. Fibroblasts arriving at the site take up the DNA and act as in vivo bioreactors, generating a high local concentration of the hormone, which in turn accelerates bone healing. Because expression of the introduced gene is limited to cells near the wound, the system avoids the side effects associated with systemic overexpression of genes. Beagles with surgically induced gaps in their leg bones regrew histologically normal bone tissue with the GAM treatment in a dose-dependent manner. According to Jeffrey Bonadio, first author on the paper and now a researcher at Selective Genetics (San Diego, CA), "In principle, the GAM technology can deploy any plasmid-gene, so a wide variety of cytokines, growth factors, and hormones could be used." Selective Genetics is currently planning clinical trials to test the technology for fracture repair in the elderly and as a bone-graft substitute.



Courtesy Bonadio et al.

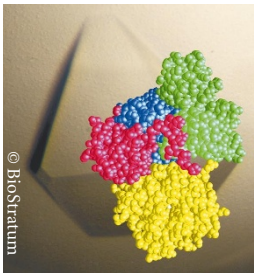
DNA cryptography

Fifty-five years after the secret message "June 6 invasion: Normandy" was first sent, researchers have encrypted the D-Day message in DNA, suggesting a potential approach for communicating secret information or discreetly tagging products or organisms. Using a simple genetic code to convert letters and numbers into three-base codons, Carter Bancroft and his team at the Mount Sinai School of Medicine (New York, NY) designed a DNA sequence encoding the D-Day message between a pair of defined primer sequences, then mixed it with human genomic DNA. After spotting the mixture onto paper and cutting away pieces appropriately sized for punctuation marks, they pasted them to a document and sent them through the US mail. When PCR amplified, the correct "encrypted" message was identified. Bancroft says the new technique has advantages over conventional microdots because, in addition to knowing where the message is hidden on a page, a recipient must know the primer sequences needed to amplify the message. At this stage, "it is. . .difficult to be sure what other sort of tagging would require the very high degree of concealment afforded by our technique," says Bancroft. That said, his laboratory is currently investigating applications in marking items such as documents, valuables, and collectibles. The findings are published in *Nature* (**399**, 533–534, 1999).

Research News Briefs written by Alka Agrawal, Alan Dove, and Andrew Marshall.

MMP-2 structure solved

The crystal structure of human matrix metalloproteinase type-2 (MMP-2), a key target for several anticancer therapies currently in trials, has now been solved to a 2.8Å resolution (*Science* **284**, 1667–1670, 1999). MMP-2 is secreted as a proenzyme that becomes partially activated upon proteolysis by activators. By breaking down connective tissue, it is thought to allow tumors to grow new blood vessels and encroach upon surrounding tissues. Researchers had previously mapped the structure of limited portions of the protein, but Karl Tryggvason and colleagues from the Karolinska Institute (Stockholm, Sweden) used a combination of x-ray crystallography and molecular replacement modeling to resolve the complete structure of the full-length proform of the enzyme. Although the active site is similar to many other MMPs, Trygvasson's structure reveals several other targets for designing more specific MMP-2 inhibitors: "One could design drugs that inhibit binding of TIMP-2 [tissue inhibitor of MMP-2] to the hemopexin domain, thereby preventing activation," he suggests. Using computer modeling, the Swedish team is currently collaborating with BioStratum (Research Triangle Park, NC) to further refine inhibitors.



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TCRs on display

Researchers have found a way to display a protein crucial for cell-mediated immunity—the T-cell receptor (TCR)—on the surface of yeast cells, opening up the possibility of engineering high-affinity forms of the protein for therapeutic applications. Using a TCR made of a single protein chain, which is unstable, instead of the usual two chains, David Kranz and his colleagues at the University of Illinois found that mutations in two regions of the TCR allowed it to be expressed on the yeast surface at high levels. As they report in *PNAS* (**96**, 5651–5656, 1999), the modified TCR could still recognize its target peptide bound to major histocompatibility complex (MHC) protein. Kranz suggests engineered TCRs could be useful in treating autoimmune diseases, such as multiple sclerosis, which result from activation of immune responses by TCR recognition of a MHC-bound self peptide. "We can engineer [TCRs with] higher affinity for the self peptide–MHC complex involved in those diseases, and use those receptors in soluble form as antagonists," Kranz ventures. This would be an improvement over antibody therapeutics, he says, since it's been difficult to make antibodies that recognize the peptide—MHC complex, whereas the TCR already has evolved to do exactly that.

Y clone?

To date, sheep, cattle, and mice have been cloned from adult somatic cells, but scientists at the University of Hawaii School of Medicine (Honolulu, HI) have broken new ground with a type of animal that had not previously been cloned—the male. Earlier somatic cell cloning experiments relied on cells from the female reproductive system, possibly limiting the technique's use for propagating valuable transgenic lines or endangered species. In an effort to overcome this barrier, the University of Hawaii team, whose report appears in *Nature Genetics* (**22**, 127–128, 1999), used tail tip cells from male mice as a source of nuclei, which were then implanted into enucleated oocytes. Three of the resulting embryos developed into live male clones of the original donor. The researchers conclude that in principle "precious animals of either sex. . .can be propagated by cloning irrespective of their fertility status." Cloning still has serious limitations—the three surviving clones represent approximately 1% of the embryos transferred, making the current approach too cumbersome for routine use.