Abstractions

LAST AUTHOR
Evolutionary biologists have long debated whether evolution is irreversible and, if so, why. But it is difficult to verify precisely which characteristics were present in early organisms and then identify the mechanisms by which they evolved into their present states. Joseph Thornton, an evolutionary biologist at the Howard Hughes Medical Institute and the University of Oregon in Eugene, and his colleagues, used a unique approach to look at the evolution of the glucocorticoid receptor (GR) in vertebrates, a protein involved in the stress response. On page 515, they show that a class of mutations that occurred in this protein hundreds of millions of years ago rendered its evolution irreversible. Thornton tells *Nature* more.

How did you find that mutations made GR evolution irreversible?
We knew that the GR's specific response to the stress hormone cortisol evolved from an ancestral vertebrate protein that responded to two other hormones — aldosterone and deoxycorticosterone — as well as to cortisol. Seven ancient mutations resulted in the GR's cortisol specificity. We expected that if we reversed them, we could engineer a protein that functioned more promiscuously, like its ancestor. Instead, we got a 'dead', or non-functioning, receptor. We found that five additional 'restrictive' mutations, which emerged during early GR evolution, clashed with the protein's ancestral conformation. When we reversed these five, together with the seven key mutations, we recreated a protein just like the GR's ancestor.

How do the two classes of mutations differ?
The seven key mutations caused the evolution of cortisol specificity, but the five restrictive mutations had little or no effect on the protein's function. They did, however, burn the evolutionary bridge the protein had just traversed.

Do restrictive mutations prevent reverse evolution in other proteins?
This is the first protein that has been studied in this way, so we don't know for sure. But I predict that the GR won't be a unique case.

What are the implications for how organisms evolve?
We came from ancestors that evolved from their own, deeper predecessors. As time progresses, the conditions that made their evolution possible are continually being erased. If we turned back the clock to start again, different chance events would almost certainly occur, leading to different futures but closing the path to the present that evolved in our world. Our biology is just one of many possible rolls of the evolutionary dice. ■

MAKING THE PAPER

Thomas Stocker

Carbon isotopes in ice cores defy a controversial hypothesis.

Around the start of the Holocene era 11,000 years ago, the levels of carbon dioxide in the atmosphere dipped slightly, only to increase again some 4,500 years later. Scientists have pondered the causes of these slight variations, which do not follow the periodic pattern of CO_2 rises and falls in the past, without finding a definitive explanation.

In 2003, US palaeoclimatologist William Ruddiman suggested that the CO_2 increase might have been a result of forest clearance by early agrarian farmers. "That hypothesis was very thought-provoking and caught the attention of many people. But it took us several years to develop the methods to produce the data," says Thomas Stocker of the Oeschger Center for Climate Change Research at the University of Bern, Switzerland.

UNIVERSITÄT BERN

Stocker heads one of ten laboratories that make up the European Project for Ice Coring in Antarctica (EPICA) consortium, launched in 1996 to construct a historical record of atmospheric CO_2 and other greenhouse gases by measuring the amounts of these gases trapped deep in the ice sheets of Antarctica.

Stocker and his colleagues looked specifically at carbon isotope 'signatures' in the ice-sheet CO_2 for clues as to its sources and sinks. For example, because vegetation and soil prefer to take up CO_2 that contains the carbon-12 isotope, rather than carbon-13, a decrease in atmospheric CO_2 accompanied by a relative increase in atmospheric ^{13}C signals that the terrestrial biosphere has caused the change. By contrast, CO_2 uptake by the ocean does not show much preference for either isotope.

But ^{13}C isotope measurements are not easy to obtain. "Each ice-core sample consists of about 10–40 grams of ice from which you have to release and capture a tiny amount of air," says Stocker. "You then have to look for variations in ^{13}C, which makes up only about 1% of the CO_2." Perhaps the biggest challenge was finding a way to extract the gas without contaminating it, he says. "We discovered, for example, that CO_2 interacts with the interior wall of the extraction device to produce artefacts."

The team's perseverance paid off and they obtained the first robust ^{13}C record of the early Holocene up to about medieval times. The next step was to interpret the data. "One advantage at our institute is that we have experimental physicists working alongside carbon-cycle modellers," says Stocker. By comparing models of CO_2 outputs from various processes with actual measurements, the team came up with an explanation for the measured CO_2 levels and ^{13}C record (see page 507).

A particular combination of three processes — land-biosphere uptake, carbonate compensation by the ocean, and coral-reef formation — are sufficient to explain the recorded ^{13}C levels, says Stocker, which means that no other processes were involved in causing the CO_2 variations. "The hypothesis that early land-use by humans modified CO_2 levels can now be confidently rejected," he says.

Stocker and his team will continue to look at ^{13}C signatures during other time periods to understand the forces that shaped the levels of atmospheric greenhouse gases and their subsequent climate effects. The team is particularly interested in the medieval period: "That is a time of intense human activity, but we cannot yet make any statement on whether such activity had any effect on CO_2 levels because we don't yet have measurements," says Stocker. ■

FROM THE BLOGOSPHERE

The clock is ticking for the H1N1 swine-flu vaccine, as reported in two blog posts on vaccine science and policy this month.

On the Spoonful of Medicine blog, *Nature Medicine* senior editor Charlotte Schubert gives an update on the vaccine's regulatory approval in the United States and reports data showing that just one dose of vaccine should be protective (http://tinyurl.com/ktovkj).

"That's better news than expected," she writes, noting that a one-dose schedule will free up vaccine supplies. She explains how the supply could be stretched even further if the United States were to implement the use of adjuvants — substances that are mixed with vaccines to improve the immune response.

Meanwhile, *Nature*'s European correspondent Declan Butler asks "Who's in the driving seat?" for the European Union's flu response. A strategy document released by the European Commission was meant to lay out the responsibilities of the Commission and the EU member states, but many issues remain unclear. Butler breaks these down for readers on The Great Beyond blog (http://tinyurl.com/ntr99e). ■

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