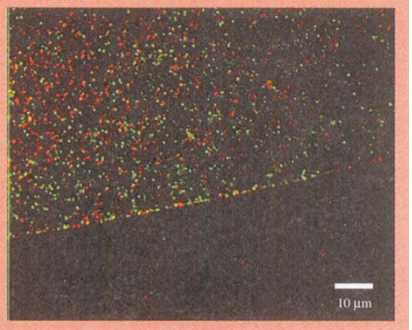
Rolling circles stay put

With the help of a novel enzyme and a clever DNA microarray-based assay, researchers at Yale University School of Medicine (New Haven, CT) and Oncor (Gaithersburg, MD) have developed a system that can detect rare single base changes in a large pool of DNA. The approach uses rolling circle amplification (RCA), a process that begins with a circularizable DNA probe binding to a linear template like a horseshoe magnet sticking to an iron bar. A subsequent DNA polymerization and ligation reaction leads to the production of a "rolling circle" of DNA, which produces a continuous, repeating linear product, rapidly amplifying the target sequence. The method permits the detection of a few mutants in a large population of wild-type cells, a capability that might be applied to detecting rare tumor cells in a biopsy. David Ward, an author on the new work (*Nature Genetics* 19:225–232, 1998), sees distinct advantages for RCA over other techniques: "In contrast to PCR, the amplification products can be spatially and optically resolved. You get physical location at the site of amplification." By attaching probes to a glass slide and using fluorescent dyes to detect products, the method might also be used to interrogate a sample for dozens of different mutations in different colors or to establish the ratio of wild-type to mutant in any allele. Oncor and two New Haven-based start-up companies, Polygenomics and Molecular Staging, are working to develop specific applications for the technology.



Gene therapy for hemophilia

Katherine High and her colleagues at the Children's Hospital of Philadelphia (Pennsylvania, PA) in collaboration with Avigen (Alameda, CA) have obtained extended and stable expression of the coagulation factor IX in a dog model of hemophilia B. Hemophilia B is one of the most common forms of hemophilia and results from a deficiency of normal factor IX. In research presented at the inaugural meeting of the American Society for Gene Therapy in Seattle, Washington, on May 28, High reported that a single intramuscular injection of an adenoassociated virus vector carrying the gene for canine factor IX resulted in stable expression of the gene for nine months. The gene therapy resulted in a dose-dependent increase in plasma levels of factor IX. The animals demonstrated improved clotting time and showed no toxic effects associated with the treatment. "This is the first example of the intramuscular administration of a gene therapy vector resulting in long-term expression of a clotting factor at levels that would be therapeutic in humans with hemophilia," says High. Avigen plan to extend the work to human trials by the end of 1998.

Research News Briefs written by Alexander Castellino, Alan Dove, Margret Einarson, and LeeAnn Leshko.

Piggyback ribozymes

Researchers at the Beckman Research Institute of the City of Hope (Duarte, CA) have found a way to sneak an antiviral agent that interferes with viral replication into virions. By attaching an antiviral ribozyme to $tRNA_3Lys$—a host cellular factor that is recruited by HIV to facilitate DNA replication—John Rossi and colleagues set out to determine whether the ribozyme could be brought in proximity to viral target sequences. Sure enough, when cotransfected with HIV proviral DNA, the chimeric $tRNA_3Lys$–ribozyme was packaged into virions bringing the ribozyme to its RNA target. More importantly, the $tRNA_3Lys$–ribozyme was effective in reducing the titer of infectious virions. Rossi cautions that "substantially increase[d] transcription of the chimeric gene in the cell [was needed] to effectively compete with endogenous (tRNA,Lys) gene for packaging into virions." He speculates that once a chimeric $tRNA_3Lys$–ribozyme is packaged into a virion "it is able to inhibit HIV replication probably through a combination of ribozyme cleavage of target HIV RNA, and blockage of the primer binding site to replication machinery." The group plans to develop this novel strategy into a gene therapy to be combined with existing ribozyme-based gene therapies to reduce HIV infectivity. The findings are reported in a recent issue of *Antisense & Nucleic Acids Drug Development* (8:185–197,1998).

A master switch for plant disease resistance

Broad-spectrum disease resistance has been made possible in tobacco by overexpressing a master regulator of plant systemic acquired resistance. Reporting in the *Proceedings of the National Academy of Sciences* (95:6531–6536, 1998), Xinnian Dong and her colleagues from Duke University (Durham, NC) have shown that transgenic *Arabidopsis thaliana* expressing just twice the normal levels of pathogenesis-related (PR) gene NPR1 have a 1000-fold increased resistance to a wide variety of pathogenic insults, including a bacterial pathogen and a fungal (oomycete) pathogen. The authors hooked up NPR1 to a cauliflower mosaic virus promoter gene to elevate levels of the regulatory protein. Northern blot analysis indicates expression of NPR1 beyond a narrow threshold suffices to turn on a battery of PR genes, which work synergistically to generate broad-spectrum resistance. Dong says that the approach is widely applicable as "NPR1 homologs are present in other plants like canola, cabbage, broccoli, tobacco, tomato, and corn."

Mouse model for heart disease

The first transgenic mouse model of cardiomyopathy has been created by scientists at the University of Chicago (IL). Their model reproduces the anatomical and clinical features of human idiopathic (of unknown cause) dilated cardiomyopathy (IDC) and could lead to new therapeutic strategies for the disease (*J. Clin. Invest.* 101:2415–2426). Jeffrey Leiden and colleagues expressed a dominant-negative mutant (Ser133→Ala) of the cAMP-response element binding (CREB) protein specifically in the developing heart. Compared with nontransgenic littermates, mice transgenic for CREB133 died prematurely (between 2 and 20 weeks) and showed marked morphological defects in both ventricles and atria. Echocardiography and hemodynamic assessment indicated decreased left ventricular function, as well as attenuated contractile responses to the β-adrenergic agonist, isoproterol—features reminiscent of human IDC. According to Leiden, "this suggests that defects in CREB and its downstream signaling components are all candidates for IDC. The mouse model provides, for the first time, a starting point for identifying genetic pathways for IDC." To extend these observations to human IDC, Leiden's laboratory is in the process of examining biopsies from human IDC tissues for defects in the CREB pathway.