Cloning for conservation

The creation of a functional chimeric fetus via nuclear transfer between two different species has been demonstrated for the first time, with significant implications for wildlife conservation (*Cloning* **2**, 79–90, 2000). Previous in vitro studies had shown the feasibility of nuclear transfer between sheep, pigs, monkeys, or rats and enucleated bovine oocytes. In the new report, a collaboration of academic and industrial researchers has taken the first steps in cloning an endangered wild Asian ox (or gaur; *Bos gaurus*) by electrofusing fibroblasts from the animal's skin with enucleated bovine oocytes. Twelve percent of the reconstructed oocytes developed into blastocysts, and 18% of these developed to the fetal stage when transferred to pseudopregnant cows. Inspection of fetuses removed at days 46 and 54 (twins), and a fetus aborted at day 202, revealed no evidence of gross external abnormalities. Microsatellite and cytogenetic analysis also confirmed that the genome of the cloned animals was *gaurus* in origin, whereas the mitochondrial DNA was bovine. According to lead author Robert Lanza of Advanced Cell Technology (Worcester, MA), one pregnancy is still ongoing and is due for delivery by cesarean in late November. "We are also working with the Spanish government to use this technology in goats to clone an extinct bucardo mountain goat from preserved cells," he says. *AM*

Meningococcal mutagenesis

A novel mutagenesis technique provides urgently needed information about the pathogenesis of meningococcal meningitis. In November's *Nature Medicine* (**6**, 1269–1274, 2000), Christopher Tang and colleagues report the use of signature tagged mutagenesis (STM) to identify genes required for *Neisseria meningitides* virulence. Tang and his team modified *N. meningitides* DNA in vitro using the transposon Tn10. The pathogens incorporate exogenous DNA into their genome through homologous recombination, allowing efficient uptake and integration of modified alleles. Screening for mutants that were unable to infect the bloodstream of neonatal rats led to the identification of 73 genes, only eight of which were previously associated with pathogenicity. "The novelty of this work is that the insertional mutagenesis was carried out in vitro, overcoming limitations imposed by currently available tools for the genetic manipulation of *N. meningitides* " says bacteriologist Ian Feavers of the National Institute for Biological Standards and Control (UK). The results provide the first comprehensive analysis of the genes involved in meningococcal meningitis and may lead to new vaccines/therapeutics. *KN*

New twist on fluorescent antibodies

A team of scientists at The Scripps Research Institute and the Skaggs Institute for Chemical Biology have alighted on a photochemical phenomenon with significant potential for fluorescent labeling of analytes in diagnostics and clinical applications (*Science* **290**, 307–313, 2000). Complexes between monoclonal antibodies (mAbs) and derivatives of the aromatic hydrocarbon stilbene produce an intense blue fluorescence when irradiated. Counterintuitively, low temperatures abolish this fluorescence (freezing normally intensifies light emission). Emission spectra from fluorescence spectroscopy suggest the formation of an excited-state complex ("exciplex") between the mAb and the stilbene derivative. Crystallographic and time-resolved spectroscopic data on the mAb–stilbene complex indicate that the fluorescence results from a dynamic interaction between the stilbene and the indole ring of a tryptophan residue in the mAb binding site. Lead author Kim Janda hopes to develop artificial nucleoside bases derivatized with stilbene (which, together with the mAb, could be used in gene sequencing) and stilbene-derivatized substances for cell labeling and tracking in the body. "Unlike traditional reporters, the fluorescence is not subject to photobleaching, and the stilbene system is robust, well characterized, and inexpensive," he says. *AM*

Differentiating ES cells

Researchers have published the first systematic analysis of the effects of various inducing factors on embryonic stem (ES) cell differentiation (*PNAS* **97**, 11307–11312, 2000). The work represents an important step toward efforts to direct ES cell differentiation toward specific cell types (e.g., insulin-producing pancreatic β-cells) for cell replacement therapies. To achieve their aim, Douglas Melton and his colleagues first tested ES cells for the expression of eight inducing factor receptors to ensure the pluripotency of ES cells in five-day old embryoid bodies (EBs). The EBs were then treated with the eight inducing factors and grown for 10 days. When the cells were assayed for 24 cell-specific genes to determine the resulting germ cell layer inductions, none of the growth factors directed differentiation exclusively to one cell type, and the researchers concluded that inducing factors both inhibit and induce differentiation of specific cell types, with a complex combination of possibly hundreds of inducing factors required for complete cell specialization. Currently, knowledge on the pathways of stem cell differentiation signaling is very limited: "This work shows how to approach the problem," says Melton. *AB*

Gene detection by array

Although sequencing of the human genome is nearly complete, deciphering its genes, estimated to represent less than 5% of the total sequence, is still a challenging problem. One popular strategy for identifying genes relies on expressed sequence tags (ESTs), which can be reassembled together to reconstruct the genome's coding regions. But this approach misses a significant fraction of genes, according to a report in November's *Nature Genetics* (**26**, 315–318, 2000). The study, by David Rank and co-workers, is based on a clever application of microarray technology to the task of gene discovery. The researchers began by designing PCR primers from predicted open reading frames in 350 megabases of genomic sequence. They amplified 9,498 500-base sequences directly from genomic DNA and spotted the amplicons onto microarrays. Hybridization with labeled cDNA from seven tissues and three cell lines showed that 51% of the arrayed sequences were expressed in at least one of the ten samples, and of these, about 15% were expressed in only a single sample. Many of the latter turned out to be novel (i.e., absent from GenBank's EST database). First author Sharron Penn says the technique "allows the confirmation of putative genes predicted by a series of gene finding algorithms." *KA*

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