three cooling-related events strengthens the contention that they are indeed related to a single, brief, global cold spell.

Given the evidence that abrupt climate excursions can wipe out the better part of a continent's mammals and open a new chapter in evolution, it's no surprise that researchers are eager to understand what causes them. Climatologists have had no trouble coming up with possible causes for the long-term warming and cooling that form the background for these sudden events. The gradual warming that peaked 53 million years ago, for example, may have resulted when volcanoes or hot springs on the ocean floor drove excess carbon dioxide into the atmosphere; the long-term cooling of the Eocene and early Oligocene may have ensued when drifting continents changed the pattern of ocean currents, which affect climate by ferrying heat around the world. Alternatively, the uplift of the Himalayas and the Tibetan Plateau could have spurred the cooling by redirecting atmospheric circulation.

K. D. ROSE AND L. KIBIUK

Progenitor primate. *Tetonius*, one of the oldest known primates.

But neither the warming spike that ushered in the Eocene nor the pulse of cooling that followed it has any obvious trigger such as an asteroid impact. Zachos and company, however, see clues in the similarities between the events. Both were short-lived, lasting only about 100,000 years. Both were embedded in long-term temperature trends having the same direction as the short-term events. And both seem to have been accompanied by changes in ocean circulation and carbon cycling.

All those parallels lead Zachos and his colleagues to suggest two separate mechanisms that may work together to produce short-lived climate excursions during a time of gradual change. One is a tendency, proposed earlier by other researchers, for a gradually changing climate to jump from one relatively stable state to another. In one example, gradual cooling might lead to the sudden onset of an ice age when the temperature reached a critical threshold below which snow no longer melts away each summer and begins to accumulate rapidly from year to year. During gradual warming, ocean circulation might create another threshold; at a certain point in the warming trend, ocean currents might suddenly reorganize, causing the warming to surge.

Threshold crossings might explain the abrupt onset of the two excursions, but not their brief duration before climate returned to a new but less extreme state. To account for the temporary overshoot, the Michigan group invokes feedbacks—the tendency of some processes, once pushed in one direction, to feed on themselves and drive even further in that direction. The greenhouse effect of atmospheric carbon dioxide might lead to feedbacks in both warming and cooling, says the Michigan group. In the short run, note the researchers, the microscopic plants of the ocean's surface waters can affect carbon dioxide concentrations by pumping carbon from the atmosphere into the deep sea in the form of a rain of organic debris. If an abrupt change in circulation during a cooling drove that pump faster by supplying more nutrients to the plants, for example, the greenhouse effect would weaken and climate would cool even further. Only when the slow overturn that links surface waters with ocean depths—a much stronger influence over atmospheric carbon dioxide than the surface waters—took control thousands of years later would the global greenhouse stabilize.

But it will take more than the two climate twitches at either end of the Eocene to firm up these speculations. To get a better reading on what causes these climate excursions, and how important they have been in the history of life, paleoclimatologists and paleontologists will be teaming up to test other seemingly gradual climate transition/extinction pairs for possible abruptness. Closer to home, climate researchers are wondering: If gradual climate change lasting millions of years was enough to derail the climate system temporarily, what is in store during the uniquely rapid greenhouse warming predicted for the next century?

–Richard A. Kerr

Additional Reading

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PROTEIN STRUCTURE

An Intimate Look at Nitrogen's Bio-Partner

Nitrogen is a star of biological chemistry—a vital player in nucleic acids such as DNA and in the proteins responsible for every organism's structure and function. It can be a reluctant performer, though: Atmospheric nitrogen, its two atoms joined by a strong triple bond, ordinarily won't link up with other elements except under extreme duress, such as the high temperatures and pressures created in a lightning bolt or in industrial syntheses. Indeed, nitrogen might never have won much of a part on the biological stage without help from a powerful costar, an enzyme called nitrogenase, which can break the strong bonds of atmospheric nitrogen and "fix" nitrogen into ammonia (a precursor of other nitrogen-containing compounds), even at room temperature and pressure. But for decades the enzyme that performs this feat has remained in the shadows, the details of its structure and workings a mystery—until now.

In this issue of *Science*, protein crystallographer Doug Rees of the California Institute of Technology and his colleagues chase away some of those shadows. On pages 1653 and 1677, they offer a close look, based on years of painstaking x-ray crystallography, at the nitrogenase complex's two components: an iron-containing protein and a larger, molybdenum- and iron-rich partner (the so-called MoFe protein). The resulting portrait is detailed enough to allow the researchers to speculate about how the enzyme plays part of its role—how it uses adenosine triphosphate (ATP), the chief energy currency of the cell, to power the nitrogen-fixing process.

The entire picture isn't in focus yet—a fact that was underscored at last month's American Chemical Society meeting in Washington, D.C. There, Purdue University crystallographer Jeffrey T. Bolin, who has been racing Rees' group to unravel the structure of nitrogenase, presented unpublished data about the structure of the metal-containing centers of the MoFe protein—data that bear out much of Rees' model but contradict some parts of it. But chemists and geneticists are already eyeing the new structure as a guide to designing synthetic catalysts that could mimic the activity of the natural enzyme, which is found only in certain bacteria that live in the soil and on the roots of a few tropical plants and legumes such as peas and alfalfa. To Harvard Univer-

sity chemist Richard Holm, it all adds up to "the protein structure of the decade."

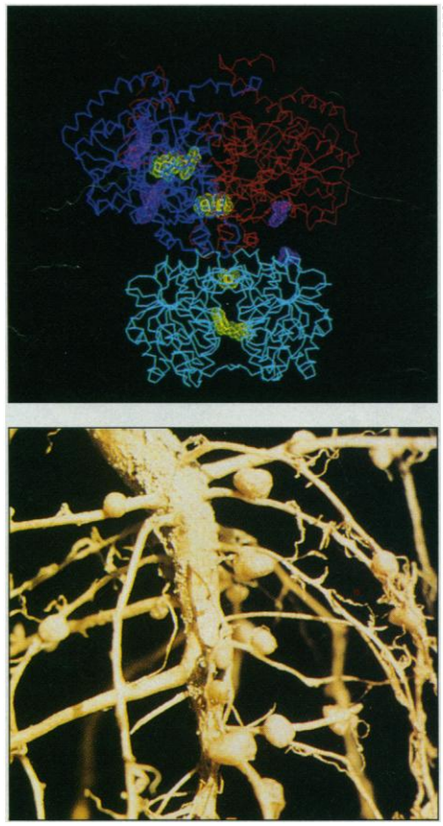
The route up this crystallographic Everest was strewn with obstacles. One is the instability of the enzyme—exposure to oxygen inactivates it within seconds. Making the complex even harder to analyze is the size of the MoFe protein: At 16,000 nonhydrogen atoms, it's twice as big as the bacterial photosynthetic reaction center, another difficult protein, whose structure won a 1988 Nobel Prize for a trio of researchers from the Max Planck Institute. Finally, proteins with metal clusters can make temperamental subjects for crystallography. To create vivid reference points in the raw x-ray diffraction data, crystallographers often add metal atoms to the protein under study. But if the protein already contains certain metals, such as iron, the added metal atoms can disrupt its structure.

Surmounting all these hurdles, says Rees, took a lot of hard slogging—finding ways to protect the enzyme from oxygen, choosing suitable metal atoms, and analyzing many different crystal forms of the protein to get as sharp a portrait as possible. All told, the task took about 10 years for the iron protein, and 2 more for the MoFe component. That's a lot longer than Rees had expected when—still a postdoc at Harvard—he first proposed the project. "If I had known then how long it would take, I might not have taken on the project," Rees said at one point in the work. But to judge from the fact that Bolin's group at Purdue has been in pursuit of the same goal for about 10 years, there's no quick route to the nitrogenase structure.

Over the years, both groups presented preliminary looks at the structure, and the latest results flesh them out. Two years ago, for example, Rees and his colleagues Millie Georgiadis, Pinak Chakrabarti, Debbie Woo, John Kornuc, and Hiromi Komiya unveiled a model of the iron protein: a butterfly-like structure with two identical subunits as the wings and an iron-sulfur cluster at the head. The refined structure confirms that general scheme but adds detail by pinpointing all but five of the protein's 289 amino acids and hinting at how the iron protein works.

Iron butterfly

Researchers had already established that the iron protein is the enzyme component that binds and cleaves ATP, using the resulting energy to drive the transfer of electrons to the MoFe protein, where they ultimately serve to reduce nitrogen into ammonia. The new structure shows that the ATP binding site lies at a kind of pivot point between the wings of the butterfly. This arrangement, says Rees, is reminiscent of proteins including the oncogene product H-ras p21 and the recombination protein rec A, which are known to change shape when they bind a nucleotide such as ATP. He thinks that the iron protein, too, probably changes shape when it binds ATP. That shape change, Rees thinks, might act to force electrons through the protein and over to the MoFe protein.



DOUGLAS REES

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The princess in the pea. Bacteria living in lumps on the roots of peas and other legumes produce nitrogenase(*top*), made up of an iron protein (*light blue*) and a molybdenum-iron protein. Metal clusters, thought to be crucial to the enzyme's working, are shown in *yellow*.

Just how the MoFe protein uses those electrons to reduce nitrogen remains more mysterious. For one thing, the new results presented in this issue offer a far less complete and detailed look at the MoFe protein than at its companion: Rees and his student Jongsun Kim concentrated their efforts on the metal-containing parts of the MoFe protein, which are thought to play a key role in reducing the nitrogen. And what they do see of the protein poses new puzzles.

Bearing out results that Bolin's group published 2 years ago, Kim and Rees found that the metal atoms are clustered in complexes that stud the protein's four subunits. One big surprise in the new data, however, is that none of those metal clusters is exposed at the surface, where nitrogen molecules could easily interact with it. It's not obvious how nitrogen gets into the enzyme or how ammonia gets out. But Rees speculates that the shape changes triggered when the iron protein cleaves ATP propagate to its larger companion, opening up some kind of route for electrons to flow between the metal centers and then to the nitrogen.

Exactly how these crucial metal atoms are arranged—a key to figuring out their role—is also unclear. Rees' model and Bolin's latest structure point to somewhat different geometrical configurations, and researchers say there's no good reason to favor one over the other. "Both are reasonable interpretations," says molecular biologist Dennis Dean of the Virginia Polytechnic Institute. "There is room for give and take, and it will be a short while before a rock-solid interpretation emerges."

Both groups say they are gathering x-ray data at still higher resolution, which should resolve the differences and sharpen the picture of how the enzyme goes about its business. But even before then, the new results may offer industrial chemists some very practical guidance. "My bet is that a lot of chemists in industry are busy working on applications of this structure," says molecular biologist Winston Brill of the University of Wisconsin, Madison, who was among the first to study the enzyme 20 years ago. They have plenty of incentive: Most of the ammonia that goes into fertilizer is still made by the 80-year-old Haber technique, a costly process that relies on high temperatures and pressures to catalyze a reaction of nitrogen with hydrogen. The new lesson from nature, says Brill, "could stimulate new approaches to getting better organic catalysts" that would work under milder conditions.

Meanwhile, molecular biologists like Brill are also taking a look backward, pleased that Rees' structure has borne out some of their earlier insights. Based on the study of mutant microbes with an impaired ability to fix nitrogen, Brill and his Wisconsin colleague Vinod Shah, for example, had proposed 15 years ago that molybdenum plays a key role in the enzyme. And University of Chicago biochemists Robert Haselkorn and Peter Lammers had predicted some of the MoFe protein's structural features based on sequencing its genes.

"As a molecular biologist, it's nice to see the stuff we anticipated proven by more physical techniques," says Dean. But, he adds, in the magnum opus that is the nitrogenase structure, there is more than enough credit to go around.

–Anne Simon Moffat

Additional Reading

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