Abstractions

SECOND AUTHOR

Formation of the Antarctic Ice Sheet was set in motion some 34 million years ago, when atmospheric carbon dioxide levels dropped drastically, causing rapid global cooling. However, it remains unclear how early glaciation events developed into a continent-sized sheet of ice. On page 690, an international team of scientists use ice-penetrating radar to pinpoint the centre of the ice sheet's growth. Beneath 3,000 metres of ice, they describe a picturesque landscape, akin to that of the Alps, that has remained unchanged for at least 14 million years. Martin Siegert, a glaciologist at the University of Edinburgh, UK, tells *Nature* more.

How did this collaboration come about?

At a meeting in France in 2002, I met lead author Sun Bo of the Shanghai-based Polar Research Institute of China, and he then visited me for a year at the University of Bristol's Glaciology Centre, where I was director. We gave him access to the largest existing data set of Antarctic ice-penetrating radar, collected by aircraft in the 1970s. He spent two years planning a field survey of the region at the centre of the ice sheet, named Dome A, that overlies the Gamburtsev Mountains. He wanted to map the subglacial landscape in this remote location and, in so doing, document the early events of glaciation, as a way of advancing China's ambitious Antarctic research programme.

Was it difficult to survey the region?

Yes. The conditions are so extreme that few people have ever visited the site. It is a desperately cold place at high altitude, set more than 4 kilometres above sea level. Sun Bo and his colleagues drove snowmobile sleds carrying radar survey equipment from the ice sheet's edge to its centre to survey an area 30 kilometres square around Dome A. This was no small achievement.

What can the Alp-like topography tell us?

That it was carved during two earlier smaller-scale glacial phases and then preserved under a large sheet of ice. The lack of erosion that had occurred since the ice sheet formed allowed Sun Bo and I to determine that these early glaciation features would have formed at about 3 °C. Because the records we have contain no evidence of Antarctic temperatures being near that level during the past 14 million years, we know that the ice sheet is at least that old.

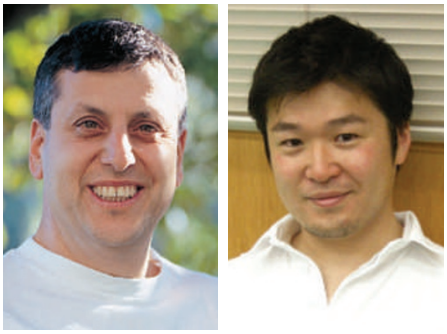
Does this work shed light on current climate change?

It provides an understanding of how little the Antarctic Ice Sheet has changed over 14 million years, during which carbon dioxide levels have been relatively stable. ■

MAKING THE PAPER

Dan Tawfik & Nobuhiko Tokuriki

Flooding the cell with chaperones speeds up test-tube evolution.



Dan Tawfik (left) and Nobuhiko Tokuriki.

For much of Nobuhiko 'Nobu' Tokuriki's tenure as a postdoc in Dan Tawfik's lab at the Weizmann Institute of Science in Rehovot, Israel, he was the only Japanese researcher in residence — earning him visits from the Japanese ambassador whenever he was in town. But Tokuriki did not mind his unique status. "I wanted to see a foreign society and culture," he says. "And I wanted to study protein evolution." For that, Tawfik's lab was the place to be.

In nature, proteins evolve new functions rapidly and efficiently. Researchers have long tried to reproduce cellular conditions in the test tube to better understand the process of protein evolution, as well as to search for new or improved enzymes with industrial or medical applications. But such methods, typically referred to as 'directed evolution', have had limited success, resulting in processes that are sluggish compared with those in nature. With Tokuriki's help, Tawfik may have found a way to greatly improve the efficiency of directed evolution by boosting the activities of the cell's quality-control officers — the chaperone proteins.

The method has its origin in work that came to Tawfik's attention about seven years ago. At the time, most scientists thought that the degree to which a mutation improved or degraded a protein's activity was what drove its positive or negative selection.

But in 2002, work by Brian Shoichet and his colleagues, then at Northwestern University in Chicago, Illinois, seemed to suggest that many mutations never actually see the light of day. Shoichet's group proposed that certain mutations would gravely affect the way that newly made proteins are folded into three-dimensional shapes, affecting their stability. Thus, the proteins would be quickly whisked away as unstable misfolded 'trash'. And even though some such mutations might be 'adaptive', or of improved function, they would never be detected. Later research indicated that this destabilizing effect of mutation is one of the major hurdles to protein evolution.

> "It's clear there are certain mutations you would never see without this method."

After Shoichet's work was published, Tawfik recalls, "I read it again and again. But it took time for its implications to penetrate into the field." Once the concept sank in, however, it was a short jump for Tawfik to the idea that chaperone proteins, which normally rescue misfolded proteins, might be able to rescue some mutant proteins and accelerate directed protein evolution.

As this idea was taking shape, Tokuriki joined Tawfik's lab, keen to work on the project. "Although it is far from perfect, protein evolution is one of the few areas where we are able to get clear biophysical and biochemical clues as to how and why evolution in the test tube happens," says Tokuriki. "This is why I chose to study this and not organism evolution."

Over the course of three-and-a-half years, Tokuriki performed directed evolution experiments on selected enzymes from the bacterium *Escherichia coli*, both in the presence of large amounts of a chaperone protein called GroEL/GroES and in its absence. This chaperone normally isolates unstable, misfolded proteins in the cell and gives them space to try to refold properly so that they can function normally. The work required each of hundreds of mutant proteins to be characterized over several bacterial generations. "Nobu can do the work of a dozen," Tawfik says.

The gruelling work revealed that, overall, GroEL/GroES rescued about one-third of the adaptive mutant proteins that, without the chaperone's aid, would have been too unstable to be viable. As a result, it allowed twice as many mutations to accumulate in proteins.

Next, the duo tested the ability of GroEL/GroES to speed up the evolution of a new, divergent function in a specific enzyme. In the chaperone's presence, directed evolution produced at least twice as many adapted variations than in its absence, and these were at least ten times more active and specific than those that evolved without the chaperone (see page 668).

Tawfik says that even though the idea was something "we thought would be stupid not to try", he was still shocked by how well it worked. Using chaperones in directed evolution will almost certainly be embraced by those trying to produce more powerful enzymes for industrial or therapeutic applications, says Tawfik, "because it's clear there are certain adaptive mutations that you would never see without this method". ■

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