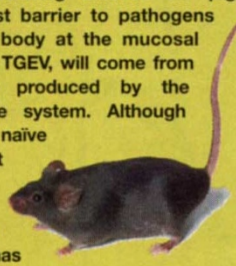
Detecting drug resistance in a tube

The control of tuberculosis (TB) worldwide begins with detection. The difficulty of identifying drug-resistant strains of *Mycobacterium tuberculosis* compounds the problem. The development of molecular beacons—reporter molecules that fluoresce upon hybridization to a specific nucleic acid sequence—may provide the first steps in finding a solution. David Alland and colleagues (see pp. 331, 359) use molecular beacons to detect and discriminate between different alleles of *M. tuberculosis* in real time, in a closed tube, in a simple PCR reaction.



Transmissible gastroenteritis—caused by the transmissible gastroenteritis virus (TGEV)—can be a devastating disease for pig farmers. The first barrier to pathogens that invade the body at the mucosal surface, such as TGEV, will come from immunoglobulins produced by the mucosal immune system. Although immunologically naïve animals may not provide this level of immune protection, passive immunity has been achieved by bottle feeding newborn animals milk containing an appropriate antibody. A more (economically) feasible alternative to passive immunity has now been achieved in a mouse model (see pp. 334, 349). A mammary gland specific promoter has allowed Castilla et al. to target immunoglobulin production so that TGEV antibodies are produced in the animal's milk. Transferring this approach to the pig has the potential of providing passive immunity to the suckling infant.

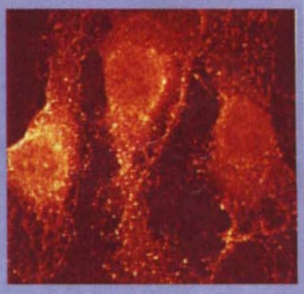
Immunizing with RNA

One approach to developing tumor specific vaccines is to enlist the support of the professionals—dendritic cells—so-called professional antigen-presenting cells. The strategy involves transfecting dendritic cells with a gene encoding a tumor-specific antigen such that it will be presented to the immune system in a manner that will elicit a cellular (CTL) response. However, even if a tumor-specific antigen can be found, the immunogenic portion of the antigen will vary between individuals. To circumvent this problem, Nair et al. (see pp. 335, 364) show that human dendritic cells can be "pulsed" with RNA, either isolated from a tumor cell or synthesized in vitro, in a manner that generates a CTL response.

Research briefs written by Philip Bernstein.

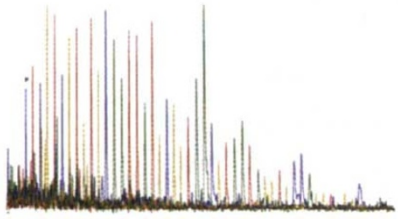
Protein import tags

While the plasma membrane provides a protective barrier to the cell's harsh environment, it also acts as a barrier for the introduction of many macromolecules, including most proteins. Certainly, this is to the benefit of the cell, but it can be a hindrance to a cell biologist or for the therapeutic delivery of protein drugs. Rojas et al. (p. 370) have used the hydrophobic region of a signal peptide sequence to deliver a protein into intact cells. This tag has previously been used to deliver only small peptides into cells; it has now been used to deliver a 41 kDa fusion protein that inhibits growth factor-induced cell signaling.



Finding folding function with phage

Escherichia coli may be the bacteria of choice for the routine expression of heterologous proteins, but it often finds creative ways of folding the protein that results in the formation of inclusion bodies in which the protein is no longer functional. Phage display allows proteins fused to the gene-3-protein (g3p) to be folded in the periplasm of the bacteria, where the cell's enzymatic folding machinery can be used. To search for bacterial proteins that might assist in periplasmic folding, Bothmann and Plückthun, have expressed a single-chain antibody that folds very poorly in the periplasmic space as a g3p fusion protein along with a library of *E. coli* proteins expressed on the same phagemid. By selecting for active antibody, the authors were able to identify the periplasmic protein Skp as a cofactor that enhances the proper folding of heterologously expressed proteins.



If the soon-to-be-sequenced human genome is to be used in medical diagnosis of disease, high-throughput methods of DNA analysis will be needed. One method, MALDI-TOF mass spectrometry has taken a giant step forward. In this issue, Fu and colleagues (p. 381) have used this technology to sequence a hot spot of mutations in the *p53* gene. Small DNA samples, similar to those used in the gel-based Sanger sequencing method, as well as the ability to perform the sequencing reactions in a single tube and automation, have allowed these authors to accurately sequence mutations in a heterozygous template. The potential for high-throughput should more than compensate for the relatively short read lengths available from individual spectra.

Bacteriostatic PNAs

As the sequencing of microbial genomes becomes more and more routine, the potential for deciphering genetic pathways and discovering new antibiotics becomes more enticing. Another tool for functional genomics, and possibly a new class of antibiotics, has been developed by Good and Nielsen (see pp. 332, 355). Bacteria have not been the targets of designed antisense oligomers to the same extent as eukaryotic cells. Using antisense peptide nucleic acids (PNAs)—DNA mimics containing a peptide backbone—the authors show that gene inhibition can be achieved in a permeable strain of *Escherichia coli*. Antisense molecules were targeted to the expressed β-lactamase RNA, which resulted in switching the phenotype from antibiotic resistant to ampicillin sensitive.

Genetically engineered herbicide-resistant crops are finding their way out of the laboratory, to the field, and into the marketplace despite lingering concerns of horizontal gene transfer. The herbicide glyphosate competitively inhibits the essential enzyme EPSPS. Overexpression of EPSPS, by introducing the gene into a crop's nuclear genome can result in herbicide-resistant plants. The altered genome, however, has the potential of being dispersed to non-transgenic plants, possibly even to weedy relatives, through the plant's own pollen. In contrast, genes incorporated into the chloroplast genome, which is maternally inherited, do not escape through pollen. By introducing the petunia EPSPS gene into the chloroplast genome of tobacco, Daniell et al. (see pp. 333, 345) have created plants that are resistant to high levels of glyphosate, which transmit the phenotype in a manner consistent with prevention of outcrossing to weedy relatives.