animal evolution to produce animal-specific functions, such as movement.

Another intriguing finding was that inactivating most of the genes on the X chromosome had no effect on embryonic or larval survival, although many of these genes (as many as on non-sex chromosome V) did produce defective phenotypes when silenced. The authors propose that this is because many X-chromosome genes are naturally switched off during development, being turned on only later. Finally, Kamath *et al.* show that the genes that produce a noticeably defective phenotype when inactivated tend to be clustered in large chromosomal regions of up to 1 million base pairs. That suggests some form of long-range coordinated regulation of these genes.

The libraries of plasmids and modified *E. coli* developed by Kamath *et al.*[1] provide a powerful tool for loss-of-function genetic screening, especially when geared towards a particular molecular or phenotypic read-out. In the second paper (which has many of the same authors), Ashrafi *et al.*[2] describe such a screen for fat-regulatory genes in *C. elegans*. As well as the dsRNA, the authors included a fluorescent dye in the worms' diet. This allows fat droplets in intestinal cells of living worms to be visualized. They then determined the amount of body fat by measuring the fluorescence intensity.

The authors identified 305 genes that reduced the amount of body fat upon inactivation, and 112 genes that increased it. By repeating the RNAi screen for these genes in worms whose body fat was already altered by mutation, Ashrafi *et al.* were able to assign some of the genes to known fat-regulating pathways. Many of these genes are conserved in humans, so might represent new targets for anti-obesity drugs. Other functional RNAi-based screens are to be anticipated, and the worm could become a major model organism for identifying new candidate drugs to treat metabolic diseases.

RNAi-based loss-of-function screens like these[1,2] are tremendously powerful. Yet they have some disadvantages compared with classical genetic screening. For instance, classical screening also looks at what happens when a gene's activity is turned up; such studies have often proved essential for ordering genes in pathways. Also, proteins are generally embedded in a network of interactions with many partners, and more highly specific phenotypes are expected if only one of the protein's interaction domains is altered by mutation, instead of the entire molecule being eliminated by RNAi. Moreover, some genes are more difficult to target by RNAi than others. And there are many noncoding RNAs, which are not translated into proteins; it remains to be seen if they are susceptible to RNAi.

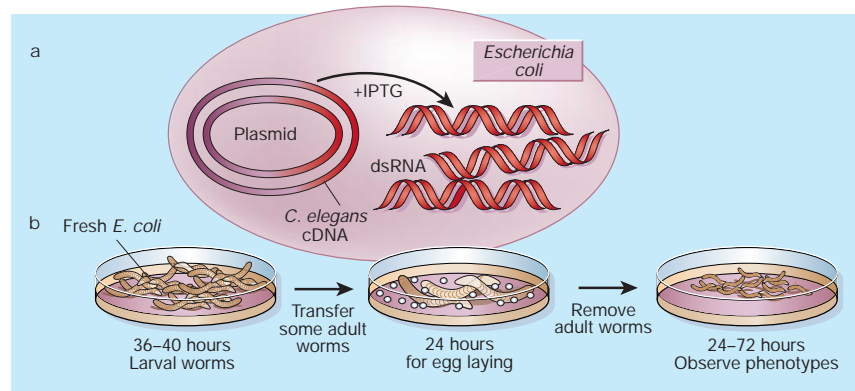But there is still a vast number of protein-coding genes, from many different organisms,

Figure 1 **Gene screening by double-stranded-RNA-mediated interference (RNAi). Kamath *et al.*[1] and Ashrafi *et al.*[2] used the following technique to silence the expression of 16,757 genes individually in** *Caenorhabditis elegans*. a, **DNA molecules (plasmids) encoding a double-stranded RNA (dsRNA) of choice are inserted into** *Escherichia coli* **bacteria. Incubation with isopropylthio-β-galactoside (IPTG) induces production of the dsRNA.** b, **Worms at the latest larval stage are placed on a lawn of** *E. coli*, **and allowed to feed. Several adult worms are then placed onto new plates seeded with the same bacteria to lay eggs. The offspring are monitored for embryonic death and post-embryonic phenotypes, such as slow larval growth or movement disorders.**

to study in detailed RNAi-based functional analyses, and this will keep the army of cell and molecular biologists busy for some time. Soon it will be possible to carry out RNAi-based screens in animal and human cells, using short synthetic double-stranded RNAs[9] or plasmid- or virus-based DNA molecules that encode hairpin RNAs[10]. With the development of phenotypic read-outs based on cell biology, the hunt will begin. ∎

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Palaeoclimatology

Cooling a continent

Peter Barrett

The effect of greenhouse gases on climate is underscored by modelling work showing that formation of the Antarctic ice sheet, 34 million years ago, occurred largely because of a fall in atmospheric CO_2 concentration.

The first continent-wide Antarctic ice sheet formed in earliest Oligocene times[1], now dated at about 34 million years ago. Given its global significance, and that the past is one of the few ways we have of peering into our climatic future, understanding that event has been among the main aims in palaeoclimatology. On page 245 of this issue, DeConto and Pollard[2] provide an account that differs from the commonly accepted explanation in invoking changes in atmospheric CO_2, rather than in ocean circulation, as the determining factor.

The previous scientific story goes back to the 1970s, when Shackleton and Kennett[3] pioneered the recovery of long-term temperature data from deep-sea sediments, in the form of oxygen isotope ratios preserved in calcium carbonate microfossils. They identi-

fied a progressive global cooling over the past 65 million years, with step-like falls in temperature at about 34 and 15 million years ago, as shown in Fig. 1, overleaf[4]. Kennett[5] linked these steps to the separation of Australia and South America from Antarctica, which opened ocean gateways that forced current systems in the Southern Hemisphere from primarily north–south flows in the Pacific, Atlantic and Indian Oceans to west–east flows around Antarctica. The inferred consequence was cooling of the Antarctic region by thermal isolation, through a reduction in heat transfer between the Equator and the South Pole, permitting sea ice to form. Subsequent further cooling allowed the formation of a large permanent ice sheet, although sediment cores from the Antarctic margin have since shown that the first cooling
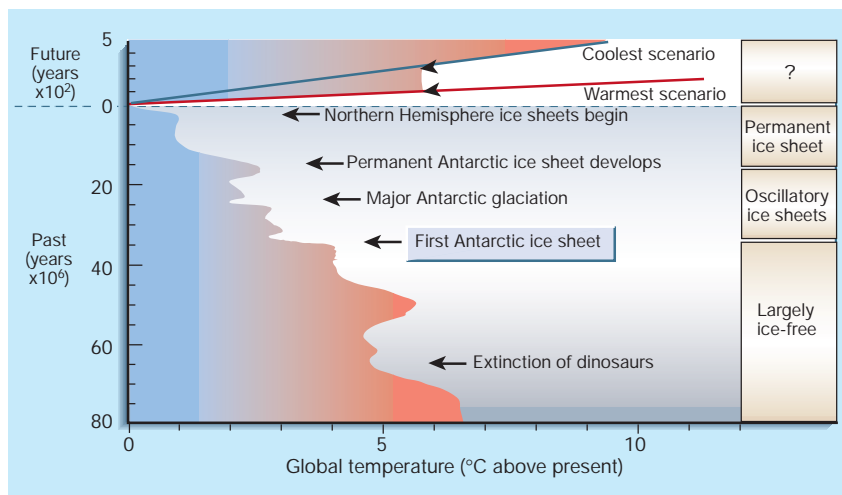
Figure 1 **Decline in average global temperature over the past 80 million years of Earth's history. This global temperature curve is inferred from deep-sea isotope data[4], the zero point being today's temperature of 15 °C. Of the significant transitions in glacial history[1] shown, the sharp cooling associated with the formation of the first Antarctic ice sheet, 34 million years ago, is ascribed by DeConto and Pollard[2] to a decrease in the concentration of atmospheric CO_2. Global temperature projections for the future, produced by the Intergovernmental Panel on Climate Change (IPCC)[14], are shown on a greatly expanded timescale. According to the warmest IPCC scenario, by the end of this century global climate will be similar to that near the end of the reign of the dinosaurs. On the coolest scenario, this climate will be achieved in about 300 years, assuming that the CO_2 concentration continues to increase at the present rate. The glacial oscillations that have occurred in the past on timescales of 40,000 and 100,000 years[1] are not shown, but each decreased the global temperature by about 5 °C.**

step, 34 million years ago, was sufficient for extensive ice growth on the continent[1].

DeConto and Pollard[2] report modelling studies of two further influences on the Antarctic climate — changes in atmospheric CO_2 levels and in the Earth's orbital parameters. They show how a decline in CO_2 content, from four times to double that of our atmosphere before the past century (a baseline termed the pre-industrial atmospheric level), can result in a climate in which winter snow eventually survives through the summer and over a large enough area to start forming an ice sheet. They also show how the exact timing of ice-sheet initiation can be affected by a combination of the Earth's orbital parameters that results in minimal summer insolation of the Antarctic.

DeConto and Pollard also checked on the likely influence, previously queried[6], of the ocean gateways opening south of Australia and South America at about the time that the first big ice sheet grew. To do this they compared the effects on simulated ice-sheet growth of an open and a closed Drake Passage, using the estimate of 20% for the reduction in southward ocean heat transport resulting from gateway opening. The Drake Passage lies between South America and the Antarctic Peninsula, and when closed represented the final barrier to circum-Antarctic circulation. The result of the experiment showed a shift in the threshold CO_2 level for ice-sheet growth from 2.4 (closed) to 2.8 (open) times pre-industrial levels. However,

there was no significant change in the pattern or magnitude of ice-sheet formation, confirming the primacy of atmospheric CO_2 as the controlling factor.

This study[2] is a fine example of how modern computer technology can be applied to major questions about climate processes and history. It also highlights areas where further research is needed, such as the relative influence of temperature and ice volume on the deep-sea isotope curve, and the effects of the roughness and hardness of the terrain beneath an ice sheet on its behaviour. But as with all models of past events, however elegant, it requires validation through concrete evidence from observations of today's world, and the records in ice and sediment layers on land and beneath the sea. For the first Antarctic ice sheet, the relevant timing and geography have been fairly well established from the evidence cited by DeConto and Pollard, and the orbital variations they call upon to trigger the first ice sheet are recorded in younger, cyclic sequences evident in cores drilled off the Antarctic margin[7]. However, a sedimentary record of the transition from 'greenhouse' to 'icehouse' Antarctica on the continent itself, with information on the extent and rate of the change in climate and vegetation, has yet to be recovered. Drilling programmes such as ANDRILL[8] and SHALDRIL[9], now under way, should provide the relevant data — for example on changes in coastal temperatures through the transition period.

What of other climate transitions in the

past? For instance, the small step at about 24 million years ago that coincides with a widely recognized fall in sea level[10] and a shift in Antarctic coastal vegetation from beech forest to tundra[11]? Or the larger step at 15 million years ago, which is widely interpreted as the transition from an ephemeral to a permanent Antarctic ice sheet[1]? These two episodes are still not understood. There is also continuing curiosity over the response of the Antarctic ice sheet during the worldwide warming about 3 million years ago. Here uncertainties need to be resolved in dating glacial deposits in the Transantarctic Mountains. On the basis of rare microfossils, some contend that the deposits date to this time[12]; others, however, have shown how the microfossils could be windborne contaminants, and the deposits could be much older[13].

As to the future, it is now clear that land-surface and ocean temperatures are rising in response to human-induced emissions of greenhouse gases — and remarkably fast on a geological timescale. The present concentration of greenhouse gases is 30% above the benchmark pre-industrial content, rising to that level in only a century, and is likely to be the highest for the past 20 million years[14]. According to the 'warmest scenario' projections of the Intergovernmental Panel on Climate Change, the projected doubling of CO_2 concentration by the end of this century is expected to result in a climate that the planet last experienced about 70 million years ago (Fig. 1).

The effects of this change are difficult to predict, but they will plainly be profound. Finding out more will require closer collaboration between geoscientists who work at extracting data on past climates from ice and sediment cores and from rock strata, and modellers who use complex mathematical representations to approximate events in the physical world. An example of such collaboration is the formation of the Antarctic Climate Evolution planning group[15].

More generally, DeConto and Pollard's study[2] brings a new understanding of the effects of CO_2 emissions on climate, and adds force to the arguments for reducing greenhouse-gas emissions beyond those agreed in the Kyoto Protocol. At the same time it is clear that regional responses to climate change through interactions between the oceans, atmosphere and ice sheets vary considerably from the global average. The science of mitigating the effects of greenhouse emissions is in some countries and cases taking priority over research into understanding climate behaviour, especially regionally. Both areas of research will need sustained support, along with an international commitment to an effective solution, if we are to survive the worst consequences of this grandest of all human experiments. ■

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Immunology

Mobilizing the army

Steven D. Shapiro

When our bodies are injured or infected, inflammatory cells migrate to the damaged area to carry out rescue and repair work. Interactions between three types of protein may form the basis of a highway to guide these cells.

Our bodies respond to injury and infection by mobilizing inflammatory cells, which, on reaching an afflicted area, kill microorganisms, eliminate any debris and regulate tissue repair. Neutrophils, which are generated in the bone marrow and circulate through the bloodstream, are the first such cells to arrive on the scene. Armed with potent protein-digesting enzymes and oxidants, neutrophils are consummate microbe killers. But if these cells accumulate and are activated in an uncontrolled way, they can cause excessive inflammation and injure the very tissues they are designed to protect. So it is important to understand how neutrophils find their way to, and so accumulate at, sites of injury. A breakthrough came recently with a paper published in *Cell* by Li and colleagues[1].

As in previous studies of tissue damage, Li *et al.*[1] infused the drug bleomycin into the lungs of mice; this causes short-lived injury and consequent inflammation of the layer of epithelial cells that lines the lungs, followed by long-lasting scarring. The authors show that when wild-type mice are treated in this way, neutrophils migrate through the lungs' capillaries, cross both the capillary wall and the epithelial layer, and enter the airspace, where they start to repair the damage. But the situation is rather different in mice lacking a particular matrix metalloproteinase[2] — an enzyme that cleaves components of the web of proteins and other molecules (the extracellular matrix) in which cells are embedded in tissues. Li *et al.* show that without this enzyme, called matrilysin or MMP-7, neutrophils accumulate in the interstitial space that separates capillaries from the epithelial tissue, and do not cross the epithelium or enter the airspace.

So MMP-7 is clearly necessary for neutrophils to migrate to sites of injury, at least in this case. But what is the key substrate for this enzyme? The authors went on to investigate, and in so doing uncovered a mechanism by which three components of the epithelial tissue interact to produce a chemical (chemotactic) gradient that attracts migrating neutrophils (Fig. 1, overleaf). When injured, epithelial cells secrete a chemokine called KC; chemokines are proteins known for their ability to contribute to chemotactic gradients, attracting cells in the direction of the gradient. KC binds to syndecan-1, an adhesive component of the extracellular matrix that contains heparan sulphate. MMP-7 is also released from epithelial cells, and cleaves or 'sheds' the syndecan-1–KC complex from the extracellular matrix, allowing a gradient of this complex to form.

All of these protein families — matrix metalloproteinases, chemokines and matrix components — had already been individually implicated in cell movement. The triumph of Li *et al.*[1] is to explain how these molecules fit together *in vivo*, and to clarify their exact functions in neutrophil migration. For instance, the ability of many cells to move and to traverse tissue barriers has long been ascribed largely to matrix metalloproteinases, with tumour cells in particular using these enzymes to clear paths, promoting local tissue invasion and distant metastasis. Extrapolating from these observations of tumour cells, it was speculated that inflammatory cells use their cadre of matrix metalloproteinases to move through tissue barriers in a similar fashion. Indeed, this has been seen to occur.

Yet confusion remained, because it was obvious that these enzymes do more than simply clear road-blocks from the path of migrating neutrophils. Moreover, cells have other ways of moving. For example, tumour cells normally take straight paths, degrading the extracellular matrix as they go. But in the presence of matrix-metalloproteinase inhibitors, such cells change both their pattern of movement and their shape as they

pick their way through microscopic gaps in the matrix (P. Freidl, personal communication). Neutrophils, meanwhile, are known to be the most 'flexible' inflammatory cells, able to weave through tissue barriers in the absence of protein-digesting enzymes[3]. All in all, then, Li *et al.*'s discovery[1] that MMP-7 is involved in neutrophil migration was perhaps not surprising. But its precise role — in 'shedding' syndecan-1–KC complexes to form a chemical gradient — was unanticipated, and shows that MMP-7 is needed for directed neutrophil migration, rather than just movement *per se*.

To complicate the picture still further, matrix metalloproteinases may also limit inflammation. Three years ago, McQuibban *et al.*[4] showed that some of these enzymes process and inactivate the chemokines to which other inflammatory cells — monocytes — respond, thereby restricting monocyte migration into sites of injury. This, together with the work of Li *et al.*, suggests a feedback mechanism whereby matrix metalloproteinases initially promote cell influx to damaged areas; later, excessive quantities of these enzymes turn off the pro-inflammatory chemokine signals.

And what of the chemokines? These proteins have long been known to act as attractants by binding to receptors on the surface of inflammatory and immune cells. But the large number of chemokines, and the lack of specificity of particular receptors for particular chemokines, has made it difficult to ascribe functions to individual molecules. So it is another significant feature of the new work[1] that, although the authors found many chemokines to be present in injured mouse lungs, a gradient of chemokine KC alone (established by syndecan-1) was both necessary and sufficient to control neutrophil migration.

Which brings us, finally, to syndecan-1. Fragments of other matrix proteins have previously been shown to be chemoattractants in cell cultures. For instance, fragments of elastin attract monocytes and fibroblast cells[5,6], whereas fragments of laminin, collagen or entactin attract neutrophils. These fragments are believed to work alone, but their significance *in vivo* is unclear. The importance of fragments of syndecan-1, however, is now apparent. Previously, it was shown that interleukin-8 (which is related to chemokine KC) must bind heparan sulphate to mediate directed neutrophil migration[7], and that metalloproteinases can shed the extracellular domain of syndecans[8]. Li *et al.* tie all this work together, showing that syndecan-1–KC complexes are snipped away from the main extracellular matrix by matrix metalloproteinases. The broader implication is that other matrix fragments may cooperate with other chemokines to create gradients that direct cell movement.

So Li *et al.*'s work completes one part of