into pieces shorter than 100 base pairs.

Researchers were quick to exploit the new possibilities. Only months after the 454 technology became available, it was applied to mammoth genomics in a paper[5] that reported 13 million base pairs of sequence — about 1,000 times more than were covered in the first ancient-genomics study with Sanger sequencing[6]. In that paper[5], published in January 2006, the authors also announced their plan to sequence the mammoth genome to completion. Miller and colleagues[1] now describe about 70% of the mammoth genome, and so go a long way to achieving that goal.

Miller *et al.* were aided immensely in their task by the fact that, unusually for extinct organisms, some specimens of woolly mammoths have been frozen in permafrost. This is an ideal setting for preserving DNA, and, moreover, for preserving hair, which is an ideal source of DNA for sequencing ancient genomes. If hair still contains DNA, almost all of it will belong to the extinct species, and will not be of bacterial or fungal origin, as is often the case with bones. Thus, the authors needed to sequence a total of 'only' 4.1 billion base pairs to obtain about 3.3 billion base pairs of mammoth DNA. They calculate that the total mammoth genome, estimated at some 4.7 billion base pairs, would have been 1.4 times bigger than the human genome.

Although the mammoth genome is larger than the human genome, the DNA substitution rate seems to be smaller — this is the rate at which one nucleotide replaces another, and so is a measure of evolutionary change. The mammoth genome differs from that of its close relative the African elephant by as little as 0.6%. This is about half the difference between human and chimpanzee, although the two elephant species diverged at about the same time as human and chimpanzee, and probably even slightly earlier (see Fig. 3 of the paper[1] on page 389). For some reason, the substitution rate in the nuclear genome of elephants is much lower than in humans and great apes, a result mirrored in the mitochondrial genomes[7], where humans and great apes also show a substitution rate more than twice as high as that in the elephant species. As nuclear and mitochondrial genomes are replicated by different enzymes, it remains unclear why both genomes evolve more slowly in elephants than in humans and great apes.

The draft mammoth genome sequence is too fragmented and error-prone to allow standard gene prediction. Nonetheless, Miller and colleagues identified several protein-coding positions that are unique to the mammoth compared with 50 other vertebrate species. The presence of such mammoth-specific differences is not surprising: it is to be expected that each mammalian species contains unique amino-acid substitutions compared with a limited number of other species. For example, the 52-amino-acid fragment of the ATP2C1 protein not only contains an amino-acid substitution unique to the mammoth, but a further two that are unique to the tenrec and the two-toed sloth, respectively. Similarly, the position in the 30-amino-acid fragment of the protein C1orf190, at which the mammoth differs from other placental mammals, also has amino-acid substitutions in the ground squirrel and kangaroo rat. Although Miller and colleagues argue that the amino-acid differences they identify have a "significantly enhanced likelihood of causing ... phenotypic effects", their analyses by no means prove that an amino-acid substitution has functional consequences or adaptive value. Such questions can be answered only by investigations of the proteins in question.

So what do we learn from the mammoth genome, except that sequencing of complete genomes from extinct species is indeed possible and that there are differences in their DNA sequences compared with those of living animals? As with many draft genome projects, not that much. But a draft genome is only the beginning of the story. The main feature of genome projects is to provide a resource for further research, as vividly shown by the thousands of times the initial human-genome sequencing papers[8,9] have been cited.

The next draft nuclear genome of an extinct species likely to become available is that of our closest relative, the Neanderthal, following on from publication of a complete Neanderthal mitochondrial genome sequence[10]. For some time yet, much work in genomics will consist of fully annotating and completing genome sequences, as indeed most published sequences of extant vertebrates, let alone that of the extinct mammoth, remain drafts. But when we look further into the future, the task will be to understand which differences at the sequence level underlie the phenotypic differences between a mammoth and an elephant, or a human and a Neanderthal, for which well-annotated genomes provide the essential basis. ■

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PALAEOCLIMATE

Greenhouse-gas fingerprints

Thomas F. Stocker and Adrian Schilt

Short episodes of warming and cooling occurred throughout the last glaciation. An innovative modelling study indicates that ocean-circulation changes produced much of the causative variation in greenhouse gases.

Much of what we know about abrupt climate change and tipping points in the climate system comes from polar ice cores[1]. But these unique data archives provide only a narrow view of the richness of climate dynamics and impacts. Moreover, the origin of the variations in the greenhouse gases associated with the pronounced climate swings during the last ice age, an interval between about 110,000 and 10,000 years ago, remains largely unknown. Hence the significance of computer models in providing a wider perspective.

On page 373 of this issue, Schmittner and Galbraith[2] present climate-model simulations for an episode of abrupt climate change during the last ice age. Their results show agreement with the palaeoclimatic record[3], not only in terms of physical climate variables, but also, remarkably, in changes in the greenhouse gases carbon dioxide (CO_2) and nitrous oxide (N_2O). The researchers conclude that the interaction of physical and biogeochemical processes in the ocean is largely responsible for the observed variations.

Schmittner and Galbraith[2] use a coupled climate model of intermediate complexity suitable for palaeoclimatic studies. The physical part of the simulation features a comprehensive ocean-circulation model coupled to an energy-balance model of the atmosphere. A marine-ecosystem module, which includes two classes of phytoplankton, simulates the distribution of nitrate, phosphate, oxygen, inorganic carbon and alkalinity in the ocean. Although the model accounts for carbon cycling in the ocean, atmosphere and terrestrial vegetation, it deals with the global nitrogen cycle only in a simplified fashion. Production of marine N_2O is diagnosed from oxygen concentrations, and the stratospheric sink and the soil source of N_2O are assumed to remain constant.

During the last ice age, the climate system exhibited a series of rapid changes known as Dansgaard–Oeschger events. These involved temperature changes of up to 15 °C in Greenland within a few decades[4]. There is good

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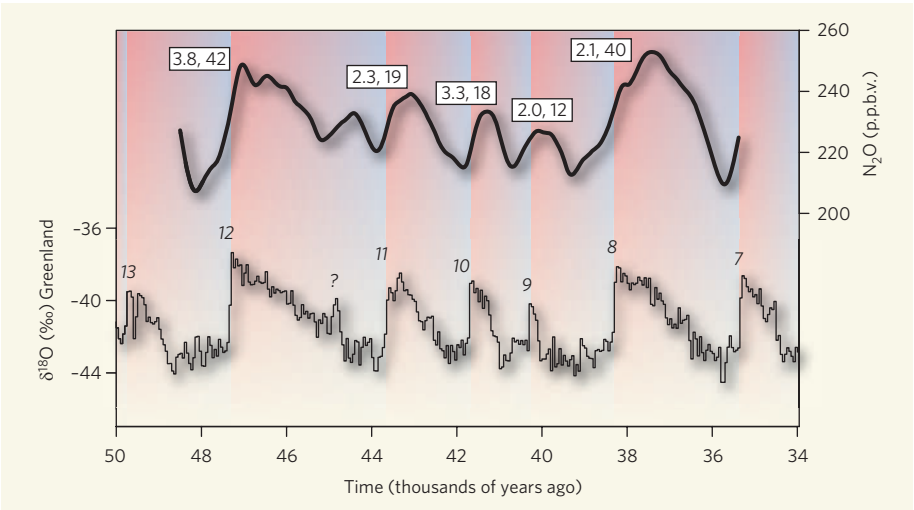

Figure 1 | Records of atmospheric N_2O and of Dansgaard–Oeschger events over a 16,000-year interval during the last ice age. The rich structure of N_2O variations[3,13] (shown as a spline through the data) correlates well with the incidence of Dansgaard–Oeschger events, the sequence of abrupt warmings recorded in a Greenland ice core plotted here as variation in a proxy, oxygen-isotope measurement, $\delta^{18}O$. Paired numbers in boxes indicate the rate of N_2O increase in p.p.b.v. (parts per billion by volume) per century and the total N_2O increase in p.p.b.v., respectively. Italic numbers denote Dansgaard–Oeschger events in this particular oxygen-isotope record[14]. From their modelling work, Schmittner and Galbraith[2] conclude that the N_2O variations are caused by changes in ocean circulation, which, in turn, influence oxygen concentration and productivity in the ocean.

evidence for the view that a major part in these events was played by the Atlantic meridional overturning circulation (AMOC), the flow of warm surface water to the far north that is balanced by the southward flow of cold water at depth. But as long as the smoking gun is missing, scientists must continue to collect circumstantial evidence from palaeoclimatic data and from model simulations to test this hypothesis. Modellers still do not have the all-encompassing climate model that would simulate a series of Dansgaard–Oeschger events in a self-contained way. They therefore need to resort to provoking such abrupt change in their models by adding and extracting large amounts of fresh water to and from the North Atlantic. The manipulation has the effect of switching the AMOC off and on, and this is what Schmittner and Galbraith do with their climate model.

The physical changes simulated by the model are in reasonable agreement with the palaeoclimatic record, although the amplitude of the temperature change in Greenland is significantly underestimated. This is probably due to insufficient sea-ice response, which is known to amplify temperature changes in the northern North Atlantic[5]. At the time when a reduced AMOC is responsible for cold temperatures in Greenland, temperatures in Antarctica start rising slowly, a phenomenon referred to as the thermal bipolar seesaw[6]. The Antarctic warming correlates strongly with the increase in the concentration of atmospheric CO_2. In this model, the increase is primarily caused by a reduction in the efficiency of nutrient use by phytoplankton in surface waters of the Southern Ocean — and hence, when they die, of the transport of carbon from the surface to depth. The response is physically driven by more deep-water formation in the Southern Ocean when deep water in the North Atlantic recedes during a shutdown of the AMOC.

A notable difference between the model results and the palaeoclimatic data appears during the end of the Antarctic warming. In the temperature records, this change extends over about 2,000 years. The model's north–south coupling seems too strong, in that the abrupt resumption of the AMOC causes a similarly abrupt cooling in Antarctica. This may be due to the absence of a dynamical response in the atmosphere, and Schmittner and Galbraith's decision to keep wind patterns constant[7].

The model does not capture the full complexity of the marine nitrogen cycle[8]; rather, a simple empirical relationship between oxygen content and changes in marine N_2O production is used. So a combination of local mixing, water-mass distribution and variability in the carbon cycle determines the concentrations of atmospheric N_2O. Also, the N_2O contribution from soils, which is known to be important, is held constant. In spite of these pragmatic simplifications, the simulations exhibit good agreement with the palaeoclimatic record, not only in amplitude but also in temporal behaviour — at least in the first phase of the abrupt climate change.

Shutdown of the model AMOC causes a cooling in the Northern Hemisphere, which results in better ventilation of the surface waters in the eastern parts of the equatorial Pacific and Indian oceans. Better ventilation increases oxygen content, thereby reducing N_2O emissions from the ocean and hence N_2O concentrations in the atmosphere. The model also seems to capture a characteristic fingerprint of N_2O changes during Dansgaard–Oeschger events first found in ice-core data[3]: longer coolings produce larger reductions in N_2O. This good agreement leaves little room for an effect from N_2O emissions on land, surprisingly so given that about two-thirds of the N_2O emissions today come from the terrestrial biosphere.

Overall then, Schmittner and Galbraith's model[2] performs remarkably well. But there is obvious room for improvement. For example, the simulation of atmospheric N_2O is poor after the abrupt cooling event has ended. The ice-core record clearly shows a peak of N_2O followed by a slow decrease that evolves over several centuries in the sequence of Dansgaard–Oeschger events (Fig. 1). Neither feature is captured by the model. As the major areas of N_2O production are located in the eastern ocean basins, where upwelling of nutrient-rich water directly responds to the wind, one wonders whether changes in wind patterns and strength, which must have occurred during Dansgaard–Oeschger events[9], might have been responsible for the rapid N_2O increase at the time of abrupt warming. Also, it is inferred from the ice-core methane (CH_4) record that the water cycle changed significantly during Dansgaard–Oeschger events[10] because the main source of CH_4 is wetlands. Therefore, a better account of soils and their changes will not only permit the simulation of CH_4, but may also contribute to an improved understanding of the centennial variations in N_2O that have not yet been captured in simulations.

Further progress will come with the generation of more data from isotopic studies of palaeoclimatic archives. Changes in the stable-isotope concentrations of greenhouse gases such as ^{13}C (CH_4), ^{2}H (CH_4), ^{15}N (N_2O) and ^{18}O (N_2O) are powerful fingerprints of different factors affecting the climate system and show their response to climate change. Because such measurements are so challenging, only a fraction of the information that is still locked up in polar ice cores has yet been revealed[11,12]. There is thus a unique opportunity for those working with physical–biogeochemical models such as that of Schmittner and Galbraith. Before more — and more detailed — isotopic data from polar ice cores become available, modellers must venture predictions of the climate changes that these data will reveal. This will be the least prejudiced approach to the problem — and so the best test-bed for our understanding of how oceanic, atmospheric and biogeochemical processes operate and interact in the climate system. ■

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CELL BIOLOGY

Nuclear order out of chaos

Tom Misteli

How cells build their internal structures remains one of the central mysteries in cell biology. If the cell nucleus is anything to go by, stochastic assembly and self-organization seem to be key.

As anyone knows who has ever put together one of those home-assembly bookshelves, most man-made structures can only be built by following a defined sequence of steps. It is difficult to set aside this preconceived notion of linear assembly when thinking about how cellular structures emerge. Yet a landmark paper by Kaiser, Intine and Dundr[1], published in *Science,* makes a powerful case for the formation of biological structures by apparently random pathways and self-organization.

The mammalian cell nucleus is a prototypical, highly organized biological structure[2]. Not only does it hold most of an individual's genetic information, but it is also the site of many essential processes, such as reading, copying and repairing the genome. To coordinate these events — and presumably to make them more efficient — the nucleus is highly compartmentalized and contains numerous nuclear bodies that have distinct functions[2].

Kaiser and colleagues[1] set out to investigate the formation of a prominent nuclear compartment called the Cajal body, a roughly spherical structure of 0.5–1 micrometres diameter. There are typically several Cajal bodies in every nucleus, often near clusters of genes encoding histone proteins or small nuclear RNAs (snRNAs)[3]. Their precise function is unclear, but the fact that they contain factors involved in small-RNA processing suggests they might be involved in modifying nuclear RNAs and in recycling RNA-processing factors[3]. Like all other nuclear compartments, Cajal bodies lack a membrane, and are highly dynamic yet stable steady-state structures, exchanging their proteins rapidly and continuously with the surrounding nuclear space[2].

How Cajal bodies, and indeed all other nuclear bodies, are formed and maintained without membrane boundaries has been a puzzle. Two fundamentally different models have been considered[4]. One idea — akin to the bookshelf-assembly strategy — is that Cajal-body proteins bind to scaffold proteins in an orderly manner to gradually build up the structure. Indeed, scaffold function has been ascribed to two prominent Cajal-body marker proteins, coilin and SMN (Fig. 1a). Alternatively, it has been suggested that nuclear bodies form through self-organization, whereby their components simply aggregate in a stochastic, largely random way (Fig. 1b).

To distinguish between these two models, Kaiser *et al.*[1] first asked a fundamental question: can Cajal bodies form *de novo*? The authors used a bacterial tethering system to immobilize individual Cajal-body proteins at an engineered random site in the genome of mammalian cells. Not entirely unexpectedly, coilin and SMN were each sufficient to form an apparently normal, functional Cajal body. Surprisingly, however, when the authors tethered minor Cajal-body components to the same genomic site, just about any Cajal-body component could initiate the formation of this structure, including all RNA-processing factors. In fact, minor components were more efficient at forming Cajal bodies than coilin and SMN.

These findings lead to two main conclusions. First, the formation of Cajal bodies does not require a specific gene locus, and could potentially occur anywhere in the genome. Second, this process does not require a strict hierarchical pathway, as any Cajal-body protein can initiate the formation of the entire structure.

These observations do not agree well with a linear-assembly model and point to self-organization as a driving force in the assembly. Several other features of Cajal bodies also favour a self-organization model. First, Kaiser and colleagues[1] show that the recruitment of any native protein to a newly formed Cajal body occurs with similar kinetics, suggesting largely random assembly. Second, previous work has shown that coilin and SMN can self-assemble[5]. This tendency of the two proteins might be crucial for the assembly process, as Kaiser *et al.* show that Cajal bodies form less efficiently in the absence of either coilin or SMN, hinting that these marker proteins serve to stabilize transient interactions among other Cajal-body components. Third, Cajal-body proteins are highly dynamic, and move rapidly between this structure and its surroundings — a requirement for a self-organization process. But perhaps most telling is the authors' finding[1] that, despite the ready availability of building blocks, the size of the structure formed *de novo* is limited, a classic hallmark of self-organization.

Are these results relevant to other nuclear structures? Probably. For one thing, Kaiser and colleagues report that tethering of PML — a marker protein of another nuclear body — leads to the formation of a PML body. Furthermore, much of what we know about nuclear bodies suggests shared principles of organization. Like the Cajal body, all known nuclear bodies represent dynamic steady-state systems that undergo rapid exchange of components[2]. Moreover, other nuclear bodies also seem to form at specific nuclear sites. Whereas Cajal bodies preferentially associate with clustered histone and snRNA genes[6], the nucleolus — the most prominent nuclear substructure — forms around sites of ribosomal-RNA transcription, and compartments enriched in factors mediating pre-messenger-RNA splicing form near the sites where RNA is transcribed. The likeliest possibility, therefore, is that nuclear bodies assemble at sites of high gene activity

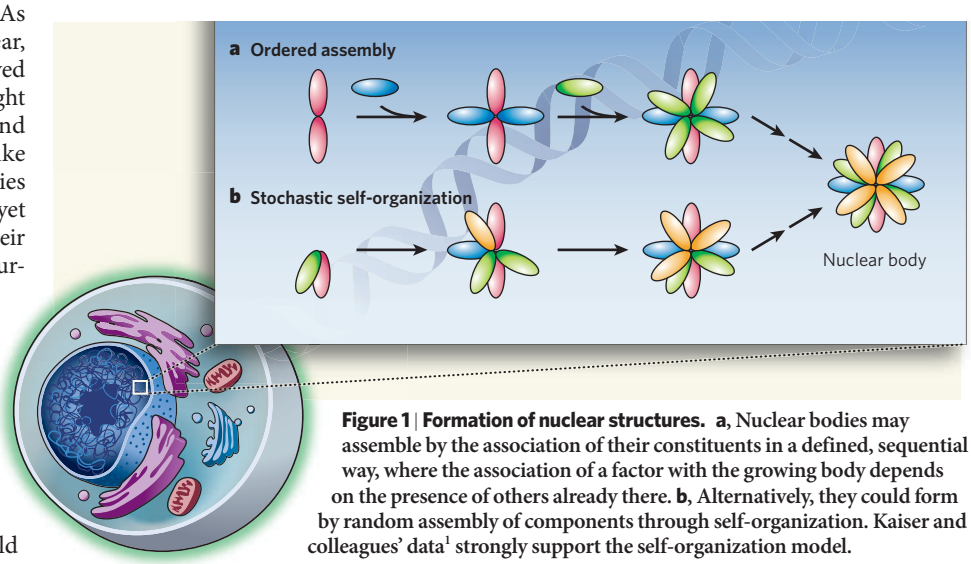

Figure 1 | Formation of nuclear structures. **a**, Nuclear bodies may assemble by the association of their constituents in a defined, sequential way, where the association of a factor with the growing body depends on the presence of others already there. **b**, Alternatively, they could form by random assembly of components through self-organization. Kaiser and colleagues' data[1] strongly support the self-organization model.