indefinitely in culture. Primary cell lines, which have not been immortalized, are not transformed by transfer of a single oncogene.

However, Weinberg, with Hartmut Land and Luis Parada of MIT, and, independently, H. Earl Ruley of Cold Spring Harbor Laboratory, have recently shown that primary cells can be transformed by two genes acting in concert. The MIT workers found that primary rat embryo fibroblasts are completely transformed to malignancy by a member of the *ras* oncogene family if it is transferred with the *myc* gene. Neither of these two genes can accomplish this result separately. In a similar fashion, Ruley showed that the same *ras* gene would transform a primary line of rat kidney cells in combination with a gene, designated E1A, from adenovirus.

Adenovirus and polyoma, both of which have DNA genomes, transform animal cells in a minimum of two steps. The E1A gene and the large T (for tumor antigen) of polyoma virus are needed to immortalize the cells. Then additional viral genes confer the characteristics of complete transformation and tumorigenicity on the immortalized cells.

The growing view is that transformation generally requires the activation of at least two oncogenes. "The results suggest," Weinberg says, "that the reason why carcinogenesis is multistep is a requirement for activating sequentially multiple genes." Some of the genes, including *myc* and E1A, appear to be needed for immortalization, whereas others, including *ras*, work later in the transformation pathway. The proposed role for *myc* in immortalization is consistent with the Leder group's finding that this gene is turned on by growth factors, implying that the *myc* product acts to facilitate cell division in some fashion.

In addition, chemical carcinogens that immortalize cultured cells may pave the way for the later action of oncogenes, according to Robert Nerbold and Robert Overell of the Institute of Cancer Research, Pollards Woods Research Station in Chalfont St. Giles, England.

Although investigators have shown that two oncogenes can collaborate to transform primary cells in culture, the results do not necessarily mean that just two events are sufficient. Additional, as yet unidentified, steps may also be required. Nevertheless, the experiments on oncogene cooperation begin to address some of the complexities of how gene changes may contribute to the multistep development of cancer.

—JEAN L. MARX

Isotopes Add Support for Asteroid Impact

Osmium isotope analysis supports an asteroid impact 65 million years ago but cannot exclude a huge volcanic eruption

Recent analyses of osmium isotopes deposited on the earth's surface 65 million years ago support the contention that an asteroid impact contributed to mass extinctions at the end of the Cretaceous Period, including that of the dinosaurs. The analyses reported in this issue (p. 613) appear to eliminate once and for all the possibility that over millions of years geochemical processes concentrated iridium, osmium, and other exotic elements from seawater or ground water. The newly determined isotopic ratios most likely resulted from some catastrophe, presumably an asteroid impact, but they do not eliminate the possibility of a mammoth eruption that spewed iridium-laden volcanic debris derived from the mantle. Other evidence weighs against such an eruption, but a consensus has not formed yet on that question.

High concentrations of iridium, osmium, and other platinum group metals in the sediments deposited at the boundary between the Cretaceous Period and the subsequent Tertiary Period prompted the suggestion that an asteroid struck the earth and caused mass extinctions at the end of the Cretaceous. Luis Alvarez, Frank Asaro, and Helen Michel of Lawrence Berkeley Laboratory and Walter Alvarez of the University of California at Berkeley began the current debate over the Cretaceous-Tertiary extinctions in 1980, when they argued that the iridium in the boundary layer must have come from an impacting body because no geological process had ever managed to bring much iridium from the iridium-rich mantle to the surface. Crustal rocks contain very little iridium, they noted, and meteorites are rich in the element. Skeptics wondered whether geochemical processes might have concentrated the dilute iridium supplied by crustal rocks and cosmic dust drifting down from space (*Science*, 20 November 1981, p. 896). The chemistry of the sediment layer at the boundary somewhat resembled that of meteorites, the skeptics conceded, but how many ways are there to make a sediment layer that chemically resembles meteorites?

Geochemical reactions cannot separate one isotope of a heavy element from another the way they can separate and enrich different elements, so researchers are turning to isotopic analysis for less equivocal tests of the impact hypothesis. Jean-Marc Luck and Karl Turekian of Yale University, whose report appears in this issue, chose to measure the ratio of osmium-187 to osmium-186. Like elemental ratios, this isotopic ratio does change. With time, rhenium-187 decays radioactively into osmium-187. And geochemical processes do change the proportion of rhenium to osmium and thus ultimately give different rocks different osmium isotope ratios. But this ratio should still provide a good test, Luck and Turekian reasoned. The present typical osmium isotopic ratio of meteorites is about 1 and that of continents is thought to be about 10, an easily distinguished difference. Rhenium decays exceedingly slowly—it takes 46 billion years for half of the rhenium-187 atoms initially present to decay to osmium-187.

Luck and Turekian's first problem was to demonstrate that if geochemical processes had indeed concentrated osmium, the osmium still carried the high isotopic ratio characteristic of the continents. To do that, they measured the osmium isotopic ratio in deep-sea manganese nodules, which can concentrate dissolved metals from seawater. The osmium-187/osmium-186 ratio in seven manganese nodules from the major ocean basins of the world ranged from 6.0 to 8.4, lower than expected for a purely continental source but well above the value of about 1 for meteorites.

Presumably, in addition to the osmium they received from the continents, recently formed manganese nodules have taken up osmium from the mantle—through hot spring activity at the mid-ocean ridge—and perhaps from cosmic dust. Hot spring activity was greater relative to continental erosion at the time of the Cretaceous-Tertiary boundary, so Luck and Turekian used the known seawater variation of the isotopes of strontium—another hot spring product—to estimate seawater's osmium isotopic ratio 65 million years ago. Their best estimate is 3.6, although Turekian guesses that this is an underestimate.

The group then measured the osmium isotopic ratio of two iridium-rich, Cretaceous-Tertiary boundary samples. One, from a marine sediment at Stevns Klint, Denmark, had a ratio of 1.65. The second, from the base of a freshwater coal bed in the Raton Basin of southeastern Colorado, had a ratio of 1.29. These results are "certainly different from anything I expected," says Turekian, who has been skeptical of the impact hypothesis. "It's very hard for me to imagine what else we could do with these numbers," he says, other than conclude that a large meteorite or meteorites hit the earth 65 million years ago. Only an iron or high-iron meteorite, with its high ratio of rhenium to osmium, could have provided ratios so far from 1, Turekian adds; on the basis of present geochemical understanding, no likely combination of continental and hot spring sources could have produced those ratios.

Other prominent geochemists agree. Says Wallace Broecker of Lamont-Doherty Geological Observatory: "The first-order result seems to me to be a very powerful confirmation of the Alvarez impact hypothesis. It is getting ever harder to explain it any other way."

The osmium isotopic results will not sweep aside all opposition to the impact hypothesis. The boundary ratios are not the same as those in stony meteorites, the most common sort. Iron meteorites might fit the osmium isotopic ratios and the abundances of other metals, but they are far rarer than stony meteorites, and an iron asteroid impact would be even less frequent than the once in 100 million years event presumed in the Alvarez hypothesis. There is also the unexplained difference between the ratios at the two sites, which Luck and Turekian prefer for the time being to explain by the impact of two different iron meteorites near the boundary—an even more infrequent event.

A current objection to the impact interpretation is that the osmium isotope test eliminates only continental rock as a source and not the earth's mantle. Geochemists had not seen this as a problem because volcanoes that tap the deep mantle for magma still produce lavas relatively poor in iridium—it tends to be left behind in the mantle. Then William Zoller and his colleagues at the University of Maryland serendipitously discovered early this year that while volcanic lavas and ash might be a poor iridium source, particles condensed from the hot gases of Kilauea volcano in Hawaii can be enriched up to one million times in iridium.

Apparently, magmas that have a fair amount of iridium and large amounts of fluorine, which is typical of such hot-spot, mantle-supplied volcanoes, can lose about 5 percent of their iridium as gaseous iridium fluoride, which becomes part of atmospheric particles upon cooling. In effect, the fluorine helps iridium distill away from the magma. Because the enrichment of iridium and other metals at Kilauea is strongly temperature-dependent, element enrichments at the boundary cannot generally be compared with the Kilauea enrichments. But researchers have noted the unexplained enhancement of selenium, arsenic, and antimony concentrations at the boundary; all are elements easily volatilized by volcanism.

"The first-order result seems to me to be a very powerful confirmation of the Alvarez impact hypothesis. It is getting ever harder to explain it any other way."

This unexpected iridium enrichment mechanism has a long way to go before anyone can use it to explain the composition of the Cretaceous-Tertiary boundary. First, there would have to have been a volcanic eruption large enough to supply the 400,000 tons of iridium thought to lie at the boundary. Scaling up from the January 1983 Kilauea eruption by a factor of 100 million, Zoller estimates that the largest eruptions in the geologic record could have supplied that much iridium, give or take a factor or 10 or so. These include the voluminous eruptions that formed the Columbia River flood basalts of about 15 million years ago and the Deccan flood basalts of northeast India, which are less accurately dated as having erupted about 65 million years ago—that is, at the time of the boundary.

The catch to a Deccan eruption source is that, at least in the case of the better studied Columbia River basalts, volcanologists are confident that many hundreds of thousands of years were required for the eruption of the bulk of the lava. Estimates of the duration of the event that deposited the iridium at the boundary range from 50 to 10,000 years. Even the most conservative estimates fall well below 100,000 years.

The next problem for a volcanic eruption is distributing the iridium around the globe. Most hot-spot eruptions, such as those at Kilauea, are modest, nonexplosive events incapable of carrying large amounts of material into the stratosphere, where winds would distribute it globally. But, as Stephen Self of the University of Texas at Arlington notes, exceptionally large hot-spot eruptions like Iceland's Laki eruption of 1783, which is dwarfed by the great flood basalt eruptions, are probably capable of lofting significant amounts of material to the stratosphere.

A more troublesome problem for a volcano may be the small spherules found around the globe at the boundary by Alessandro Montanari, Richard Hay, and Walter Alvarez of UC Berkeley and colleagues at the Lawrence Berkeley Laboratory. They argue that the chemical and mineralogical composition of these spherules requires that they condensed from vaporized rock under conditions expected after a large impact, not the milder conditions of an eruption. In addition, Alvarez says, no volcano could have carried such large particles—they average 0.3 millimeter in diameter—high enough in the atmosphere to transport them to the central Pacific and to Italy, where they were found. Only a huge impact, which could blow a hole through the atmosphere to space, would allow that, he says.

The controversy over a possible asteroid impact 65 million years ago is not over. Proponents of other explanations of the composition of the Cretaceous-Tertiary boundary have fewer options than ever, but they believe they have not run out of them entirely. Geochemists will be measuring the osmium isotopic ratios of more samples, but they will also be looking for isotopes of other elements to settle the question. Either way, by impact or eruption, the end of the Cretaceous still appears to have been one heck of a catastrophe.—**Richard A. Kerr**

Additional Reading

1. A. Montanari *et al.*, *Geology* (November 1983), vol. 11.
2. W. H. Zoller, J. R. Parrington, J. M. Phelan, *Science*, in press.