40°C (*5*, *6*). These differences were driven at least in part by atmospheric CO_2 concentrations about five times the preindustrial value (*7*). Researchers have long documented (*8*) that the water cycle associated with Eocene greenhouse climate must have been substantially different from that of today. However, although studies have documented shifting patterns and overall intensification of water cycling, particularly at high latitudes and during short-lived, extremely hot hyperthermal events (*9*–*12*), strong evidence for changes in the tropics has been lacking.

Clementz and Sewall provide an innovative approach to fill this gap by looking for the imprint of atmospheric water cycling on the oceans. Imbalances in evaporation and precipitation rates over the tropical and subtropical oceans affect the chemistry of marine surface waters, most notably producing salinity contrasts between relatively fresh tropical waters, and salty subtropical surface waters. The same process also leads to differences in the stable isotopic composition of these water masses, with the heavy isotope ^{18}O more concentrated in subtropical regions of net evaporation, and less concentrated in the tropical surface ocean. The strength of this isotopic variation is directly related to the local imbalance in precipitation and evaporation fluxes, and hence represents a proxy for the intensity of water cycling within Hadley cells.

The primary challenge to applying the water isotope proxy to greenhouse climate conditions millions of years ago is that the isotopic composition of paleo–surface waters can be measured only indirectly. The most common way to do this is to measure the oxygen isotope composition of fossil shells produced by marine microorganisms, which are widely available and reflect the isotopic composition of the water in which they formed. One problem, however, is that their isotopic composition is also strongly influenced by water temperature. Clementz and Sewall circumvent this challenge by taking advantage of biology: They measure the O isotopic composition of carbonate in the tooth enamel of fossil sirenians (sea cows, dugongs, and manatees), which like all mammals presumably maintained their body temperatures at constant values. Together with certain favorable behavioral characteristics, inferred by analogy with modern sirenians—such as large home ranges and a preference for shallow-water habitats—the homeothermic conditions of sirenian tooth enamel formation allow the authors to reconstruct paleoseawater O isotope values without many of the uncertainties inherent to other methods. The results demonstrate enhancement of the isotopic contrast between the tropical and subtropical surface oceans, supporting the hypothesis that contrasts between low-latitude regions of net evaporation and precipitation were enhanced during the Eocene CO_2 greenhouse. The isotopic results are backed up by climate model simulations designed to replicate the climate of the Eocene, which suggest a pattern of intensified water-cycle change at the fossil collection sites that is largely consistent with the isotopic data.

The new results offer compelling evidence that the tropical engine of the water cycle revved faster during past greenhouses, but the implications for understanding past and future climate states still hinge on a number of unknowns. Both the natural range of sirenians and the availability of fossil collections limit the distribution of samples in this study to coastal regions. As a result, the isotopic data do not directly represent the massive gyres and regions of the intertropical convergence zone where the most intense precipitation-evaporation imbalances occur. Records directly representing these regions would strengthen the case for globally significant changes in tropical water cycling in the Eocene. In addition, the surface-water proxy approach applied here does not clarify the dynamic role of enhanced water cycling in the Eocene climate, and it does not account for important factors such as the relationship between faster cycling and cloudiness, water transport to the extratropics, and precipitation intensity. These are challenging problems that both the modern and paleoclimate communities will continue to struggle with, but the work of Clementz and Sewall should help assure us that when the wheels of the water cycle spin faster, they are driven by the tropics.

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MOLECULAR BIOLOGY

Time-Lapse Transcription

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RNA transcription rates can randomly vary in a single cell over time.

Measurements that involve averaging values from large numbers of cells can obscure the fact that processes such as gene expression are fundamentally dynamic and can vary greatly from cell to cell (*1*). Similarly, static snapshots of individual cells can reveal instantaneous differences between cells but cannot reveal often important changes over time; for example, it is impossible to tell whether a yellow light follows a green light using only static pictures of traffic lights. On pages 472 and 475 of this issue, Suter *et al*. (*2*) and Larson *et al*. (*3*) report on using a time-lapse approach to document the dynamics of a key cellular process, RNA transcription. Using carefully constructed experimental systems and sophisticated analyses, they tracked changes in RNA transcription in single live cells. Their results provide new details about the dynamics of key steps in transcription.

The transcription of a gene into mRNA is fundamental to the parsing of the genetic code and is highly regulated by the cell. In eukaryotes, transcription requires a large number of biochemical steps, including the initiation of the process and the subsequent elongation and maturation of the RNA molecule. The transcriptional activity of individual cells may deviate from the averages measured in most experiments. In part, that is because a typical cell contains only a few copies of most genes—or even just one copy—and these few genes determine the production of the RNA transcript for the entire cell. In some cells, small fluctuations in the biochemical reactions that determine

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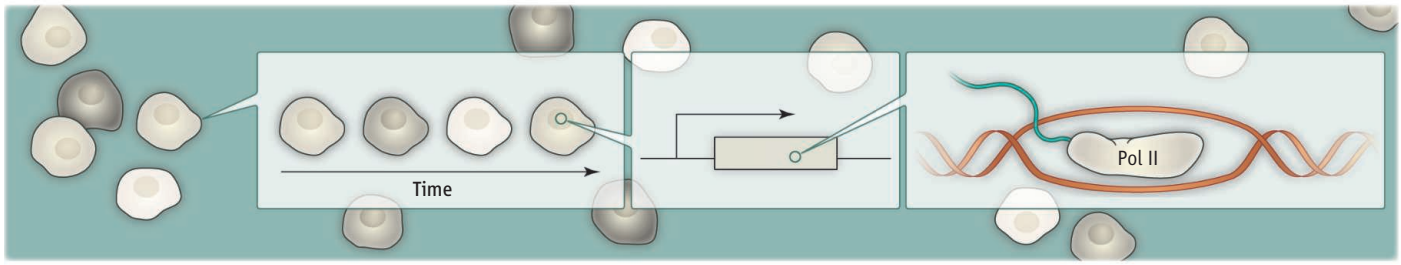

Time-lapse transcription. Even genetically identical cells (**far left**) can have widely varying levels of gene expression. This variability arises from fluctuations in gene expression in a single cell over time (**first box**). These fluctuations can result from random activation and inactivation of the gene itself (white rectangle, **center box**), potentially involving the sequences in the gene's promoter (black arrow). Ultimately, the stochastic behavior of the molecules responsible for transcription, such as RNA polymerase II (Poll II, **right box**), must be responsible for gene-level variability.

whether a gene is competent for the initiation of transcription can lead to "bursts" in transcription (*4*–*6*). Suter *et al*. show that individual genes have unique bursting dynamics, and they begin to explore the biochemistry that underlies this phenomenon.

To conduct their study, Suter *et al*. created a number of populations of mouse fibroblasts, each of which had a particular gene engineered to express a readily observed bioluminescent protein in place of its usual product. They found that genes can show large temporal fluctuations in the synthesis of the protein. These can only be explained if transcription takes place in a burst-like rather than continuous fashion. By using careful mathematical data modeling, they determined the rates of gene activation, RNA production when active, and gene inactivation. Each individual gene they studied exhibited markedly different values for all of these parameters. This shows that there are no "global" parameters that are inherent to the transcriptional process (such as a global rate of transcription when active).

In an effort to begin answering the questions of whether and how gene expression dynamics are controlled, Suter *et al*. used artificial promoters to study expression. They found that increasing the number of binding sites increased the RNA burst size and, to a lesser extent, the rate of gene reactivation, but did not affect gene deactivation. This suggests that the biochemical steps that control deactivation and initiation are not the same as those that control reactivation, and echoes the results of earlier ensemble distribution measurements (*5*, *7*). Going further, Suter *et al*. used a model-based technique to reconstruct the most likely time sequences for changes in the gene state and RNA transcript numbers for each cell. Their analysis of the distribution of the time periods when genes are active and inactive strongly suggests that gene inactivation is controlled primarily by a single rate-limiting step. In contrast, reactivation requires the completion of several sequential or parallel processes with comparable rates.

Larson *et al*. show that single-molecule methods have the potential to reveal even more detailed biochemical insights. In their study, they added a sequence to genes of interest that, when transcribed, binds to a coexpressed fluorescent protein. This allowed them to observe nascent RNAs in live single yeast cells and to infer the working schedule of individual RNA polymerases as they completed rounds of transcription. Through the use of signal-processing techniques, they show that the time between transcription of the tag sequence and the departure of the full transcript from the transcription site, which reflects the RNA polymerization process, is remarkably consistent between events. Once initiated, RNA polymerization in the yeast genes they studied proceeded at a steady rate, unencumbered by random major pauses.

The inherently stochastic behavior of gene expression observed in these experiments is ultimately a manifestation of particular molecular events in transcription. Measurements like those done by Suter *et al*. reveal aspects of gene activity dynamics that complement insights gained from new ensemble techniques that map out the position of polymerases, transcription factors, and chromatin modifications on DNA (*8*). Combining these powerful techniques should lead to clearer explanations for the molecular basis of gene activation and deactivation. We anticipate that single-molecule, live-cell studies like those carried out by Larson *et al*. will have a particularly strong impact on our understanding of the oft-observed phenomenon that the transcription of mRNAs proceeds more easily after the first transcription event. Although this facilitated transcriptional re-initiation accounts for a substantial fraction of the transcript pool in many organisms (*9*), it has been difficult to study because very few ensemble techniques can distinguish transcripts produced by an initial round of transcription from those that come later.

Quantitative measurements of the stochastic behavior of genes, especially as a function of their regulatory apparatus, will pave the way to understanding the biochemical constraints encountered by organisms as they develop reliable genetic programs. Although there are a few examples that demonstrate how variability can be exploited as a mechanism for cellular decision-making (*10*–*14*), a more common theme in biology is that the variability in gene expression observed by Suter *et al*. is undesirable, and that mechanisms must be in place to produce reliable biological outcomes. This is likely to be especially true in situations when cells must coordinate their activities, as in development (*15*), and a loss of reliable control of expression may accompany disease. Little is currently known about what such mechanisms might look like because of the scarcity of quantitative details of the dynamics of transcription. The tools highlighted here may ultimately allow us to construct and validate models of these mechanisms.

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