help to delay the onset of cancer. Moreover, involvement of the insulin-mediated pathway indicates that drugs that ameliorate insulin resistance in type 2 diabetes might be beneficial in preventing cancer, even in non-diabetic patients. One could also envisage using dietary restriction as a possible therapy in some specific cancers, and to predict which tumours would be vulnerable to such treatment on the basis of their mutation profiles, in particular in the genes encoding PTEN and PI3K. Limited food intake, together with a PI3K-pathway inhibitor, might have synergistic effects on cancer regression in tumours that have mutations in *PI3K* and/or *PTEN* genes. In addition, depriving tumours of nutrients locally by means of anti-angiogenic drugs, which would interfere with the tumour's blood supply, may be yet another way to apply the beneficial effects of dietary restriction. In general, developing such additional strategies to mimic restriction could be particularly useful, because — as we all know — restricting food intake is notoriously difficult to implement in humans, and may even be psychologically damaging. ■

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BIOGEOCHEMISTRY

Less nickel for more oxygen

Mak A. Saito

The availability (or lack) of oceanic trace elements is providing fresh lines of explanation for turning points in Earth's history — the Great Oxidation Event being one such momentous occasion.

About 2.4 billion years ago, the oxygen content of Earth's atmosphere increased in what is called the Great Oxidation Event (GOE). This marked the beginning of the most significant series of chemical changes Earth has ever experienced, setting the stage for oxidative weathering of the continents, successive changes in ocean chemistry, and the eventual rise of multicellular life.

Yet the sequence of events leading up to the GOE is not well understood. Most researchers agree that the evolution of oxygenic photosynthesis within a group called the cyanobacteria was the source of the molecular oxygen that caused the GOE[1]. But the timing of the rise of these bacteria is uncertain[2,3], and there may have been a period of inertia — due, for example, to chemical reactions with methane that consumed oxygen[4] — that prevented a swift increase in atmospheric oxygen. It remains a matter of debate how these two phenomena might have induced the GOE: an early rise of cyanobacteria and slow crumbling of chemical resistance[3,4]; or a late rise of cyanobacteria leading to rapid initiation of the GOE[5].

On page 750 of this issue[6], Konhauser *et al.* report evidence for an alternative driving mechanism of the GOE, one that would have decreased microbial methane production in the oceans and paved the way for increased oxygen abundances. The authors find significant decreases in the nickel-to-iron ratios in ancient rocks, known as banded iron formations, that provide records of element concentrations in the oceans (Fig. 1). They estimate that a major decrease in the oceanic inventory of nickel must have occurred around 2.7 billion years ago. This, they conclude, led to a cascade of events in which methanogens, with their gluttonous appetite for nickel to feed three nickel-containing metalloenzymes, would have become starved of the element and so have produced much less methane. With the decrease in chemical inertia associated with methane[4], the stage was set for cyanobacterial oxygen to accumulate, leading to the GOE. Moreover, although Konhauser *et al.* don't go into detail, the decline in atmospheric methane, a powerful greenhouse gas, is believed to help account for the initiation of a planetary-scale glaciation known as Snowball Earth that is thought to have begun between 2.3 billion and 2.2 billion years ago[4,5].

Figure 1 | Record site. This is a view of Dales Gorge, northwest Australia, one of the banded iron formations sampled by Konhauser *et al.*[6].

I. PATERSON/ALAMY

The idea that significant changes in seawater trace-metal abundance have occurred during Earth's history is becoming popular[7,8]. For example, it is thought that iron and cobalt were abundant in ancient oceans, whereas zinc and copper were probably extremely scarce owing to precipitation with sulphides[8]. When the oceans became oxygenated, it is likely that this scheme was reversed, with iron and cobalt becoming scarce through oxidation and precipitation as oxyhydroxides, and zinc and copper becoming much more abundant upon the oxidation of sulphide to sulphate in sea water. These predictions of broad changes in ocean chemistry are mirrored by the physiological and genomic traits of archaea and bacteria, relative to those of the later-evolving eukaryotes[8,9].

Nickel has largely been left out of this intriguing story. On the evidence of chemical modelling[8], it seems that nickel was not as strongly affected by the variations in sulphide and oxygen during Earth's history. But such a conclusion does not take into account the possible involvement of external factors. Konhauser *et al.* show how such a factor might have come into play, with the cooling of Earth's mantle resulting in decreased eruption of nickel-rich rocks and causing an estimated 50% fall in the oceanic nickel inventory.

Konhauser and colleagues' thinking[6] may come as a surprise to those familiar with the chemistry of the modern oceans. Trace metals — as their name suggests — are extraordinarily scarce in sea water. In vast regions of the modern oceans, photosynthesis is limited by low iron availability, with iron concentrations often being less than 0.05 nanomoles per litre[10]. Yet, of the trace metals required by life, nickel is one of the more abundant in sea water, with surface water concentrations of 1–2 nanomoles per litre[11]. In this modern context, the idea of a nickel famine seems odd. But the nickel requirements of methanogens are reported[6] to be in the hundreds of nanomoles per litre, suggesting that methanogens cannot live in the modern oceans and are perhaps relegated to

sedimentary, coastal and freshwater environments, where nickel is more abundant.

By connecting changes in mantle temperature to nickel fluxes and methanogens, Konhauser and colleagues' study is particularly satisfying. Instead of relying on the uncertain timing of the rise of cyanobacteria to explain the GOE, that event can instead be tied to a specific mechanism recorded in the banded iron formations. In addition, this 'nickel famine' mechanism is consistent with evidence[12] of 'whiffs of oxygen' that occurred more than 50 million years before the GOE. But I cannot help but wonder whether there is a reason — such as the slow chemical kinetics of nickel ions — why methanogens could not evolve a high-affinity nickel-uptake mechanism similar to those that exist for the uptake of iron, zinc and cobalt[13–15].

Finally, there is another context in which the research of Konhauser *et al.* is set — the exciting endeavour of trying to understand how the elemental cycles (of nickel, carbon, iron, nitrogen and so on) have 'co-evolved' with microbial life. Many of the changes in element cycling were probably caused by the rise and fall of specific microbial metabolisms, while also strongly affecting the trajectory and composition of life on Earth. Life and the cycling of elements have both been changing throughout Earth's history, often influencing each other profoundly along the way. One of the sobering realizations of studies such as this is that, although natural selection provides a clear, single positive-feedback mechanism for the continuation of life, elemental cycles are instead influenced by an aggregate of mechanisms, including biological evolution, chemical reactions, changes in ocean circulation and geological events. If, as Konhauser *et al.* suggest, a single geological change can starve a major oceanic microbial community, and thereby change the trajectory of life on Earth, it suggests that there is a fragility to Earth's elemental cycles that we are only beginning to uncover. ■

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STEM CELLS

Low-risk reprogramming

Martin F. Pera

New techniques circumvent a roadblock to the production of embryonic-stem-cell-like lines from adult tissue. Such reprogrammed cell lines should be much safer to use for therapy.

Shinya Yamanaka's amazing discovery[1] that cells from differentiated tissues can be reprogrammed into induced pluripotent stem (iPS) cells — cells that can potentially differentiate into any cell type — has transformed research in stem-cell biology and regenerative medicine. The breakthrough provided both a deft approach to the production of patient-specific stem-cell lines with which to study disease, and a practical means of developing large banks of stem-cell lines suitable for tissue matching in transplantation therapy. But the original protocols for producing iPS cells relied on the integration of foreign 'reprogramming' genes into the host-cell genome, a process associated with risks including mutation, dysregulation of native gene expression, and the development of cancers after iPS-cell transplantation. Four studies[2–5], including two in this issue[2,3], now show that iPS cells can be produced without any permanent modification to the host-cell genome.

The first-generation iPS cells were produced from culture-grown mouse and human somatic (non-germ) cells, most often skin fibroblasts[1,6,7]. The protocol involved the introduction into the host cell of reprogramming genes crucial for the establishment or maintenance of the pluripotent state using various lentiviral or retroviral constructs as vectors. The constructs integrated into the host genome at multiple sites.

Although in most cases the foreign genes eventually became inactive in the iPS cell lines, this was not always the case. Moreover, even if expression of the transcription factors that the genes encoded was stopped, the integrated foreign DNA remained in the host genome. This could, in principle, disrupt host genes, alter gene expression at nearby genomic loci or, if subsequently reactivated in the differentiated cells, result in these cells becoming cancerous. Indeed, chimaeric mice generated from normal cells and some iPS cell lines developed tumours[8]. Work with viral vectors that integrate into the host genome also left open the daunting possibility that integration and genetic modification of the host cell per se might be required for reprogramming.

Subsequently, several groups showed that, depending on the host cell type, reprogramming can be achieved using fewer foreign genes. But the goal of completely eliminating the need for genomic integration of foreign sequences remained a priority.

Given the intense activity in the field of reprogramming, many groups pursued solutions to this particular obstacle. Two groups[4,5] report using non-integrating adenoviral vectors or plasmids to achieve transient expression of reprogramming factors without disturbing the host genome. But such an approach presents two immediate problems: the requirement for prolonged expression of the pluripotency factors to achieve reprogramming, and the difficulty of repeatedly delivering the full complement of factors using a different vector for each one.

To address the first problem, the authors[4,5] used hepatocytes — which, compared with other cell types, are more amenable to both reprogramming and infection with adenoviruses — and introduced the genes into the cells repeatedly over a period of days. The second problem was solved in one study[5] by borrowing a clever trick from the foot-and-mouth virus. By inserting a virally derived oligopeptide sequence called 2A as a spacer between four reprogramming genes, the researchers made a multiprotein expression vector.

The key feature of the 2A sequence is its ability to undergo self-splicing and be removed from a peptide undergoing translation, possibly through a mechanism in which the ribosome skips over one codon without forming a peptide bond, thus allowing the production of several peptides from one transcript. Using this approach, several reprogramming genes can be introduced and efficiently expressed using a single adenoviral or plasmid construct.

Both groups obtained mouse iPS cells that express cell-surface markers and genes characteristic of embryonic stem cells, and that could undergo differentiation *in vitro*. *In vivo*, these cell lines contributed to teratomas (benign tumours containing cells from various differentiated tissues) in chimaeric mice, although their ability to form germ cells was not assessed. The main drawback of this approach was its relative inefficiency: at least 100-fold fewer iPS colonies were obtained than with the retroviral or lentiviral vectors. Given that the production of iPS cells from human cells is generally less efficient than from mice, it is questionable how practical it would be to use these methods[4,5] for human cells. But the studies established one point clearly — viral integration is not necessary for reprogramming.

Two other elegant studies[2,3] provide an alternative, more efficient, strategy that involves virus-free integration of reprogramming genes, followed by their removal. Woltjen