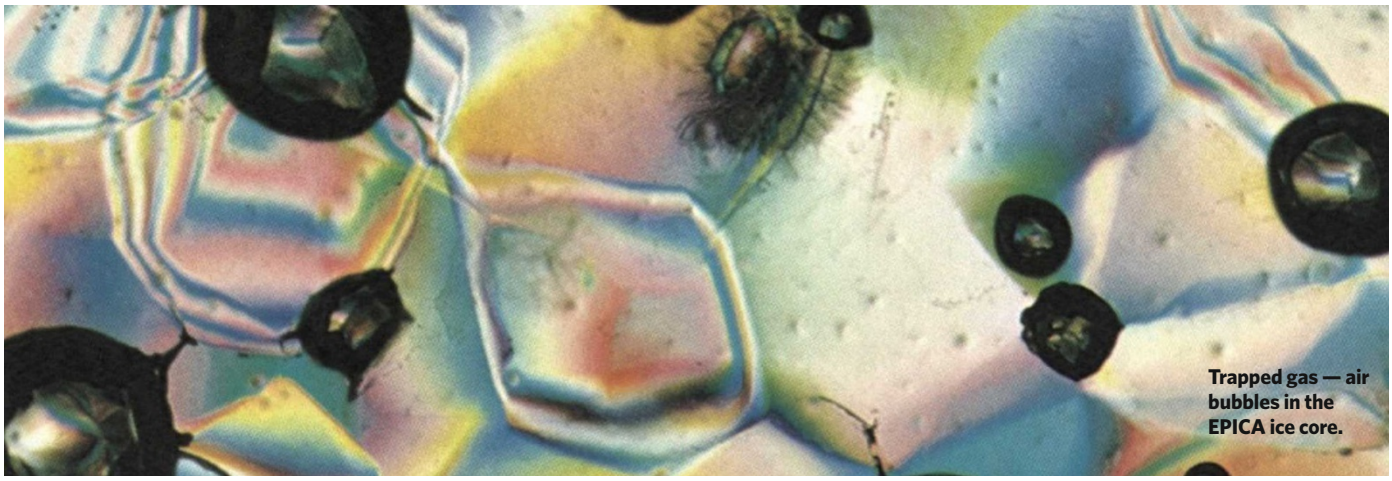
NEWS & VIEWS



THE EPICA COLLABORATION

Trapped gas — air bubbles in the EPICA ice core.

PALAEOCLIMATE

Windows on the greenhouse

Ed Brook

Data laboriously extracted from an Antarctic ice core provide an unprecedented view of temperature, and levels of atmospheric carbon dioxide and methane, over the past 800,000 years of Earth's history.

Palaeoclimatologists are scientific detectives. Using indirect clues from concentrations of stable isotopes and trace elements, and from fossils and other components of the geological record, they infer changes in climate long before they themselves were on the scene. Direct evidence of past environmental conditions is rare, which makes it all the more valuable where it does occur. In this issue[1,2], members of the EPICA (European Project for Ice Coring in Antarctica) collaboration present the latest, and longest, record from perhaps the most valuable of these archives: the atmospheric gases trapped and preserved in ice cores extracted from Earth's polar regions.

Polar ice cores provide us with the long view of the cycling of greenhouse gases such as carbon dioxide and methane. Their potential is being realized by a relatively small band of international scientists who are gradually drilling further down into the ice cap and progressively analysing older ice cores. Until recently, the Vostok ice core from eastern Antarctica set the benchmark[3] — an iconic 440,000-year data set that became a central backdrop for discussions about modern climate change.

That ante was upped in 2005 by a 650,000-year record[4,5] from EPICA's 'Dome C', another drilling site in eastern Antarctica where much older ice could be extracted. An 800,000-year reconstruction of temperature change from the core already existed[6]. Now, after years of careful work and collaboration, Dome C has yielded a complete reconstruction of the history of atmospheric carbon dioxide (Lüthi *et al.*, page 379)[1] and methane (Loulergue *et al.*, page 383)[2] over the past 800,000 years.

The fundamental conclusion that today's concentrations of these greenhouse gases have no past analogue in the ice-core record remains firm. The general long-term behaviour of methane and carbon dioxide, following patterns driven ultimately by slow changes in Earth's orbit, continues throughout the older sections of the records. The remarkably strong correlations of methane and carbon dioxide with temperature reconstructions also stand (Fig. 1, overleaf).

The data further reinforce the tight link between greenhouse gases and climate, a link maintained by as-yet only partially understood feedbacks in the Earth system. Variations in methane levels are most probably caused by variations in the influence of temperature and rainfall on wetlands in the tropics and boreal (high-northern-latitude) regions. Carbon dioxide variability is almost universally viewed as an oceanic phenomenon, a consequence of the large pools of carbon sequestered there. Changes in ocean circulation, biological productivity, carbon dioxide solubility and other aspects of ocean chemistry have been implicated, but the exact mix of mechanisms is not clear.

In considering these extended records[1,2] in detail, intriguing nuances emerge. Their most prominent feature is a sawtooth-shaped variability on 100,000-year timescales. As reported last year[6], the amplitude of the 100,000-year temperature cycle reconstructed at Dome C seems to have changed fundamentally about 450,000 years ago (Fig. 1). Warm phases (interglacials) in the later period have been warmer, whereas cold phases (glacials) seem similar throughout the record. The carbon dioxide record generally shares this pattern, with muted cycles in its older part. Methane also follows the trend, though not as strongly: relatively high methane maxima in the oldest interglacial cycle approach those of later warm periods.

A curious facet of the extended carbon dioxide record is unusually low levels of the gas during the two earliest glacial–interglacial cycles. Lüthi *et al.* speculate[1] that, taken as a whole, the carbon dioxide record is hinting at a longer-term cycle in mean levels of the gas that takes 400,000–500,000 years to complete. The eccentricity of Earth's orbit — its deviation from a perfect circle — does vary with a 413,000-year period. But whether this or some other mechanism explains any variation awaits the retrieval of an even older ice core.

The extended records also provide information about shorter-term, millennial-scale climate change taking place within the longer cycles. Data from ice cores in Greenland covering the past 110,000 years show that variations in methane levels were extremely closely coupled to episodes of abrupt warming and cooling in the mid-to-high latitudes of the Northern

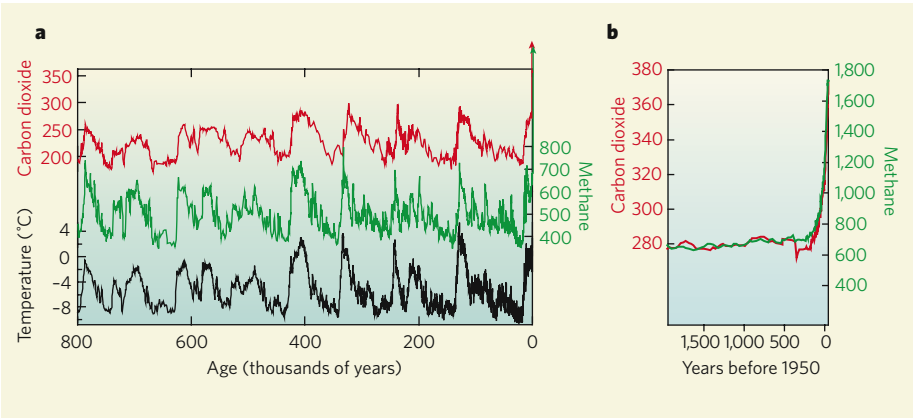


Figure 1 | A long look back. a, The 800,000-year records of atmospheric carbon dioxide (red; parts per million, p.p.m.) and methane (green; parts per billion, p.p.b.) from the EPICA Dome C ice core[1,2], together with a temperature reconstruction (relative to the average of the past millennium) based on the deuterium–hydrogen ratio of the ice[6], reinforce the tight coupling between greenhouse-gas concentrations and climate observed in previous, shorter records. The 100,000-year 'sawtooth' variability undergoes a change about 450,000 years ago, with the amplitude of variation, especially in the carbon dioxide and temperature records, greater since that point than it was before. Concentrations of greenhouse gases in the modern atmosphere are highly anomalous with respect to natural greenhouse-gas variations (present-day concentrations are around 380 p.p.m. for carbon dioxide and 1,800 p.p.b. for methane). **b**, The carbon dioxide and methane trends from the past 2,000 years[13,14].

Hemisphere[7,8]. No older records from Greenland exist at present; indeed, records extending back further than about 200,000 years are not expected to be found there owing to high accumulation rates and the flow of older ice towards the margins of the ice sheet. But assuming that the close coupling between Greenland's temperature and levels of atmospheric methane holds before 110,000 years ago, jumps in the Dome C methane record provide a Southern Hemisphere proxy for abrupt warming in the Northern Hemisphere. Loulergue *et al.*[2] identify 74 such jumps in their data and, following this logic, conclude that abrupt warming and cooling in Greenland and the Northern Hemisphere has been a characteristic of the climate system over at least the past 800,000 years.

Again using methane as a proxy for Greenland's temperature patterns, it can be shown that on millennial timescales carbon dioxide concentrations rose during times when Greenland was cold. At the same time, Antarctica warmed[9]. This pattern has been attributed to the effect of changes in ocean circulation on the carbon cycle and climate[10]. Lüthi *et al.*[1] identify examples of this kind of variability in ice as old as 750,000 to 780,000 years, another indication that these millennial patterns pervade the palaeoclimate record.

These new benchmark data[1,2] for greenhouse-gas variability pose questions as to what a much longer record might show. One such question is whether the 400,000–500,000-year cycle speculated on by Lüthi *et al.*[1] is a real effect. Another is whether the 100,000-year cycles in carbon dioxide and methane, now so clearly established, give way to 40,000-year cycles before about 900,000 years ago; such behaviour might be predicted by comparison with climate reconstructions from ocean sediments[11]. If that is the case, what caused the shift? Was it a reduction in mean concentrations of greenhouse gases 900,000 years ago? This commonly cited theory[12] is just one of many competing hypotheses[11].

The international community of ice-core scientists, under the auspices of the umbrella group IPICS (International Partners in Ice Core Sciences), has set itself the immediate target of establishing a continuous 1.5-million-year record to attempt to answer these questions. The search for the right sites is beginning, and is likely to take several years. The best places are undoubtedly in eastern Antarctica, most probably in remote, high regions where snowfall rates and temperatures are extremely low. Meeting the challenge of drilling those cores should open up a further window on goings-on in the greenhouse. ■

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1. Lüthi, D. *et al. Nature* **453,** 379–382 (2008).
2. Loulergue, L. *et al. Nature* **453,** 383–386 (2008).
3. Petit, J. R. *et al. Nature* **399,** 429–436 (1999).
4. Spahni, R. *et al. Science* **310,** 1317–1321 (2005).
5. Siegenthaler, U. *et al. Science* **310,** 1313–1317 (2005).
6. Jouzel, J. *et al. Science* **317,** 793–796 (2007).
7. Severinghaus, J. P., Sowers, T., Brook, E. J., Alley, R. B. & Bender, M. L. *Nature* **391,** 141–146 (1998).
8. Huber, C. *Earth Planet. Sci. Lett.* **243,** 504–519 (2006).
9. Ahn, J. & Brook, E. J. *Geophys. Res. Lett.* **34,** L10703 (2007).
10. Schmittner, A., Brook, E. J. & Ahn, J. in *Ocean Circulation: Mechanisms and Impacts* (eds Schmittner, A., Chiang, J. C. H. & Hemming, S.) 315–334 (AGU, Washington DC, 2007).
11. Clark, P. U. *et al. Quat. Sci. Rev.* **25,** 3150–3184 (2006).
12. Berger, A., Li, X. S. & Loutre, M.-F. *Quat. Sci. Rev.* **18,** 1–11 (1999).
13. MacFarling Meure, C. *et al. Geophys. Res. Lett.* **33,** L14810 (2006).
14. www.cmdl.noaa.gov/infodata/ftpdata.html

SIGNAL TRANSDUCTION

The rhodopsin story continued

Gebhard F. X. Schertler

Determination of the architecture of an invertebrate photoreceptor protein, squid rhodopsin, is a notable event. It illuminates the mechanism of invertebrate vision and a ubiquitous intracellular signalling system.

Many invertebrates have excellent visual systems[1]. Squid, for example, are formidable hunters that rely on their acute visual abilities to catch their prey. As in vertebrates, the properties of the photoreceptor protein rhodopsin contribute significantly to those abilities. So one reason for the attention that will be paid to the paper on page 363 of this issue[2], in which Murakami and Kouyama present a high-resolution crystal structure of rhodopsin from the squid retina, is that it will help in understanding invertebrate vision. But the paper's significance extends far beyond that.

Rhodopsin is located in the cell membrane of photoreceptor cells. When activated by light, it undergoes a conformational change that triggers the action of a heterotrimeric GTP-binding protein (G protein) lying just beneath the cell membrane. Rhodopsin, therefore, is a member of the superfamily of G-protein-coupled receptors (GPCRs), all of which contain seven structural domains that each span the membrane. However, invertebrate rhodopsin signals through the α-subunit of a G_q type of G protein (rather than transducin, the α-subunit for vertebrate rhodopsin), leading to activation of phospholipase C and eventually the opening of a calcium channel (rather than activation of cyclic GMP phosphodiesterase, leading eventually to closing of a cation channel, which occurs in vertebrates[3]).

In other words, Murakami and Kouyama[2] are the first to determine the structure of a G_q-coupled GPCR. The wider significance of the paper is that many hormone and neurotransmitter receptors signal through G_q, including vasopressin and oxytocin receptors as well as serotonin and acetylcholine receptors in the brain. And many drugs, such as antihistamines and angiotensin antagonists, target G_q-coupled receptors.

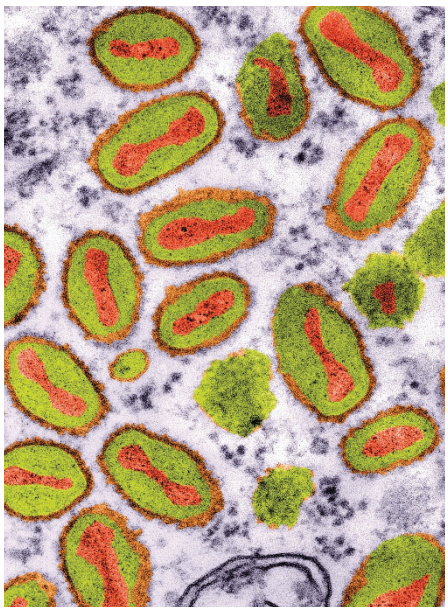
The functional unit of squid rhodopsin

underlying mechanism is as follows. When a cell becomes infected with the virus, it displays apoptotic features, including the presence of phosphatidylserine in its outer membrane layer. Thus, when the virus buds off from the cell, it inherits this as part of its envelope. Consequently, cells probably 'mistake' the unusually large vaccinia virus for an apoptotic body (the debris of dying cells) and engulf it.

Mercer and Helenius[3] find that vaccinia virus seems to enter its host cell through an endocytic process called macropinocytosis, which normally mediates fluid uptake. Like virus budding, virus uptake also exploits apoptotic mechanisms. The authors show that vaccinia virus initially binds to cytoplasmic protrusions called filopodia that extend from the surface of the target cell. It moves along them towards the cell body, and then somehow sends signals into the cell, stimulating extensive membrane blebbing. During blebbing, the actin network that forms the scaffolding of the cell beneath the cell membrane becomes detached (Fig. 2). Blebbing is transient, and soon after, the network reassembles in the same cellular location, and the blebs retract[5].

The authors find that virus internalization by this macropinocytosis-like process is mediated by the blebs, as bleb retraction and re-formation of the actin network coincide with virus entry. Moreover, the drug blebbistatin, which inhibits blebbing[6], blocks virus entry. In addition, virus internalization requires several proteins (including actin, PAK1, Rac1 and various lipid- and protein-kinase enzymes) that are involved in membrane blebbing[3]. Thus, blebbing might participate in endocytosis, probably when the bleb is retracting and the actin system is re-forming, as the bleb could fold over, or invaginate — a process that would resemble macropinocytosis.

The possibility that blebbing and macropinocytosis are two entirely independent processes, both of which are stimulated by vaccinia virus, is equally valid. Specifically, blebbistatin inhibits the myosin II protein, which is required for blebbing. But it can also inhibit myosin-II-independent processes such as macropinocytosis[7]. So Mercer and Helenius's observations raise the question of whether macropinocytosis should be subdivided into at least two types: the traditional type in which membrane ruffling (small, dynamic folds of the membrane; Fig. 2b) precedes vesicle formation; and the type that involves blebbing. Multi-modal macropinocytosis would fit well with the increasing number of other types of endocytosis that are being identified[8].



K. BOLLER/SPL

Figure 1 | Source of infection. False-colour electron microscopy image of vaccinia virus.

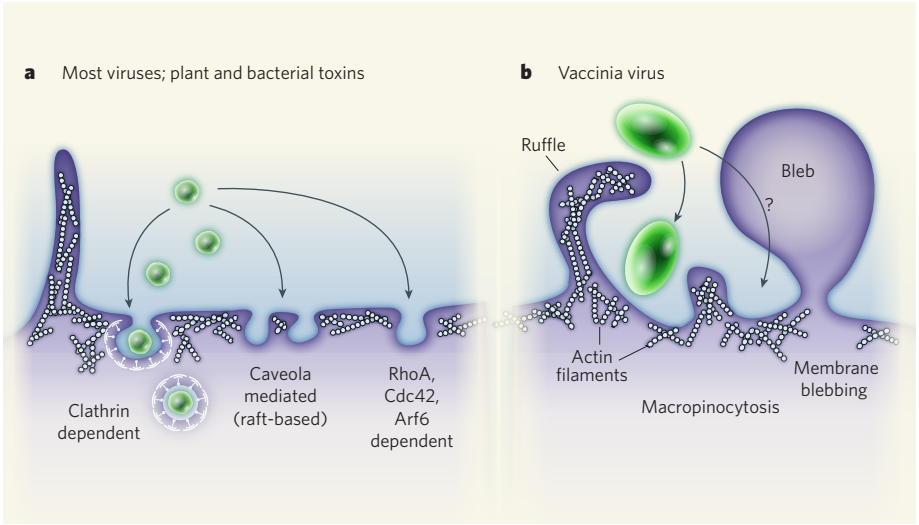


Figure 2 | Vaccinia virus chooses to be different. a, Most viruses, as well as plant and bacterial toxins, enter host cells through the classic method of endocytosis, which involves membrane invagination and pinching off of the membrane to form an intracellular transport vesicle. Three different forms of this type of endocytosis are shown. **b**, Mercer and Helenius[3] find that vaccinia virus enters by an endocytic mechanism resembling macropinocytosis. On the cell surface, the virus triggers membrane blebbing, which might also lead to the formation of membrane invaginations that will evolve into transport vesicles. The actin network, which is normally present beneath the cell membrane and is involved in various endocytic processes, is absent in the bleb, but re-forms during bleb retraction.

That opportunistic pathogens exploit various mechanisms for entry and replication within host cells is also documented in a study[9] of the bacterium *Pseudomonas aeruginosa*. This pathogen induces the formation of very large membrane blebs in epithelial cells, entering the blebs and replicating there. The blebs are quite translucent, and do not seem to contain cytoskeletal elements such as actin. Moreover, the bacteria are highly motile within the blebs. But the exact mechanism of bacterial entry into them remains elusive.

Discoveries often raise new questions, and Mercer and Helenius's work[3] is no exception. First, what is the exact relationship between blebbing and macropinocytosis? Cholesterol, for example, is required for both virus infection and macropinocytosis. Is it also required for blebbing? Is virus-induced blebbing cell-type-specific? What happens in polarized cells, in which membrane components and structural elements vary in different parts of the cell, as opposed to the non-polarized cell lines that were studied here? As the active form of the Arf6 protein inhibits virus infection, one might also wonder how Arf6 is involved in this process. Much is to be learnt about the mechanisms and pathways underlying the internalization of opportunistic pathogens such as vaccinia virus. Ironically, further knowledge about endocytosis itself is likely to come from studies of pathogens. ■

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1. Marsh, M. & Helenius, A. *Cell* **124,** 729–740 (2006).
2. Sandvig, K. & van Deurs, B. *Gene Ther.* **12,** 865–872 (2005).
3. Mercer, J. & Helenius, A. *Science* **320,** 531–535 (2008).
4. Henson, P. M., Bratton, D. L. & Fadok, V. A. *Curr. Biol.* **11,** R795–R805 (2001).
5. Charras, G. T., Hu, C.-K., Coughlin, M. & Mitchison, T. J. *J. Cell Biol.* **175,** 477–490 (2006).
6. Straight, A. F. *et al. Science* **299,** 1743–1747 (2003).
7. Shu, S., Liu, X. & Korn, E. D. *Proc. Natl Acad. Sci. USA* **102,** 1472–1477 (2005).
8. Sandvig, K., Torgersen, M. L., Raa, H. A. & van Deurs, B. *Histochem. Cell Biol.* **129,** 267–276 (2008).
9. Angus, A. A. *et al. Infect. Immun.* **76,** 1992–2001 (2008).

Correction
In the News & Views article "Palaeoclimate: Windows on the greenhouse" by Ed Brook (*Nature* **453,** 291–292; 2008), the wrong credit was used for the picture on page 291. The figure in fact came from the 1978 doctoral thesis of W. Berner (University of Bern).