GENOMICS

# Clone of the cave bear

**For the first time, biologists have developed a strategy for the efficient cloning and analysis of genomic DNA from ancient species—an approach with the potential to unlock secrets of our own genetic ancestry.**

Some things age gracefully—unfortunately, DNA isn't one of them, and researchers hoping to uncover genetic information about long-dead species have been thwarted by degradation that leaves genomic DNA severely fragmented. "Mitochondrial DNA has been recovered successfully from a wide range of samples 50,000 years and older," explains James Noonan, a postdoctoral fellow working with Edward Rubin at Lawrence Berkeley National Laboratory. "But genomic sequence has been recovered in very small amounts, and that's under special circumstances, where the material's frozen… or else it comes from a very dessicated environment."

The main problem is that most researchers, recognizing the scarcity of ancient genomic material, have tried to amplify it first—but the DNA is generally far too degraded to amplify. Noonan and Rubin, working with Svante Pääbo's group at the Max Planck Institute, decided on a more direct approach. They began by extracting total DNA from cave bear tooth and bone samples, dated as being more than 40,000 years old. This DNA was enzymatically repaired, and then directly ligated into vector backbones to generate genomic libraries. The researchers benefited from the recent completion of the dog genome sequence—dogs and bears are closely related, with over 90% genomic sequence identity—and used this data as a framework for analysis of their sequence information.

Most of what was cloned came from environmental fungi or microbes, but a considerable amount of sequence was identified that lined up quite well against annotated dog sequence, revealing putative cave bear exons and regulatory sequences. "If we had the will, and the money, and the time, we could sequence the entire genome from these libraries," says Noonan. This is not the goal, however, and the team sees this study as a trial run for a more ambitious and relevant project. "The cave bear was useful because it's the same age as Neanderthal remains and [has] about the same level of preservation. They come from the same kind of environment," says Noonan, and after optimization of their techniques, the team will immediately begin work on reconstructing the Neanderthal genome, for direct comparison against humans and chimpanzees. Noonan sees great promise in this approach, and suggests it may even work with far older specimens. "If you look back at the literature in the ancient DNA field, you will find everybody saying that this is impossible, that you will never get significant amounts of genomic DNA from any sample… but you can! And you can do it using methods that you use to sequence modern genomes."

**Michael Eisenstein**

RESEARCH PAPERS

Noonan, J.P. *et al.* Genomic sequencing of pleistocene cave bears. *Science*; published online 2 June, 2005.

CHEMICAL BIOLOGY

# Uncaging an antibiotic

**The use of a 'caged' version of a protein synthesis inhibitor allows its release at a high concentration in a restricted region and paves the way for use of this technique to precisely inhibit biological processes.**

Regulation of protein synthesis is important in many biological processes, but it is difficult to study because of a lack of methodologies to interfere selectively with protein synthesis with temporal and spatial precision. To address this problem, a team of researchers led by Timothy Dore at the University of Georgia and Erin Schuman at the California Institute of Technology used a caged version of the antibiotic anisomycin, an inhibitor of protein synthesis that blocks the peptide bond–forming reaction in eukaryotic ribosomes.

This caged compound, *N*-([6-bromo-7-hydroxycoumarin-4-yl]methyloxycarbonyl) anisomycin (Bhc-Aniso), comprises a photolabile protecting group covalently bound to the antibiotic anisomycin. Exposure to light cleaves away the protecting group, thus 'uncaging' the protein synthesis inhibitor.

For the work described in *Chemistry & Biology*, the team synthesized three different caged anisomycins and found that Bhc-Aniso had the fastest uncaging kinetics and was sufficiently sensitive to two-photon excitation for biological use. They tested Bhc-Aniso in an *in vitro* protein translation system, CHO cells, neurons and HEK293 cells, and found that photoreleased anisomycin can inhibit protein synthesis with spatial specificity. In cells expressing a green fluorescent protein reporter, the maximum decrease in fluorescence was within 100 μm of the center of the uncaging spot, whereas cells outside of the uncaging spot had constant fluorescence or a slight increase as more reporter was synthesized over the course of the experiment.

The successful use of Bhc-Aniso in neurons will allow researchers to study the role of local protein synthesis in this system. "When and where in the neuron is protein synthesis used to bring about changes? How does protein synthesis regulate synaptic strength and axonal outgrowth? These are questions we'd like to answer," says Schuman.

This protecting group potentially has broad-ranging applications in the study of other physiological processes. According to Dore, "Anything that has an amine, an alcohol, a phosphate, a ketone, an aldehyde or a carboxylate can be attached to Bhc… It's a general protecting group." This strategy could, for example, be used in drug development by administering the caged drug and then studying how the system responds when the drug is selectively activated in various cellular locations.

So far, Dore has developed several caging groups, and he plans to apply them to biological systems: "We are working on strategies and using different caging groups to enable proteins, RNA, drugs—any biological effector you can imagine—to be activated in a spatially and temporally controlled manner, hopefully using two-photon excitation, which would potentially enable subcellular localization of the release."

**Irene Kaganman**

RESEARCH PAPERS

Goard, M. *et al.* Light-mediated inhibition of protein synthesis. *Chem. Biol.* **12**, 685–693 (2005).