of guidance cues. Cyclic nucleotide levels are subject to sophisticated temporal and spatial regulation, introducing ample potential for precision, and crosstalk among signal transduction pathways. Converging signaling mechanisms also simplify customized solutions to the problem of assessing relative signal strength and thus interpreting the same local environment in neuron type– and neuron state–specific manners. In this context it is interesting to note that spinal cord neurons that behave very differently in their natural environment in the embryo exhibited similar responses in vitro. Probably, the complex in vivo environment provides unique spatial compositions of guidance cues, thus eliciting context-related responses.

These results raise a number of issues for further study. One is the molecular nature of the effector mechanisms that mediate attraction or repulsion. Attractive candidates are the small G proteins (heterotrimeric GTP-binding proteins) cdc42, Rac, and Rho, which regulate actin cytoskeleton morphogenesis and growth cone activity (*9*). RhoA can mediate growth cone collapse, is negatively regulated by cAMP, and may also mediate repulsion; cdc42 participates in chemotaxis and may also mediate attraction (*10*). A further issue is whether receptor complexes rather than single-receptor proteins may mediate attractive and repulsive responses (*11*). Finally, to what extent and how do the mechanisms described in these studies affect guidance in situ? The recent report that, after crossing the midline, commissural axons become insensitive to attraction by the floor plate is consistent with the possibility that attraction by Netrin-1 may be switched to repulsion in vivo (*12*). In a similar scenario, guidance by guidepost cells may involve initial attraction, followed by repulsion to promote further navigation of the growth cone toward the next guidance cues. Direct experimental testing of these possibilities is needed. It will be particularly important to determine how growth cones handle complex sets of guidance clues, and how parallel systems that use cAMP and cGMP interact. By recruiting other signaling components, contact-mediated guidance may add significant complexity to the attractively simple mechanisms uncovered by these in vitro studies.

It seems a safe bet that these discoveries will promote significant further progress. Similar switch mechanisms may operate in synapse formation, nerve sprouting, and synaptic plasticity in the adult (*13*). Clearly, however, the most exciting possibility raised by these findings is that the inhibitory signals that prevent nerve regeneration in the adult central nervous system could be attenuated by pharmacological interventions that raise cyclic nucleotide levels in injured axons, thus promoting their regeneration.

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PERSPECTIVES: NUCLEAR STRUCTURE

Duplicating a Tangled Genome

Peter Cook

The 46 DNA molecules that make up the diploid human genome are incredibly thin and very long, with a width of ~2 nanometers and a combined length of ~2 meters. All 46 molecules must be packed into a nucleus only ~10 micrometers wide, a packing problem analogous to folding a kite string that stretches from New York to Chicago into a sphere 10 meters across!

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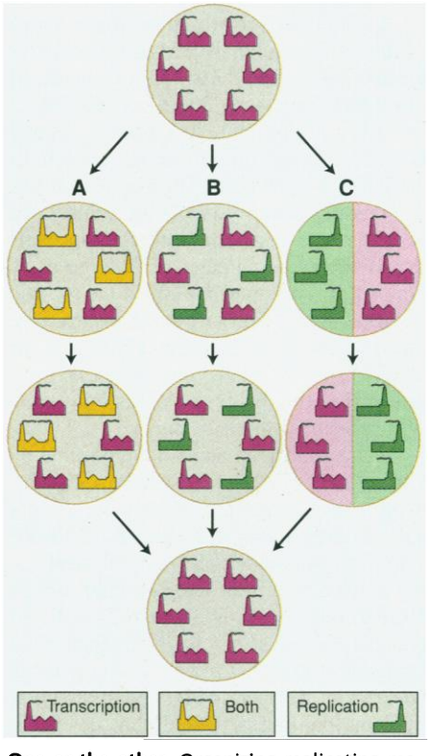
Real-sized DNA strings are probably packed into real-sized nuclei by a combination of random bundling (like pasta in a bowl), coiling into higher order spirals, and looping by attachment to an underlying nuclear skeleton or "matrix" (*1*, *2*). Yet this tangle must still allow transcription of individual genes and the replication of all chromosomes. Somehow one complete new genome must be sorted out for inheritance by each daughter cell. One milestone in the elucidation of such structure-function relationships within these complex tangles was the isolation by Berezney and Coffey of the nuclear matrix in the 1970s (*3*). Now a report from Berezney's lab, on page 1502 of this issue, illuminates the order in the nuclear tangle during the process of DNA replication (*4*).

The new work builds on several observations. First, the cellular machines that replicate DNA (composed of DNA polymerase and associated proteins) do not act alone. Instead, tens (sometimes hundreds) are housed in enormous "factories" (with diameters of 0.1 to 1 μm); individual machines in each factory reel in loops of DNA as they replicate them (*5*). Second, transcription machines that copy DNA into RNA (RNA polymerases and associated proteins) are concentrated in analogous factories (*6*). Third, transcription happens continuously; it starts when a cell is born and goes on until it divides. In contrast, DNA is replicated only during the middle third of each cell cycle. Replication begins in many factories located in transcriptionally active regions and ends in a few large factories in less active regions (*5*, *7*). Fourth, structure-function relationships are remarkably stable. For example, clusters of



One or the other. Organizing replicating machines in nuclei. (A) Replicating machines are installed in functioning transcription factories. (B) Existing transcription factories are decommissioned and replaced by dedicated replication factories. (C) Zoning regulations ensure dedicated replication factories are grouped together.

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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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