RNA forms a hybrid with the cytosine-rich DNA template leaving the other DNA strand (the nontemplate strand) on its own (*6*). The RNA in the hybrid is sensitive to ribonuclease (RNase) H, an enzyme that specifically snips the RNA in DNA-RNA hybrids. In their new work, Tracy *et al.* (*1*) now demonstrate that after B cells from mouse spleen are induced to undergo CSR, RNA-DNA hybrids form within the S regions of the DNA. They also provide data indicating that these RNA-DNA hybrids are important for CSR in vivo.

To isolate S region RNA-DNA hybrids, the investigators used RNase A (which cleaves single-stranded RNA), followed by DNase I (which cleaves all DNA), to digest all of the nucleic acids of B cells activated by cytokines and lipopolysaccharide to induce CSR. In the residual material (which should contain RNA that was present in RNA-DNA hybrids) they detected a heterogeneous RNA population containing S region sequences. The RNA species were found in B cells that had been induced to express the germ line transcripts, and the S region sequences in the RNA-DNA hybrids were derived from the intron regions of these transcripts. Treating activated B cells with RNase H before DNase I eliminated this RNA population.

To test whether the RNA-DNA hybrids are required for CSR, Tracy *et al.* (*1*) created transgenic mice that constitutively express RNase H in splenic B cells (and presumably in many other cells). The immune system of these mice is normal in a variety of tests, although they have greatly reduced levels of S region RNA-DNA hybrids. When the mice were immunized, their B cells showed an impaired capacity for class switching relative to the B cells of wild-type mice, as assessed by levels of antigen-specific serum IgG and examination of DNA recombination within S regions.

These results raise several interesting questions. What structural features of the S region are required for formation of the stable hybrid during transcription? Although mouse and human S regions are G-rich, and runs of guanosines are known to form unusual structures, this may not be a crucial feature because the S region upstream of the Ig C$\mu$ gene in the frog *Xenopus* is A-T rich (*7*). It is possible that the stretches of purine-pyrimidine asymmetry, the repeated sequences, and the numerous palindromes (sequences that read identically in both directions) in the S regions of mouse, human, and *Xenopus* are essential for CSR.

How does the RNA-DNA hybrid regulate class switching? Tracy and co-workers suggest that it could serve as the recognition target for a hypothetical endonuclease that initiates CSR by creating double-strand breaks (*8*) (see the figure). This is an interesting hypothesis, although none of the available data indicate that germ line transcripts are required for formation of double-strand breaks in S regions.

Although the authors do not determine whether splicing of germ line transcripts is required for formation of RNA-DNA hybrids, it is possible that splicing may stabilize the hybrids by removing extra nucleotides incapable of participating in their formation. Answers to these and other questions raised by the Tracy *et al.* study will bring us to a much better understanding of the mechanism of CSR.

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PERSPECTIVES: ECOLOGY

# Black-Footed Ferret Recovery

**Andy Dobson and Annarie Lyles**

The black-footed ferret, *Mustela nigripes*, is a nocturnal carnivore indigenous to the western United States (see the figure) that dines almost exclusively on prairie dogs. By the 1970s the black-footed ferret was believed to be extinct, the initial decline in its numbers matching that of prairie dogs, which were poisoned, shot, and otherwise extirpated to improve range-land habitat for cattle. The discovery of a remnant population of black-footed ferrets in Wyoming in 1981 launched a struggle to save the species that brought together an interdisciplinary team of veterinarians, zoo-biologists, wildlife managers, and behavioral ecologists. The cooperative efforts of at least 35 private and government agencies have succeeded in rebuilding a healthy captive population of ferrets large enough to provide recruits to seed new wild colonies.

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At the dawn of the 21st century, an important conservation goal has been achieved: The black-footed ferret has been returned to the wild and is now breeding in at least four locations in the Rocky Mountain region of the western United States. The successful restoration of black-footed ferrets (as well as gray wolves) means that the mammalian fauna of this region is as diverse now as it was 100 years ago. Obviously, formidable challenges remain in the restoration of this region. In particular, invasive plant species and agriculture have completely altered the flora and invertebrate fauna of vast areas; similarly, huge demands for freshwater have severely damaged riverbank habitats. The struggle to save the black-footed ferret has yielded fascinating lessons about captive breeding, behavioral conditioning for reintroduction, disease management, and prairie dog politics that can be applied to saving other endangered animals.

The captive breeding program for black-footed ferrets began in October 1985 with six ferrets from the last known wild population at Meeteetse in Wyoming. Two of these ferrets were infected with canine distemper; this spread to the other four, and all six died. Intense efforts were then launched to capture as many of the last remaining free-living ferrets as possible. These were vaccinated against distemper soon after capture, and all 18 captured ferrets survived to form the sole captive breeding population for the species. No young were born in the population's first year in captivity, but in the second year two females produced a total of eight kittens, of which seven survived. These were followed by 34 kittens in 1988, 58 in 1989, and 66 in 1990.

The captive breeding program aims to manage a captive population of 240 ferrets (90 males and 150 females) of prime breeding age (1 to 3 years old) housed in several facilities to reduce the risk of a catastrophe such as a fatal disease outbreak. One of the main goals of this program is to maintain 80% of the genetic

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variability in the founder population for 25 years. At present, thanks to careful genetic management, the mean inbreeding coefficient of just over 0.125 has increased very little since the first few captive generations (an inbreeding coefficient of 1.0 means that the entire founder population is genetically identical, and of 0.5 that its members are full siblings). The population has passed through a severe bottleneck, and probably somewhat less than 25% of the original 19 founders' genes have made it into the current population. Even though all animals are fairly closely related, sharing 11 to 12% of their genome (so everyone is effectively at least a cousin), population biologist Jonathan Ballou of the National Zoological Park in Washington, D.C., notes that "no effects of inbreeding have been detected in any of the measurable fitness terms." Today, half the population resides at the National Black-Footed Ferret Conservation Center (the former U.S. F&W Sybille breeding facility) near Meeteetse in Wyoming. The rest are in zoos and conservation centers including the National Zoos Conservation and Research Center at Front Royal, Virginia, the first zoo to establish a captive colony.

As the population is small, it is important that all individuals contribute to the breeding program. Artificial insemination techniques and cryopreservation of sperm were successfully developed at the National Zoo by a team led by JoGayle Howard. The procedure uses a tiny slender laproscope to deposit sperm directly into the uterus of a female ferret. Cryopreservation has allowed the development of a Black-Footed Ferret Genome Resource Bank, a frozen repository of sperm from the genetically most valuable males. In addition, pseudo-pregnancies were reduced after Howard discovered that young males have delayed reproductive readiness. In the 12 years since 1987, the captive breeding program has produced over 3000 ferrets. This has allowed reintroduction of captive ferrets to the wild at a number of sites, initially in Wyoming then in South Dakota, Montana, and Arizona. Other potential sites have been identified in Colorado, Utah, and New Mexico.

A number of methods have been used to prepare captive black-footed ferrets for release. These techniques have significantly improved the survival of ferrets after their release into the wild. Although behavioral training for release is widely believed to be beneficial, in practice benefits are generally a matter of faith rather than science. Benjamin Beck, the American Zoo and Aquarium Association's reintroduction advisor, observes that the efforts to precondition feeding behavior in the black-footed ferret provide "one of the few sets of data I know of that really demonstrate the effect of prerelease training of captive-born animals on postrelease survival." One of the most successful techniques is to rear black-footed ferrets in large enclosures placed on top of existing prairie dog colonies. Ferrets reared in this way disperse over a much smaller area than animals reared in isolation in more artificial pens (*1*). This results in four times as many successful reintroductions from outdoor pens as from cage-reared animals.

Astrid Vargas at the U.S. Fish and Wildlife BFF Conservation Center in Laramie, Wyoming, has shown that black-footed ferret predatory behavior has both innate and learned components (*2*). Predation usually occurs underground at night; most of the behavioral cues are therefore olfactory. The ferrets search burrows for sleeping prairie dogs, then position themselves on top of a dormant prairie dog, which they wake with a light tap followed by a sudden grab for the throat of the waking animal. This response seems to be innate because even naïve black-footed ferrets will bite in the correct location. However, the ability of ferrets to locate prairie dogs and assume the position for attack seems to require experience. Black-footed ferrets that have been exposed to prairie dogs in large pens before release are much more successful at capturing prairie dogs than either purely naïve individuals or those trained to catch hamsters in small artificial pens.

**A furtive friend.** The black-footed ferret, once believed to be extinct in the United States, has made a remarkable recovery thanks to captive breeding programs that have yielded sufficient animals to ensure seeding of wild colonies after the captive ferrets have been released.

CREDIT: MICHAEL DICK/ANIMALS ANIMALS

Three states (Colorado, Montana, and New Mexico) now have on-site breeding and preconditioning pens. This should maximize the success of reintroductions at these sites. Unfortunately, reintroduction at the original Wyoming site has had to be curtailed after an outbreak of sylvatic plague that killed not only the ferrets, but also their prairie dog prey. Plague is occasionally introduced to prairie dog colonies by coyotes and badgers, and usually leads to the death of 99% of the colony. Canine distemper remains a second major disease threat to black-footed ferrets. Although a vaccine is potentially available for reintroduced animals, the company that has manufactured it has so far been unwilling to market the monovalent form that can be used in black-footed ferrets because the market is so small. This puts a further constraint on the future success of reintroduction efforts.

The ultimate fate of black-footed ferrets in the wild still depends on the fate of prairie dogs. Currently only one of five species of prairie dog, the Utah prairie dog, is listed as threatened. However, all of the other species have experienced large-scale reductions in their range—the range of black-tailed prairie dogs contracted by 98% during the last 100 years. The National Wildlife Federation recently petitioned to have the black-tailed prairie dog placed on an emergency list as a threatened species. The U.S. Fish and Wildlife Service refused the emergency listing, but did agree to review the status of the species to see if listing was warranted. Hopefully, this will lead soon to listing of the blacktailed prairie dog as a threatened or endangered species. There are very few prairie dog colonies in excess of the 10,000 individuals required to sustain a viable black-footed ferret subpopulation of around 10 breeding pairs. This has delayed reintroduction of more ferrets according to Della Garrell, the captive program coordinator of the Cheyenne Mountain Zoo. Moreover, the distances between these big prairie dog colonies are too large to be effectively traversed by reintroduced black-footed ferrets as they disperse.

Thus, the future preservation of prairie dogs and black-footed ferrets will be strongly tied to the future use of public and private lands in the western United States and northern Mexico. Although the Bureau of Land Management has made some progress in protecting prairie dog colonies on private lands in Utah, other areas remain subject to frequent abuse by off-road vehicles and leases for grazing cattle. Unfortunately, most prairie dog colonies tend to be on private lands where they are subject to the whims of landowners. Most ranchers see prairie dogs as a threat to their livestock, although the evidence for this is equivocal (*3*). David Wilcove, senior ecologist with the Environmental Defense Fund, sees both the prairie dogs and ferrets as an important opportunity, rather than a threat to cash-strapped cattle ranchers, most of whom make only a modest one dollar per acre per annum. "In the long term, the biggest obstacle to recovering the black-footed ferret is the shortage of really large prairie dog colonies. There aren't many places left in the West that have enough prairie dogs to sustain a viable population of ferrets." Wilcove and his colleagues are examining the possibility of using economic incentives to encourage ranchers and farmers to maintain prairie dog colonies on their property. As he says "Wouldn't it be great if we could declare a cease-fire in our century-old war against prairie dogs? I'm confident there's enough room for both people and prairie dogs in the American west." Hopefully some of these prairie dog colonies may prove large enough to be sites for future introductions of captive black-footed ferrets, allowing their range to be extended even further.

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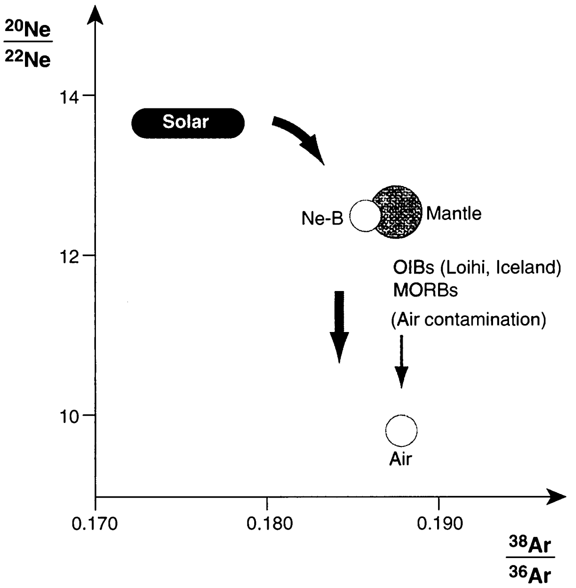
PERSPECTIVES: GEOCHEMISTRY

# Earth's History Trapped in the Mantle

**Ichiro Kaneoka**

The isotopic compositions of noble gases in the mantle have been assumed to be the result of two processes: the incorporation of nuclides from various sources during the formation of the solar system, and the loss or addition, during the evolution of Earth, of radiogenic components that decay or accumulate at defined rates. If this assumption is correct, measurements of the isotopes of the noble gases should be able to constrain models of Earth's birth and evolution. Indeed, the ratios of isotopes in Earth's atmosphere are quite different from those in extraterrestrial materials. And so, if noble gases from Earth's atmosphere have been incorporated in the mantle—either early in Earth's history or over time—the assumed starting composition of the mantle would change. There has been considerable debate whether the nonradiogenic noble gases in the mantle reflect mostly solar-like or mostly atmosphere-like compositions. As reported on page 1036 of this issue, Trieloff *et al.* (*1*) have analyzed typical oceanic island basalts from Loihi, Hawaii, and Iceland with high-precision mass spectrometry in an effort to settle this problem, with strikingly clear results that point to a terrestrial atmospheric origin for these trapped gases.

The evidence so far has been mixed. Solar-like Ne isotopes have been reported in mid-ocean ridge basalts (*2*) and oceanic island basalts (*3*), supporting the idea of solar-type noble gases in the mantle (*4*). In addition, the $^{3}$He/$^{4}$He ratio in mid-ocean ridge basalts (*2*) and oceanic island basalts is one order of magnitude higher than that of the atmosphere, which suggests the presence of pristine components in the mantle with a likely solar origin, to which radiogenic $^{4}$He was added over time, causing a drop in the ratio. These solar components would have originated directly from the nebular gases from which the solar system was formed.



**Isotopic ratios.** The arrows indicate a conjectured sequence describing the evolution of noble gas isotopic ratios during the formation of Earth. OIBs, oceanic island basalts.

For the heavier noble gases Ar, Kr, and Xe, however, the situation is quite different. In the mid-ocean ridge basalts, atmosphere-like compositions have been reported for nonradiogenic isotopes on the basis of precise analyses of an anomalously gas-rich basalt called popping rock (*5*). Clear observation of excess $^{129}$Xe and $^{131-136}$Xe in the same sample precludes the possibility of air contamination at shallow depths because excess $^{129}$Xe is the decay product of the extinct nuclide $^{129}$I (half-life, 16 million years) and excess $^{131-136}$Xe is the fissiogenic product of both $^{244}$Pu (half-life, 83 million years) and $^{238}$U (half-life, 4.47 billion years). Although the isotopic compositions of oceanic island basalts are also indistinguishable from those of the atmosphere for nonradiogenic components, no clear excess $^{129}$Xe or $^{131-136}$Xe has been identified. Hence, such observations have been suggested to be the result of air contamination (*6*). Recent statistical work on data for mid-ocean ridge and oceanic island basalts and diamonds also suggests atmosphere-like nonradiogenic isotopes of heavier noble gases (*7*), and these too have no associated excess $^{129}$Xe or $^{131-136}$Xe.

Trieloff *et al.* (*1*) analyzed noble gas isotopes in a dunite (a mantle rock rich in olivine) from Loihi, Hawaii, and in volcanic glasses from Iceland by crushing the rock slowly in steps to release trapped gas and then performing high-precision mass spectrometry of the gas. They have observed a correlated excess of $^{20}$Ne and $^{40}$Ar and found evidence for excess $^{129}$Xe and $^{131-136}$Xe. Although many samples from Hawaii and Iceland have been analyzed to date, this is the first evidence that island basalts from these areas show clear excess $^{129}$Xe and $^{131-136}$Xe. Furthermore, the nonradiogenic isotopic compositions of Ar, Kr, and Xe are indistinguishable from those of the atmosphere, which suggests that the source of these oceanic island basalts had atmosphere-like components of heavier noble gases. Trieloff *et*

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