from man-made transmitters could influence lightning discharges.

Thus the outcome of this piece of research is in agreement with Robert Browning's sentiments: "How strange it seems, and new!" although Samuel Johnson held a different view: "All is strange, yet nothing new". It is fitting, in this instance, that Lord Macaulay should have the final word: "His theory is therefore this, that God made the thunder, but that the lightning made itself". □

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Gene regulation

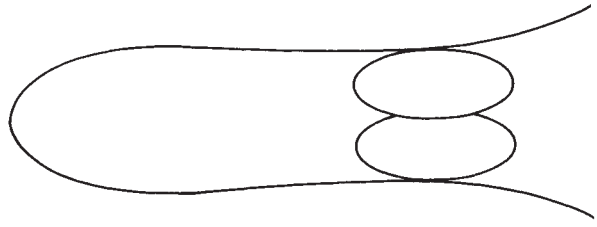
Why should DNA loop?

Robert Schleif

For its central functions of DNA storage and retrieval, DNA can be quite adequately approximated as a one-dimensional line of nucleotides. Recent experiments show, however, that this approximation will not suffice to explain transcriptional regulation. In some regulatory systems, the DNA loops out of one dimension to bring two specific well-separated DNA sites into close proximity (see figure). The evidence for looping in several prokaryotic systems[1-9] as well as one eukaryotic one[10], has recently been reviewed in *Nature*[11]. In all cases, sequences required for transcriptional regulation of a particular gene are found at a site more than 100 nucleotides away from the RNA-polymerase binding site, and a protein or proteins bound at this site is (are) believed to contact a protein or proteins bound close to the transcriptional start site. In the arabinose system[4] and in an artificial construct involving λ phage repressor and operators *in vitro*[12,13], looping has been demonstrated between repressor molecules bound cooperatively at separated operator sites. Does this mechanism explain eukaryotic enhancer function? And if so, why might such a mechanism have evolved?

Enhancers, stretches of DNA perhaps 40 nucleotides long, are required for correct regulation of transcription of a gene. They were first identified in animal viruses, but have since been found to be associated with many eukaryotic promoters. They can be located hundreds or sometimes thousands of nucleotides from the promoter and in many cases the major portion of the DNA in between is irrelevant to enhancer function. Before discussing the reasons for DNA looping, I will explain why it is that proteins which regulate transcription bind to DNA at all.

Regulating transcription of a gene requires that a protein sense the presence or absence of a suitable signal and communicate this status to the transcription machinery. To permit more than a handful of genes to be regulated simultaneously within a cell, the potential influence of a gene-regulating protein must be restricted to the appropriate gene. Therefore regulatory proteins are built to bind to DNA near a promoter so that they can modulate the activity of RNA polymerase(s) only at that promoter.



DNA looping promoted by proteins bound at two sites. If the two proteins, which can be identical or different, possess appreciable affinity for one another, they can hold the DNA in a looped state. Such looping facilitates gene regulation by permitting more than two proteins plus cofactors to affect transcription frequencies from a promoter by altering the binding or activity of RNA polymerase. The looping permits binding with low protein concentrations, facilitates rapid dissociation of each individual protein and, by virtue of the cooperativity inherent in such a system, generates nonlinearity that may be useful in gene regulation.

Two general reasons can be advanced for DNA loops. The first is geometrical. Only two or perhaps as many as four proteins may bind DNA next to and affect the activity of a protein such as RNA polymerase bound to a promoter. For a gene with a complex regulation pattern, how can more than two signals from proteins bound to DNA be sent to an RNA polymerase molecule? Looping is one solution, in which proteins next to a promoter-bound RNA polymerase molecule can modulate its activity, and proteins bound to DNA hundreds of nucleotides away can touch by looping to the adjacent proteins or RNA polymerase itself and also affect transcription initiation. Proteins may also be able to affect the looping and hence the regulation by binding in the middle of the loop at positions well away from the ends.

A second reason for looping is the cooperativity generated by the multiple site binding. For the purposes of this discussion, consider a single bivalent protein that binds to two DNA sites and forms a DNA loop. This is merely an extreme of the general case in which two different proteins bind to the two sites and bind to each other to form the DNA loop. The cooperativity inherent in these cases derives from the fact that if the protein is bound to one site, its other DNA-binding region automatically is relatively near the second DNA site. That is, the presence of the protein on one binding site increases the local concentration of the binding protein to the other site and stimulates the binding of the protein to the second site.

The cooperativity resulting from loops can generate a high effective affinity of the protein for its binding sites. Consequently, only small amounts of a regulatory protein are necessary to occupy a DNA site almost completely. Such an effect may be essential in reducing the total necessary amounts of the thousands of regulatory proteins required to be within a cell or nucleus. In addition, the cooperativity that can generate nearly complete binding to DNA does so with sites whose intrinsic affinity is relatively low. Consequently, the dissociation rates of the protein from either of the sites forming the loop will be high. Such a high dissociation rate may permit rapid transcriptional responses as well as facilitate, for example, the passage of DNA polymerase during DNA replication. Finally, the cooperativity might allow effective regulation to result from small alterations in the concentration of a regulatory protein. That is, a small decrease in the concentration of a regulatory protein could produce a large decrease in the occupancy of its binding sites.

Two points can be made with respect to the actual formation of loops that bring together domains of proteins bound to DNA. One extreme is to place the burden of looping on the DNA as implied in the above discussion. The opposite extreme is to let the protein do the looping. The interacting protein sites can be put on long flexible arms so that the appropriate domains can interact without much bending

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of the DNA. This may be the case for the yeast *HIS3* (ref. 14) and *GAL1* (ref. 15) gene enhancers. The second point is that in some cases bending DNA into a loop may be energetically unfeasible without the intrinsic bending provided by supercoiled DNA. One such example is the arabinose system, which can loop *in vitro* only when the DNA is supercoiled[3]. Not only does supercoiling help bring distant points together, but it can also assist formation of complex wrapped structures, such as occurs for the integration complex of the int, xis and IHF proteins of phage λ (ref. 16). □

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Climatology

Hundred-kiloyear cycle queried

Alan C. Mix

ONE of the major research issues of the past decade in palaeoclimatology has been to determine the cause of the 100-kyr cycle, which has seemed to dominate the apparent fluctuations of the ice ages preserved in the geological record. In a recent paper[1], Bill Ruddiman and Maureen Raymo of Lamont–Doherty Geological Observatory add a new twist to this picture. Their detailed study of hydraulic-piston-cores taken by the Deep-Sea Drilling Project confirms earlier suggestions[2] that the 100-kyr, ice-age cycle is a recent aberration, prevalent only during the past 800 kyr.

Milankovitch hypothesized[3] in 1941 that the Earth's climate changes in response to the changing geometry of the Earth's orbit. He predicted climatic cycles with periods of about 23 kyr (due to precession of the equinoxes, the circular wobble of the Earth's axis), 41 kyr (due to variations in the tilt of the Earth's axis) and 100 kyr and 400 kyr (due to the varying eccentricity of the orbit). Recently, palaeoceanographers studying microfossils from deep-sea sediments have developed timescales accurate enough to demonstrate convincingly[4] the presence of these 'Milankovitch cycles' during the past several hundred thousand years.

The striking thing about this record is that the preserved 100-kyr ice-age cycle is the strongest, even though the direct eccentricity-forcing is the weakest of the orbital effects. In addition to their small, direct influence on collection of sunlight by the Earth, eccentricity variations modulate the strength of the precession effect, which controls where on the Earth's orbit the seasons occur. For example, when the Earth's orbit is perfectly circular it does not matter where summer or winter occurs. When the orbit is eccentric, however, the Earth–Sun distance varies throughout the year. Hotter summers would occur in the Northern Hemisphere when June occurred at a point closer to the Sun, as it did 11,000 years ago.

The 100-kyr climate cycle has generally been explained[5] by the relatively slow growth and fast melting of large ice sheets. Through this nonlinear mechanism, many believe that the ice sheets respond to the modulation of precession by eccentricity, and thus get their 100-kyr cycle indirectly. This theory relies on the internal characteristics of ice sheets, their long (and nonlinear) time constants, to account for the observed 100-kyr cycle.

This theory is now questioned. First, strong 100-kyr climate cycles seem to have occurred before the late Cenozoic ice ages[6,7], implying that ice is not necessary to generate a 100-kyr rhythm. And second, the new evidence of Ruddiman and Raymo ironically suggests that the 100-kyr cycle is a rare occurrence for ice ages. This study confirms that the 100-kyr rhythm of glaciation appeared within the past million years, and became dominant only within the past half-million years. A much longer interval of the late Cenozoic ice ages, from ~2.4 to 0.8 Myr, was almost completely dominated by the 41-kyr tilt cycle.

The major question addressed by Ruddiman and Raymo is what caused the shift in rhythmic response of the ice ages to orbital forcing? They believe that evolution in character of the ice ages must result from some change in the external boundary conditions of climate (the configuration of land, sea, ice and atmosphere). They speculate that the rapid tectonic uplift of the Himalayas and parts of western North America during the past few million years has increased the sensitivity of the system, probably by inducing downstream meanders in the jet stream. These atmospheric waves would cause cold spots over eastern North America and Europe exactly where the large Northern Hemisphere ice sheets were located. Another theory, due to Pisias and Moore[2], suggests that the growth and decay times of smaller, land-based ice sheets of the early record were shorter than those of the more recent, larger, marine-based ice sheets. This might account for the growth of the 100-kyr cycle through time, but leaves open the question of why the more recent ice sheets were bigger than the earlier ones. Ruddiman's and Raymo's suggestion may solve this problem.

As noted by Ruddiman and Raymo, many uncertainties remain that will keep geologists and climatologists busy for years. First, of course, is the problem of developing timescales for geological sections accurate enough to read the cyclic patterns of climate change. Because Ruddiman and Raymo tuned part of their time scale to the 41-kyr tilt cycle, it is not surprising that the 41-kyr cycle dominated their time series. It is unlikely, however, that their tuning could have erased a 100-kyr cycle from the record.

Second, exactly when the major uplift of the Himalayas occurred and how fast it was is unclear. Although some believe[8] that much of the uplift took place within the past few million years, others[9] believe the Himalayas reached essentially full height more than 20 million years ago; details of the last 3 Myr are sketchy at best.

Third, how sensitive to topography are waves in the high-latitude wind fields? Different computer models give conflicting results for the cases of no-topography versus present-topography[10,11]. No models have been run for intermediate cases of continuous uplift. Of course, the addition of the ice sheets adds topography to the system, and these boundary conditions have yet to be explored fully in general-circulation models of the atmosphere.

Finally, the observation of 100-kyr climate cycles before the ice ages[6,7], if correct, remains a mystery. If this rhythm is present during much of time before the onset of Northern Hemisphere glaciation, it may be that the interval of 41-kyr cycles from 2.4 Myr to 0.8 Myr found by Ruddiman and Raymo is the anomaly, and that the resumption of 100-kyr cycles at 0.8 Myr is simply the return to normal behaviour. At this point, there are more questions than answers. With the addition of long time series on climate change coming from the Ocean Drilling Program the rapid development of climate models, and the refinement of tectonic reconstructions, the next few years should see interesting developments (and more surprises) in this field. □

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