

loops—which are replicated together by one factory—stay together for many cell generations; they are again replicated together by new factories in the descendant cells (8). Thus, the nuclear structure can dictate exactly when and where factories are to assemble.

Where do replication and transcription take place within the nuclear tangle? To answer this question, Wei *et al.* (4) permeabilized cells and allowed them to make DNA and RNA in the presence of tagged building blocks, which are incorporated into the nucleic acids; then they bound antibodies to the tags, to which fluorescent labels can be directed so that the newly made DNA appears green and new RNA is red.

After this process, the nuclei in young cells contained several hundred red foci, each marking a transcription factory (only six are shown for the nucleus in the figure). These foci are so faint and small that they can only be seen with sensitive light microscopes pushed to their limits of resolution. How do the replication factories then get assembled during the middle third of the cell cycle? Two simple models present themselves. In one (path A in the figure), replication machines (green) are installed in some of the preexisting (red) transcription factories; in this case we would expect to see some factories (9) that would fluoresce both green and red, thus appearing yellow. Once the replication machines had finished their job, they might be transferred to other transcription (red) factories, turning them yellow; then, when all DNA had been duplicated, no more yellow factories would be seen.

In an alternative model (10) (path B in the figure), existing transcription (red) factories would be decommissioned and completely new replication (green) factories would be constructed in the vicinity. Then, all factories would appear either green or red, but never yellow.

The results, surprisingly, fit neither model: Green foci tended to lie next to green foci, and red ones next to red foci (path C in the figure). This suggests that nuclei are divided into zones, distinct replication (green) and transcription (red) areas restricted to one or the other function (only one zone of each type is shown on path C in the figure). As all DNA is duplicated eventually, groups of red factories must all be decommissioned together when a region is first zoned green, and then recommissioned together when it is rezoned red.

These results beg several questions. How strict are the zoning regulations, and who polices them? What structure underpins a zone? A chromosome? How are factories coordinately decommissioned and recommissioned? As always in this difficult field, the devils are in the details: Did

the conditions used during permeabilization aggregate sticky nucleic acids? How well was the native structure of the tangle preserved during analysis, and were many faint foci missed? Such devils can only be confronted by technical improvements in sample preparation and microscopy, or by imaging active factories in living cells (11).

Whichever path turns out to be correct, these new results confirm that DNA strings are not packed completely randomly like pasta in a bowl; something organizes the replicating and transcribing regions into foci. The simplest possibility is that a factory ties the string into a rosette of loops, while polymerases in the factory reel in the loops during replication and transcription. As always in biology, function depends on structure, and vice versa.

This paper also highlights how little we know about replication and transcription machines, usually depicted in textbooks as small, lone complexes that track along individual segments of DNA. Rather, these results suggest that many machines reel in many different DNA loops simultaneously. At a time when we will soon know the exact sequence of the billions of bases in the human genome, we know almost nothing

about how those bases are strung in three-dimensional space and how the resulting structure facilitates gene function. Common sense suggests that there must be some underlying order within the apparent tangle, and by studying sites of activity the Wei *et al.* report goes directly to the heart of the structural problem.

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PERSPECTIVES: PALEOCLIMATE

Glacial Puzzles

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One of the most perplexing and enduring puzzles in paleoclimatology has been the cause of the 100,000-year rhythm of the major glacial-interglacial cycles during the past 1 million years. The smaller warmings and coolings superimposed on this pattern are more-or-less linear responses to variations in the distribution of solar heating caused by changes in Earth's orbital position, known as "Milankovitch" variations, after an early investigator (1), but no such simple orbital mechanism has been found that can explain the 100,000-year cycle. Although Earth's orbital eccentricity, and hence average distance from the sun, varies with a 100,000-year cycle, the resulting changes in solar heating are believed to be too small to be climatically significant.

How can such a strong climate response arise from such a seemingly weak forcing? And why did the 100,000-year cycle only appear about 800,000 years ago? From about 3 to 1 million years ago, smaller ice

sheets varied at an almost metronomic 41,000-year rhythm, the period of changes in orbital tilt. Hypotheses seeking to explain this 20-year-old paradox have generally fallen into one of three camps: mechanisms that posit that Earth's ice-atmosphere-ocean climate system maintains an internal oscillation near 100,000 years that can get phase-locked to external orbital forcing (2), mechanisms that invoke highly nonlinear responses of this system to weak forcing by eccentricity (3), and mechanisms that instead invoke temporal variations in the inclination of Earth's orbit relative to the solar system (4), another orbital parameter that varies with a periodicity of about 100,000 years. Each of these explanations has difficulty accounting for some aspect of the climate record, and hence none have achieved broad acceptance. Recently, a fourth type of hypothesis has been proposed (5), one that elegantly avoids some of the shortfalls of earlier models and draws attention to simple relations apparent in the most recent and accurate ice volume and insolation records [see, for instance, (6)].

Inspired by the observation that models of ocean thermohaline circulation have multiple steady states, Paillard (5) investigated a

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climate system with three steady states and a set of predefined rules for moving between them. The three climatic regimes are interglacial, mild glacial, and full glacial; transitions between regimes occur whenever a specific threshold is passed. Very simply, if incoming solar radiation (insolation) at high summer latitudes falls below a specific threshold, an interglacial to mild glacial transition occurs. If insolation then stays below a critical threshold for an extended period of time, a mild glacial to full glacial transition will occur. The model returns to interglacial conditions only from a full glacial state by exceeding a defined insolation threshold; it cannot go directly from a full glacial to a mild glacial or from a mild glacial to an interglacial state. This simple linear differential model, which is forced by insolation and which allows ice volume to change continuously, shows an impressive match to the geologic record of ice volume change (see figure). In particular, by showing a strong interglacial response at marine isotope stage 11, a time of weak eccentricity forcing, this model does not fall victim to the classic "stage 11 shortfall" problem of the oscillator and nonlinear response models.

How realistic are the assumptions of this multiple-state model when compared with our best geologic records of the ice ages? We know that one of the defining characteristics of the "100,000-year world" is terminations: rapid and abrupt shifts from extreme glacial to extreme interglacial conditions that are not observed earlier in the Pleistocene. These terminations, which conclude in an interglacial-glacial couplet, require for their genesis the buildup of unusually large, presumably unstable ice sheets. The inherent instability of such ice sheets can then explain the extraordinary rapidity of the deglaciation. This "extra" ice appears to grow only during extended periods of low summer insolation at high northern latitudes (see figure). When insolation next increases to high levels (that is, above a certain threshold), terminations are triggered much as described by Paillard's model.

From insolation and ice volume records (see figure), it is also apparent that interglacial-glacial cycles are "quantum" in the sense that they are either four or five pre-

cessional cycles long depending on the particular interactions of obliquity and eccentricity with precession. This characteristic is captured by Paillard's model. What the model does not appear to capture is the lack of large ice sheet growth and, subsequently, a termination, during marine isotope stages 7 to 8 and 13 to 14 (see figure). The data suggest that a mild glacial to mild interglacial transition can occur, a sequence of events precluded by Paillard's initial model.

This brings one back to the big question, the one asked by our students: What causes the 100,000-year cycle? I would answer that it is a pseudoperiodic cycle varying in length from about 80,000 to 120,000 years. It is caused by the periodic buildup of large

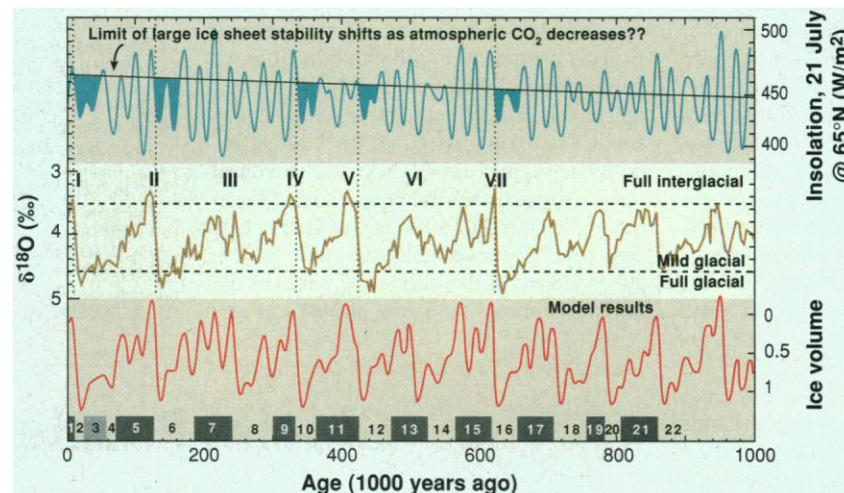
far more perplexing mystery may be how one explains the first 2 million years of the major Northern Hemisphere ice ages when global ice volume and deep-sea temperature change varied predominantly at the 41,000-year period of orbital obliquity. If the canonical view that summer radiation at high latitudes exerts the ultimate control on ice sheet mass balance were correct, then far more precessional variance would be observed in high-latitude climate records of the late Pliocene and early Pleistocene. No existing climate model has yet been able to successfully reproduce the almost sinusoidal growth and decay of the ice sheets at 41,000 years observed during the first two-thirds of the Northern Hemisphere ice ages.

Hence, even though the "41,000-year world" is often held up as the textbook example of orbital control of climate, the mechanisms by which that control is exerted are far from obvious. The 41,000-year world may prove even more mysterious than the 100,000-year world, which has baffled scientists for decades. Still, Paillard has made an important and insightful contribution to our understanding of the pacing mechanisms of climate change over the past 700,000 years and has pushed the study of the 100,000-year cycle in an important new direction.

Our understanding of the mechanisms of climate change, the "rules" that govern transitions between states, is still far from perfect. However, they involve ocean-atmosphere-ice processes that can change atmospheric temperatures, CO_2 , methane, ocean circulation, and sea surface temperatures, on time scales as short as centuries, even as they are paced by orbital changes that occur over many millennia. Only with the accumulation of more data and thoughtful modeling like Paillard's can we hope to solve the puzzle of paleoclimate.

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Glacial cycles. Summer solar radiation in the Northern Hemisphere [after (7) (top)], the oxygen isotopic record for ice volume (8) (middle), and Paillard's (5) modeled ice volume over the last 1 million years (bottom). The model reproduces the oxygen isotope records. Most of the full glacial episodes correspond to extended times of low summer insolation (shaded peaks, top plot). Marine isotope stage designations are shown at the bottom.

ice sheets during times of unusually low summer insolation at high latitudes that occur roughly every 100,000 years as dictated by the interaction of precession with eccentricity and, to a lesser degree, obliquity. A cycle ends abruptly, with a termination, when insolation increases above a threshold value that causes the ice sheet to become unstable and melt rapidly.

Why then did the 100,000-year cycle begin, apparently abruptly, about 800,000 years ago? Along with Paillard, I suspect that a gradual secular decrease in the strength of Earth's greenhouse is to blame (shown schematically by the fiducial line on the insolation curve in the figure). If atmospheric CO_2 concentrations were higher in the past, then, regardless of orbital configuration, it may never have been cold enough, for long enough, for massive ice sheets to grow on North America and Scandinavia.

Furthermore, given that precession so completely dominates the insolation regime at high latitudes in summer (see figure), a