the dye moved from dense water towards lighter water, the team was able to identify an upwelling rate of around 100 metres per day, at least 10,000 times larger than the average value of one centimetre a day that Munk estimated, and up to 100 times larger than other indirect measurements[7].

The results presented by Wynne-Cattanach *et al.* provide compelling evidence that upwelling near the sea floor can be vigorous. However, many open questions remain. Although the data suggest that upwelling near the ocean floor is substantial, the error bars on the upwelling rate were large, and it is not clear how such results generalize to other locations. Furthermore, the observational sampling was not sufficient to determine the turbulent diffusivity, a key parameter that characterizes the overall rate of mixing. Diffusivities have previously been measured using shear-probe instruments (which estimate turbulence by sensing shear forces at centimetre scales)[6] and by tracking the movement of dye-like tracers. But measurements made using these two methods have been inconsistent, and their differences have been a huge motivation for studies of ocean mixing[8].

Wynne-Cattanach and colleagues' observations also shift attention to the processes (currents, waves and eddies) that bring water (and tracers such as salt and carbon) in and out of contact with the sea floor. As well as connecting regions of upwelling and downwelling, these flows maintain differences in density near the ocean floor (which turbulence acts to destroy) — a role that has fuelled past debate on the efficacy of sea-floor mixing[9]. Owing to a paucity of observations, scientists' understanding of these processes is only in its infancy, although results obtained in the past few years are beginning to shed some light on them[10].

Understanding the complex nature of bottom-mixing processes and their interactions with other flows constitutes a major challenge in oceanography, and one that has global implications. To address this challenge, more observations are needed, with support from detailed numerical modelling experiments that are conducted on a regional scale. Finally, the ultimate challenge lies in incorporating these complex small-scale processes into numerical climate models. Without dynamic representations of mixing, such models will struggle to reproduce deep-ocean circulation, heat and carbon exchanges between the surface and deep ocean, and their impact on the climate system.

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## In retrospect

# Two decades of deep ice cores from Antarctica

### Kenji Kawamura & Ikumi Oyabu

In June 2004, the results of an ambitious Antarctic ice-drilling project brought insight into hundreds of thousands of years of climatic changes. The extraordinary sample still has much to offer climate research — even as its successor is being drilled.

Twenty years ago this month, a large group of researchers reported the first results from the European Project for Ice Coring in Antarctica (EPICA). This was an ambitious bid to archive information about Earth's past climate by sampling layers of Antarctic ice that had built up over hundreds of thousands of years[1]. The initial drilling procured ice that was up to 740,000 years old — the oldest ice ever extracted from Antarctica and twice as old as that in earlier records[2,3]. The core revealed the atmospheric temperature and dust content over Antarctica during the past eight glacial–interglacial cycles, as well as changes in carbon dioxide and methane that accompanied the melting of continental ice sheets in the Northern Hemisphere about 430,000 years ago. The study (and those inspired by it) provoked a discussion about the fundamental drivers and interactions of climatic changes, and about how the past can inform our understanding of current and potential changes.

The best kept climate records are arguably the ice sheets on Antarctica and Greenland. These represent an archive of essential climatic information, such as air and ocean temperatures, as well as the factors that affect these variables, including atmospheric composition. The first ice core to penetrate to a depth of more than 2,000 metres was drilled in the late 1960s at Byrd Station in West Antarctica[4] (Fig. 1). This sample provided 83,000 years of information about temperature and two important atmospheric greenhouse gases ($CO_2$ and methane), among various other parameters. Then, in the 1970s, drilling began in Vostok in East Antarctica, a project that culminated in a 3,623-m core, offering climatic and atmospheric records over the past 420,000 years, covering four glacial cycles[2]. Together with another ice core from Dome Fuji in East Antarctica[3] (reaching 2,503 m and 340,000 years), these records offered strong evidence that the climate is spatially uniform over the vast Antarctic plateau.

But global ice volumes suggest that the largest glacial–interglacial transition occurred around 430,000 years ago, so the Vostok record ends at a crucial point in Earth's history. Before this time, the amplitudes of glacial cycles seem to have been smaller, suggesting that this transition brought with it key changes in climate feedbacks (such as the effects of $CO_2$) or forcings (such as quasi-periodic changes in Earth's orbital parameters)[5]. A longer record was needed and — thankfully — EPICA had already set its sights on drilling Dome C, a summit with an ice thickness of around 3,300 m. The first results from the EPICA Dome C (EDC) ice core were reported in *Nature*[1] while the team was still drilling the deepest part of the ice, and subsequent drilling extended the record to 800,000 years. The Dome Fuji record was similarly lengthened to cover 720,000 years (ref. 6).

Beyond the EPICA paper's achievement in extending the Antarctic climate and atmospheric greenhouse-gas records, it is an excellent showcase of the ability of a large multidisciplinary and multinational community to provide reliable data on a rapid timescale. Roughly half the paper is dedicated to establishing that the ice layers are in chronological order (by comparing different types of data) and then determining the age of each layer. This attention to detail was essential
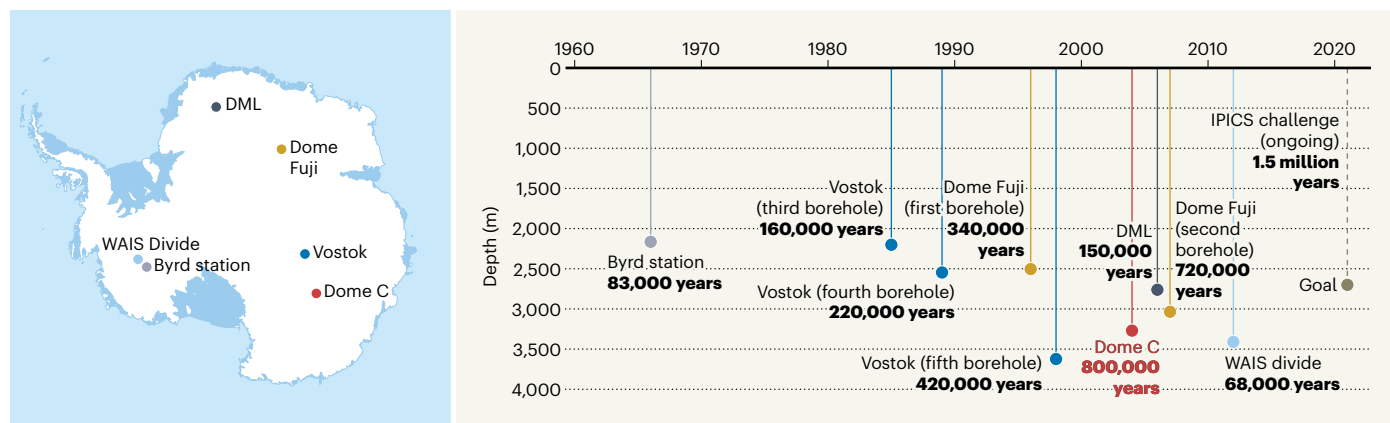
**Figure 1 | A timeline of deep ice-core drilling in Antarctica.** Scientists have been drilling deep ice cores (with depths of more than 2,000 metres) since the late 1960s. The cores contain layers of Antarctic ice that have built up over hundreds of thousands of years, providing information about Earth's past climate. In 2004, the European Project for Ice Coring in Antarctica (EPICA) reported a core from Dome C comprising layers that date back 740,000 years (ref. 1). Subsequent drilling extended the record to 800,000 years. The undertaking has inspired many climate studies since then, prompting a challenge from the International Partnerships in Ice Core Sciences (IPICS) to sample 1.5 million years of Earth's climate history. DML, Dronning Maud Land; WAIS, West Antarctic Ice Sheet

because the deepest 15% of the ice sheet is subject to ice flow that could have disturbed the ice layers. The latter half of the paper chronicles Antarctica's temperature over these early glacial cycles, which involved interglacial periods that were longer and less warm than the five most recent periods, including the Holocene (the current interglacial age).

The EPICA paper inspired numerous discoveries, including the revelation that the relationship between $CO_2$ and Antarctic temperature has remained remarkably stable throughout the past eight glacial cycles[7]. This, in turn, led to suggestions that the Southern Ocean has a key role in regulating atmospheric $CO_2$ (ref. 8), and that the way in which $CO_2$ affects warming (and vice versa) had a profound impact on the glacial cycles leading up to the Holocene[9], but did not determine the relative amount of ice across the globe in each cycle. The EPICA authors also prompted debate about how human existence has affected glacial cycles, by comparing a long interglacial period 410,000 years ago with the current one, and questioning a suggestion that Earth would have entered the next glacial period by now if humans didn't exist.

One of the most notable conclusions to emerge from these studies came in 2008, when it was revealed that present greenhouse-gas levels are unprecedented compared with those of the past 800,000 years (ref. 7, 10). This was made possible by the great passion of many young scientists (mostly graduate students) who came together to make the most of this extraordinary archive of Earth's past atmosphere. One of us (K.K.) was fortunate enough to take part in developing methods and measuring the $CO_2$, methane and nitrous oxide levels in the EDC core as a postdoctoral researcher at the University of Bern. Coffee breaks were spent in fervent discussion about the emerging data, and how to get the most out of them – with a poster of *Nature*'s 10 June 2004 cover looming large on the door of the laboratory.

Yet, in spite of all these advances, there remains much to explore in the deep ice cores from Antarctica. For example, it is unclear whether the $CO_2$ and warming feedback cycle was a prerequisite for the melting that occurred 430,000 years ago, because modelling efforts have not succeeded in reproducing this particular deglaciation by assuming constant $CO_2$ levels[11]. This challenge could be overcome with improved modelling, combined with a better understanding of how $CO_2$ increases coincided with sea-level rises and of what sets the pace of glacial cycles[12], which might be possible with the Antarctic ice cores – if their timescale uncertainty is further reduced[13]. A more accurate timescale might also help to settle debate about the temperature difference between glacial and interglacial periods, which has emerged from a suggestion[14] that previous estimates are out by a factor of two.

Fresh light on the old cores could also help to solve a puzzle about the climatic connections between the polar regions. Changes in the Northern Hemisphere have effected changes in the Southern Hemisphere, over multiple glacial periods, through a mechanism called the bipolar see-saw, which was first evident in the comparison of the Byrd ice-core temperature record and Greenland records[4]. Subsequent deep ice cores drilled by EPICA at Dronning Maud Land (DML)[15] and by the US National Science Foundation at the West Antarctic Ice Sheet (WAIS) divide[16] provide higher resolution than other cores, and were obtained with the aim of better understanding this see-saw effect for the most recent glacial period.

Bipolar connections that occurred in earlier glacial periods cannot be revealed by the Greenland cores, but they could be investigated with the help of the Antarctic cores alone, using methane data as a proxy for the Northern Hemisphere climate[1,7,10]. So far, this has been possible only for large events because of limited data resolution, but a relatively new technique to measure methane continuously could be applied to the old ice cores to extend the analysis to smaller events. Similarly, the cores could be used to reconstruct mean ocean temperatures from atmospheric noble gases using a technique[17] that was not available 20 years ago. Last but not least, whether biological processes that occur in the ocean have changed with glacial–interglacial variations is also a hotly debated topic[18], and one that the Antarctic ice cores could help us to understand.

Although the scientific possibilities afforded by EPICA are almost unlimited, the International Partnerships in Ice Core Sciences (IPICS) set an even grander challenge in 2004 – to obtain an ice core spanning 1.5 million years. Back then, one of the organization's key aims was to retrieve a continuous ice core that can provide the data required to understand the causes and mechanisms behind a 40,000-year periodicity that occurred in the early glacial cycles and changed into the current 100,000-year cycle around 1 million years ago[19].

Two decades on, several national and international efforts are under way to achieve the ambitious goal[20]. The Beyond EPICA project has already drilled to a depth of more than 1,800 m, but others are not far behind, including Japan's Third Dome Fuji project (which started drilling last year) and Australia's Million Year Ice Core project. Drilling and analysing these cores will come with challenges, but the data will no doubt lead to some very exciting science. The time is nigh for yet another leap in ice-core research.

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## Biotechnology

# Programmable enzymes for large genome edits

Connor J. Tou & Benjamin P. Kleinstiver

RNA-guided recombinase enzymes have been discovered that herald a new chapter for genome editing — enabling the insertion, inversion or deletion of long DNA sequences at user-specified genome positions. **See p.984 & p.994**

A long-standing aspiration for biologists has been to develop programmable methods to rearrange long DNA sequences in genomes. This capability would allow kilobase-scale DNA sequences to be inserted, inverted, deleted or moved to user-specified genome locations in cells in a single step. Recombinase and transposase enzymes that mediate genomic rearrangements of large sequences have therefore been studied intensively, but it has been challenging to reprogram these enzymes to precisely target user-specified genomic sites. Two papers[1,2] on pages 984 and 994 now report the characterization of recombinases that are guided by a 'bridge' RNA molecule, and can thereby be reprogrammed to enable new genome-editing capabilities.

Recombinase enzymes that target large DNA donor sequences for integration into genomic target sites consisting of about 30–50 base pairs are particularly attractive for genome-modification technologies[3]. These proteins recognize DNA sequences by forming extensive protein–DNA interactions (Fig. 1a). However, this mode of recognition is complex, making it difficult to reprogram the enzymes to bind specifically to other sequences.

The identification of thousands of recombinases that collectively target a diverse range of sequences[4], combined with emergent protein-engineering approaches[5,6], has substantially expanded the recombinase toolbox. Nevertheless, recombinases that could be completely reprogrammed have

been elusive. The discovery of recombinases that use easily customized RNA molecules to recognize DNA targets could obviate the key challenges of the existing toolbox, just as the reprogrammable guide RNAs of DNA-cleaving CRISPR–Cas enzymes ushered in a new era of genome editing.

DNA sequences that move around the genome, known as mobile genetic elements, are of particular interest in this context. Insertion sequences (ISs) are some of the simplest and most compact mobile genetic elements. Studies have revealed that IscB and TnpB — proteins encoded by ISs from the IS200/605 family — are ancestors of the Cas9 and Cas12 enzymes, respectively[7,8], and are also DNA-cleaving enzymes (nucleases) that use a guide RNA to target specific DNA sequences. Moreover, the transposase enzyme TnpA, which is often co-expressed with TnpB, has been used for programmable genome editing[9]. Given that RNA programmability underpins diverse functions of the enzymes encoded by ISs, it seems possible that recombinases might have evolved to use RNA in a similar way.

It has been speculated that the non-protein-encoding ends of ISs in the IS110 family help to regulate expression or activity of the encoded recombinase[10]. Failed attempts to reconstitute IS110-mediated DNA excision and integration outside host organisms hinted that a host-expressed nucleic acid might be needed[11]. Furthermore, IS1111, a relative of IS110, has been hypothesized to use a non-coding RNA to select its target site[12], leading to speculation that IS110 might also use an RNA for this purpose.

Durrant *et al.*[1] now demonstrate that IS110 elements encode a recombinase and a non-coding RNA, termed a bridge RNA (bRNA), that directs the recombinase to target sites (Fig. 1b). The bRNA consists of two regions: a donor-binding loop (DBL) that recognizes
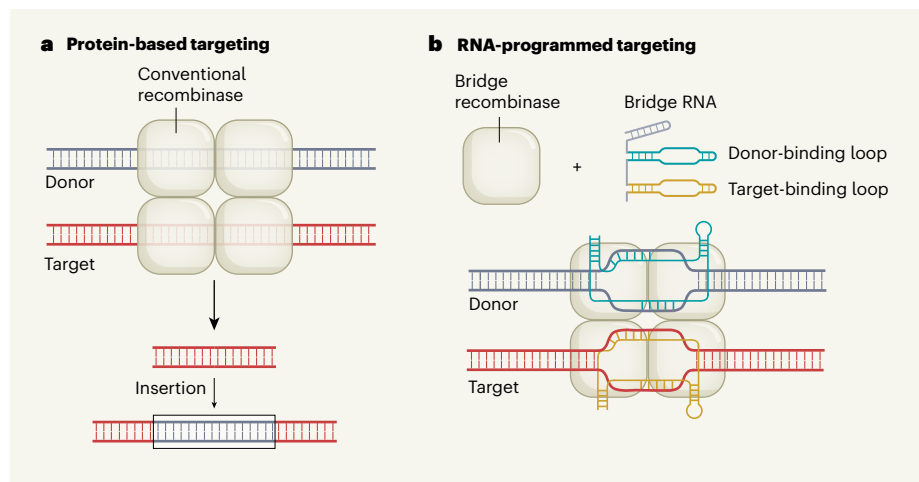


**Figure 1 | Comparison of conventional and bridge recombinase enzymes.** Recombinase proteins catalyse reactions in which long sections of DNA are inverted, deleted or cut out of donor DNA and inserted into target DNA. **a**, Conventional recombinases recognize DNA sequences by forming extensive protein–DNA interactions. In insertion reactions, four recombinase molecules bind specifically to the sequence that will be cut from the donor, and to the target sequence into which the donor's DNA will be inserted. However, this mode of sequence recognition is complex, making it difficult to engineer the proteins to bind specifically to other sequences. **b**, Three papers[1,2,15] now report recombinases that use an RNA molecule (called a bridge RNA in refs 1 and 2) to recognize DNA. Hiraizumi *et al.*[2] report that this RNA contains two loops that bind to donor and target sequences as shown. The loops can be independently engineered so that the recombinase carries out inversions, deletions and insertions of user-specified sequences.