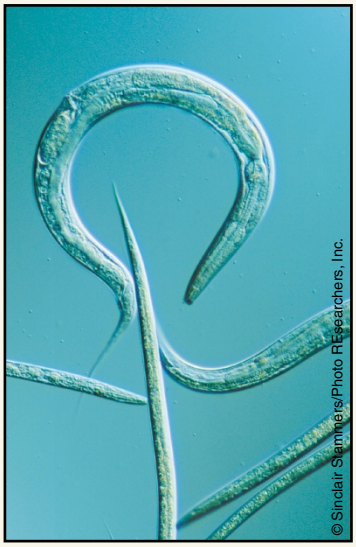
Functional genomics fed to worms



Julie Ahringer and her colleagues have devised an RNAi-based approach to generate high coverage loss-of-function phenotype libraries in the nematode worm *Caenorhabditis elegans* (*Nature* **421**, 231–237, 2003). They achieve targeted gene inactivation by feeding the worms bacteria expressing double-stranded RNA homologous to the genes of interest. With this approach the function of approximately 86% of all 19,427 predicted genes in *C. elegans* was inhibited. Important conclusions from the global analysis of the resulting phenotypes include: genes with orthologs in other eukaryotic genomes are more likely to have detectable RNAi phenotypes; single-copy genes are also more likely to have an RNAi phenotype; and genes involved in basic metabolism and maintenance have a higher incidence of nonviable RNAi phenotypes. The study provides a platform for investigating *C. elegans* biology, for extending the use of RNAi-based technologies to functional genomic studies of metazoan genomes, and for directly providing insights into gene function and phenotype determinants in humans, given that over 50% of *C. elegans* genes have homologs in the human genome. *GTO*

Knockout xenopigs!

In an advance for xenotransplantation, David Ayares and colleagues have generated piglets lacking α1,3-galactose (α1,3Gal) epitopes, one source of the immunological barriers that have slowed the development of animal organ xenografts (*Science* **299**, 411–414, 2003). The piglets are the product of three successive rounds of cloning. First, the authors generated cloned piglets in which one allele of the gene encoding α1,3-galactosyltransferase (which synthesizes the α1,3Gal epitopes) had been disrupted by homologous recombination (*Nat. Biotechnol.* **20**, 251–255, 2002). Fibroblast cells from these animals were transfected with a construct designed to disrupt the second allele, and then selected with a toxin that kills cells bearing α1,3Gal epitopes. Nuclear transfer using cells that survived the selection yielded three fetuses lacking the epitopes. A third round of nuclear transfer with cells from these animals led to the birth of four apparently healthy double-knockout piglets. Disruption of the second allele was traced to a spontaneous mutation that inactivated the enzyme, not to integration of the targeting construct. *KA*

Research News Briefs written by Kathy Aschheim, Laura DeFrancesco, Meeghan Sinclair, and Gaspar Taroncher-Oldenburg.

NMR speed demon

In the last three decades, the field of protein structural mapping has greatly benefited from nuclear magnetic resonance (NMR) techniques. However, despite advances in NMR technology, only modest improvements have been achieved in resolution and coverage of large molecules. An ingenious approach has now been developed by Thomas Szyperski and Seho Kim at the University of Buffalo to dramatically reduce the sampling time required to obtain high-resolution, multidimensional NMR spectra (*J. Am. Chem. Soc.* **125**, 1385–1393, 2003). They describe an acquisition scheme that maximizes the amount of information extracted from a conventional NMR scan by sampling the subspectra resulting from the chemical shift multiplets generated during the experiment. Fourier transformation (FT) of these linearly combined subspectra provides a precise reading of the chemical shifts in the analyte and is equivalent to adding several dimensions in multidimensional NMR, a process that requires the collection of exponentially increasing numbers of spectra. This could save from days to weeks in analysis time for large proteins that require five- or higher dimensional FT NMR acquired with high digital resolution. The approach may not only increase the throughput of NMR studies, but also allow real-time NMR of molecular processes such as protein folding. *GTO*

Measuring parts of the whole

A new approach promises to facilitate quantitative analysis of specific interactions in protein complexes. Ruedi Aebersold and coworkers from the Institute for Systems Biology (Seattle, WA) have combined isotope-coded affinity tag (ICAT) technology with a purification step to examine protein complexes (*Nat. Genet.* **33**, 349–355, 2003). In their approach, a protein complex of interest is purified; in the first example, RNA polymerase II (Pol II) preinitiation complex is isolated using a promoter DNA affinity purification step. For comparison, they also perform a control purification in which the complex is not enriched. The isolated proteins are then labeled with a 'heavy' or 'light' version of the ICAT label and digested to peptides. The peptides are separated using sequential chromatographic steps, and analyzed by electrospray ionization–tandem mass spectrometry. Components of the complex are identified by their increased abundance in the specific purification compared with the control. They also applied the approach to detect changes in the abundance and composition of immunopurified yeast transcription factor STE12 protein complexes from yeast cells grown under different conditions. *MS*

DNA damage detection on film

In a recent issue of the *Journal of the American Chemical Society* (**125**, 1431–1436, 2003), researchers at the University of Connecticut report on a rapid, *in vitro* system for detecting DNA damage. Detection is based on the propensity of chemically damaged DNA to undergo electrochemical oxidation more readily than double-stranded DNA. Previous work had shown that electrodes coated with ultrathin (20–40 nm) DNA films could detect disruptions in the DNA double helix caused by exposure to styrene oxide (a genotoxic metabolite of styrene generated on exposure to cytochrome P450 or myoglobin). In the present report, the authors create films with alternating layers of DNA and enzymes. Using square-wave voltammetry to measure current generated when damaged DNA reacts with $Ru(bpy)_3^{3+}$ ions, they show that genotoxic levels of styrene oxide induced by oxidizing chemicals can be detected within minutes on the same film. The system mimics toxicity reactions that can cause DNA damage in the liver and offers a quick screen for checking the genotoxicity of new compounds. *LD*