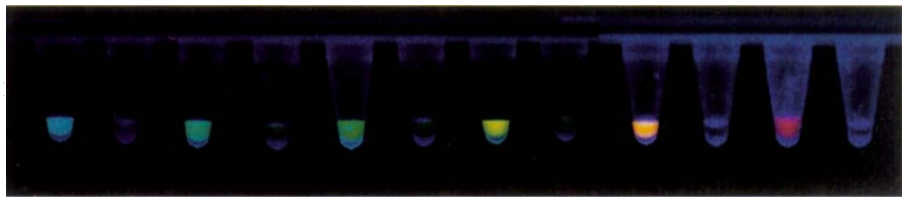


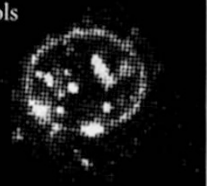
Researchers have developed molecular beacons—hairpin RNAs that fluoresce upon hybridization to their target sequence—as probes that allow real-time hybridization analysis. Tyagi and colleagues (see p. 49) generate variously labeled probes that should allow multiplex sequence identification of alleles that differ by as little as a single nucleotide.

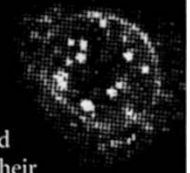
Monitoring bacterial mRNA expression

As the number of bacterial genomes sequenced continues to rise, the ability to identify genes expressed in response to different environmental stimuli will facilitate the engineering of those bacteria producing important metabolites as well as the discovery of novel antimicrobial targets against virulent strains. Messenger RNA in prokaryotes is not polyadenylated and, therefore, it can be a difficult task to separate the low percentage of these transcribed genes from the bulk ribosomal RNA. By targeting immobilized arrays of defined sets of oligonucleotide probes against a subset of bacterial mRNA, de Saizieu et al. (see pp. 23 and 45) demonstrate that differential expression patterns of mRNA can be visualized among the high background of ribosomal RNA.

Nuclear targeting DNA vectors

Gene transfer protocols require the efficient uptake of DNA into the cell nucleus. To facilitate this process, Sebestyén et al. have covalently modified DNA to contain the canonical SV40 nuclear localization peptide. Although modified DNA shows good expression and enhanced nuclear uptake in a permeabilized cell model, cultured cells did not take it up into their nucleus after microinjectiion into the cytoplasm (see p. 80).

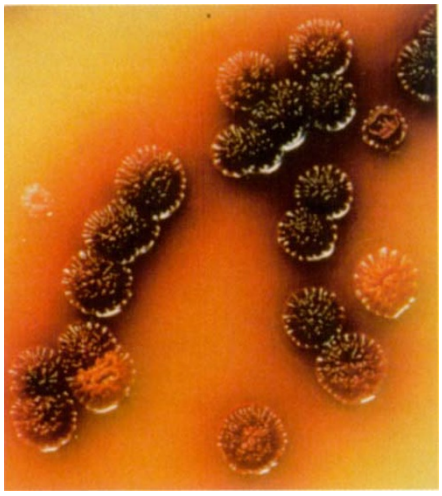




Research briefs written by Philip Bernstein.

Sequencing by hybridization

Along with the prospect of obtaining the sequence of the entire human genome comes the expectation that disease diagnosis will rely increasingly on gene identification. High-throughput methods, with a sensitivity approaching that of nucleotide sequencing, will be required. To these ends, Drmanac et al. (see p. 54) have used 10,000 7-mer probes to identify both homozygous and heterozygous mutations of exons 5–8 of the tumor suppressor gene, *p53*, using a sequencing by hybridization format.

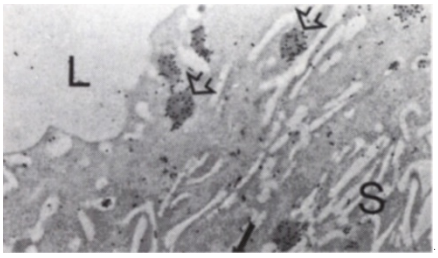


Many secondary metabolites produced in a variety of microorganisms are closely related to compounds that have important commercial uses. While engineering the bacteria to produce the desired compound may appear to be simple, more often a variety of enzymes in overlapping metabolic pathways are involved. The chemotherapeutic agent, epirubicin, is produced by a multistep semisynthetic protocol. By introducing a variety of genes from different microorganisms into the deoxysugar biosynthetic pathway of *Streptomyces peucetius*, Madduri et al. (see pp. 19 and 69) have used this combinatorial approach to effectively enlist these bacteria for the fermentative production of an important antitumor drug.

Start making antisense

Antisense molecules, which bind to a specific target RNA to inhibit its translation, are moving from laboratory tools to potential therapeutic agents. The first step in their design—target identification—continues to be a major bottleneck. Two different approaches are described this month (see pp. 24, 59, and 64). Patzel and Sczakiel describe the relationship between the secondary structure of an antisense RNA—as determined by computer modeling—and the effect of its hybridization rates on the inhibition of gene expression. Although antisense RNA that slowly hybridized to the target HIV *gag* mRNA formed more stable complexes, it is the faster hybridizing RNA species that produces a greater antisense effect in cells. In a different approach, Ho et al. use semi-random oligonucleotides to select the most accessible site on the RNA, as determined by ribonuclease H cleavage. This strategy was used to determine optimal targets for the angiotensin type-1 receptor. The authors show that the selected targets result in repression of expression in cell culture as well as a physiological effect when these therapeutic molecules are injected into rat brains.

Milking the bladder



Gene cloning made possible the development of protein therapeutics. The expression of these genes in the mammary gland of transgenic animals has facilitated their large-scale production in milk. Using animal bioreactors, as opposed to cell-culture technology, allows cheaper, more efficient production of these molecules. While milk is an efficiently harvested bodily fluid, it is only produced in transgenic female animals after the animals first lactation, which necessarily requires an investment of time. To examine the potential of using another easily collectable bodily fluid—one that is less complex in composition than milk and produced sooner and more often—Kerr et al. have developed transgenic mice that produce growth hormone in their urine, using the animal's bladder as a bioreactor (see pp. 21 and 75).