

What could activators do to facilitate initiation? A popular model is that acidic activators facilitate the binding of the general factors TBP and/or TFIIB to the promoter, but it has little direct support and is in fact contradicted by much experimental data. In direct DNA-binding studies, TBP, TFIIB and the rest of the transcription machinery bind with reasonable affinity to DNA in the absence of activators. However, the success rate of transcription initiation is likely to be very low, with only a fraction of promoter templates used.

An alternative model is that activators increase the efficiency of polymerase initiation by interaction with TFIID, TFIIB or both⁹. Recall that activator, TFIID and TFIIB are all required during assembly of the preinitiation complex to achieve high levels of transcription¹¹. In the absence of activator, TFIID and TFIIB might assemble non-productively to form inactive initiation complexes^{9,14} (Fig. 2); this might be due to inherent properties in the proteins themselves and/or to factors binding to TFIID-TFIIB and inhibiting further assembly of the complex. In the absence of transcription inhibitors, these non-productive complexes may bind polymerase and the remaining general factors, but RNA synthesis would occur from only a fraction of them. In the presence of activator, TFIID-TFIIB would assemble in a productive manner giving a high percentage of active initiation complexes. If so, one would predict the existence of mutations in TFIIB or TFIID which would shift the equilibrium towards either the productive or the non-productive complexes. But whatever the mechanism for activation, it seems certain that identification of the target is only the first step in a detailed understanding of the mechanism of gene control. □

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Not just a lot of hot air

Mark Chandler

THE 1980s were warm, at least when temperatures are globally averaged and compared with the instrumental record for the past 125 years. The past century, as a whole, was also warm compared with the preceding 600 years starting just before the Little Ice Age. Moreover, the past several millennia are warm when held up against the previous 2.5 million years. Even so, most climatologists now predict that the warming that will occur by the middle of the next century will be greater than any experienced on Earth since before the development of the genus *Homo*. With that possibility in mind, a slightly more evolved group gathered at a recent convention* to discuss the characteristics and processes of past climates whose warmth matched or exceeded that anticipated for the near future.

Modellers and those who look at the geological record all provided a steady stream of evidence that, although carbon dioxide has almost certainly fluctuated throughout the Earth's history, the oceans have been important in regulating past global warming. Such news would come as little surprise to those who have been studying the role of the oceans in the glacial-interglacial cycles of the Pleistocene (the past 2 million years). But the importance of ocean transport in the Earth's warm periods has long been overshadowed by concern over the effect of CO₂. Now begins the formidable task of unravelling the exact role of the oceans and greenhouse gases, along with all their associated feedbacks, in the global warmth that dominated the past half-billion years.

Atmospheric carbon dioxide and ocean heat transport are the two primary candidates for regulating surface air temperature. Carbon dioxide arose as the new "paradigm" for past global warming (T. Crowley, Applied Research Corporation) when Barron and Washington¹ used a then state-of-the-art computer climate model to find if increased levels of CO₂ could yield a planetary warming of the magnitude indicated for the mid-Cretaceous (100 million years ago). Their experiments and more recent simulations of the Eocene (L. Sloan, Univ. California, Santa Cruz) show that, indeed, global warmth of the magnitude experienced throughout the past 200 million years can be achieved by increasing CO₂ to levels similar to those predicted by models of the global carbon cycle or estimated from geochemical proxies in the geological record. What the models also show, however, is that the latitudinal distribution of

surface air warming caused by increasing CO₂ is inconsistent with the patterns determined from palaeoclimatic data: CO₂ warms the Earth at all latitudes whereas the data consistently show warming to be greatly amplified towards the poles but marginal in the tropics (D. Poore, US Geological Survey, Reston; J. Zachos, Univ. California, Santa Cruz).

Intensified ocean circulation, taking heat from low to high latitudes, is viewed as the most likely mechanism for stabilizing the tropical temperatures under conditions of increased CO₂. Numerical experiments^{2,3} supported this, but indicated little further influence over global warming until a series of more detailed simulations showed otherwise. In particular, melting of the reflective ice caps as more heat is carried to the poles increases the amount of solar radiation absorbed by the globe. Indeed, it turns out that the increased ocean heat transport alone — that is, without any change in CO₂ — might have been responsible for the warm climates of the Mesozoic (245–65 million years ago) and Cenozoic (65 million years ago till now)⁴. Thus the question shifted from "what was the mechanism of past global warming?" to "which was the mechanism of past global warming?". Furthermore, climatological discussions, once built on the concept of the past as a key to the future, are now concerned with the validity of that concept.

Certainly there are many differences between ancient climates and the climate of the near future: the continents and oceans are distributed in completely different ways; continental topography has altered; the extent of ice over land is different; and vegetation patterns are not the same. Projects such as PRISM (Pliocene Research, Interpretations and Synoptic Mapping) are examining the most recent warm periods, seeking to minimize those differences in order to provide the best possible comparison between past and future (Poore). But PRISM's initial sea-surface temperature data for the North Atlantic⁵, and our model simulations using the PRISM data as boundary conditions (D. Rind and M.C.), now add more evidence suggestive of an oceanic source for the Pliocene warming, 3 million years ago. Furthermore, new sediment core carbon-isotope analyses (M. Raymo, Massachusetts Institute of Technology) reveal that middle-Pliocene CO₂ levels were at most 100 parts per million greater than they are at present (just over 300 p.p.m.). This is far below the 1,300 p.p.m. that our models at the Goddard Institute imply would be necessary to achieve

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the mid-Pliocene temperatures.

So if the oceans behaved so differently in past warm climates, is the past irrelevant to the predictions of future global warming driven by increasing trace gases? With different boundary conditions and different initial forcing mechanisms, different climates might be expected. There are several reasons not to be dismissive. Evidence of altered forcing by greenhouse gases in the Earth's history is ever more compelling. Ice-core records, carbon isotopes from palaeosols and marine microfossils, and carbon-cycle modelling all indicate that CO₂ change has influenced climate on geological time-scales. In addition, terrestrial data continue to accumulate showing that continental interiors, far from the oceans' influence, were substantially warmer, particularly during winter, in the Mesozoic and early Tertiary (K. Gregory, Lamont-Doherty Earth Observatory). Models have so far failed to reproduce this winter warming and increasing the amounts of greenhouse gases in the models is currently the only reasonable way to begin to reconcile this discrepancy (Sloan).

Although both the oceans and green-

house gases are behind the Earth's past warm periods, in a system as interspersed with feedback mechanisms as the climate, forcing factors may be far removed from resulting climatic states. The challenge now is not in determining cause and effect alone, but in understanding the series of processes connecting one transient state with the next. Global circulation models, working from the fundamental physical equations, may one day do the job, but not until atmospheric water and ocean features are simulated more realistically. Recognizing that the devil is in the details would ensure that the road to hell is not just paved with good conventions. □

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ENZYMES

Snapshots along the pathway

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ON page 693 of this issue¹ a group led by Bauke Dijkstra describes a study of the reaction mechanism of the enzyme haloalkane dehalogenase through the crystallographic analysis of intermediates in the reaction. This is an ingenious piece of work, and highly informative for it provides snapshots of the enzyme in action.

Protein crystallography has provided a wealth of detail about the static structure of enzymes and has helped to delineate substrate binding sites, but it has told us little about the kinetic aspects of enzymatic reactions. Information about the intermediate states in a reaction is usually inferred from the structure of a stable complex of an enzyme with an appropriate model compound, a transition state analogue for instance. In the past few years technological developments such as intense X-ray synchrotron radiation sources and fast data acquisition techniques have provided the first glimpses of enzymatic reactions in progress. But Dijkstra and colleagues have now succeeded in monitoring an enzymatic reaction using conventional crystallographic methods. They have followed the breakdown of 1,2-dichloroethane by haloalkane dehalogenase through a series of stages: first free enzyme, then complexes with the substrate, with a covalently bound intermediate and with one of the breakdown

products remaining in the active site.

The difficulty in following transient processes with protein crystallography stems from the fact that the observed electron density distribution is an average over the time of the experiment and over all of the molecules in the crystal. The turnover time for a normal enzymatic reaction (well under 1 second) is much shorter than the data acquisition time. The alternative, investigation of stable enzyme-inhibitor complexes, does not provide all the answers as the study of the serine proteases shows — many complexes later, the details of the catalytic mechanism remain controversial^{2,3}.

Shortening data acquisition time from days to minutes and even seconds has become possible by combining synchrotron radiation with diffraction data collection by the polychromatic Laue method. Experiments by Hajdu *et al.*⁴ with glycogen phosphorylase *b* demonstrated that data collected in only a few seconds could provide an interpretable electron density map, showing clearly the binding of maltoheptose. But although in principle slow enzymatic reactions could then be visualized, synchronized action of all molecules in the crystal is required. This task has turned out to be rather difficult and, in current practice, only reactions with a half-life of the order of minutes are in-

vestigated. To that effect, Schlichting *et al.*⁵ used a photolabile derivative to unravel the details of the GTP hydrolysis by Ras p21 protein. The GTP derivative was co-crystallized with the protein and the release of GTP was triggered by flash photolysis.

The attempts to slow down reactions include the use of poor substrates, change of temperature or pH, and the use of caged substrates⁶. For instance, Sweet and colleagues⁷ applied synchrotron Laue photography to follow a deacylation step of a transient, trypsin-bound ester intermediate. By selecting a poor substrate and exploiting the pH dependence of the reaction to capture the intermediate and then release it slowly, they were able to identify the water molecule involved in the nucleophilic attack during deacylation.

An example of the application of a partially defective mutant to identify an intermediate state was provided by Strynadka *et al.*⁸. Following the observation that the Glu166→Gln mutation in RTEM-1 β -lactamase of *Escherichia coli* affects the release of covalently bound intermediate of β -lactam breakdown, they soaked the crystals of this mutant in penicillin G, collected high-resolution data using monochromatic synchrotron radiation, and characterized the intermediate state. From this structure and that of the free enzyme, they proposed a detailed reaction mechanism and identified the water molecules involved in the catalysis.

Dijkstra's group have now extended the use of standard crystallographic methods to follow the reaction catalysed by haloalkane dehydrogenase; this is one of many examples now emerging of a protein that has a fold in common with other proteins without having a significant similarity in sequence. The enzyme is one of the α/β hydrolase fold proteins, which are thought to have evolved from a common ancestor⁹. Their conserved catalytic nucleophile-histidine-acid triad, reminiscent of serine proteases, is adapted to hydrolyse vastly different substrates. Although the topological location of the triad is conserved, the nature of the nucleophile and the acid are not. Serine, cysteine or aspartate have been observed in the nucleophilic position, while both aspartate and glutamate have been found as the acidic member of the triad⁹. It is presumed that the ester hydrolysis by esterases of this family follows a mechanism similar to that delineated for serine proteases. But the possible enzymatic mechanism of dehalogenase, with an aspartate nucleophile, was less clear. Two possible mechanisms were consistent with the three-dimensional structure of the free enzyme. One involves a covalently bound intermediate, while the other is a general base catalysis by an activated water molecule.