oldest landscapes on Earth (e.g., Western Australia), soils are extremely low in total phosphorus, and drylands are dominated by evergreen and slow-growing shrubs (*5*). Conversely, recently glaciated regions (e.g., most of the Northern Hemisphere) have more fertile soils rich in nitrogen (*6*) and are dominated by vegetation with contrasting ecological strategies. Such differences are likely to modulate the effects of increasing aridity and the respective thresholds. Also, rock-derived nitrogen can contribute substantially as a nitrogen source for some terrestrial ecosystems globally (*7*). This suggests that disruption of the nitrogen cycle in response to aridity might be site dependent. Thus, including geology along with the dimensions of soil characteristics is likely to change threshold identification.

Water-table depth (defined as the combination of hydrological and topographical features in the landscape) can alter the direct effects aridity and soils have on vegetation attributes (*8*). Access to the water table allows the maintenance of higher vegetation cover and lower tree mortality risk in response to drought. If, under low-precipitation conditions, tree seedlings develop their rooting systems with sufficient speed and depth, then the trees can thrive in the landscape (*9*). Water-table depth directly affects the reach of rooting systems at the global scale (*8*) and indirectly modulates the effects of drought conditions on tree mortality (*10*).

The available technology for time-series monitoring does not permit the measurement of sufficiently long time frames to assess vegetation shifts after thresholds have been crossed. Use of the so-called "space-for-time substitution" method serves as an alternative to overcome such limitation (*11*). The idea is to take a snapshot in time and use the data to assess how vegetation changes across environmental gradients. As shown in Berdugo *et al.* for drylands, this approach has been used to show the likelihood of catastrophic shifts in nature (*12*–*14*) but is limited in assessing how and at what speed shifts can happen. Scientists must map the trajectories and pathways of changes in ecosystems to provide information on how abrupt and irreversible the shifts can be. Furthermore, researchers require a better understanding of the ecological mechanisms that drive such trajectories so as to predict more realistically changes in the structure and functioning of different ecosystems.

In drylands defined only by climatic variables, vegetation cover can be highly heterogeneous. Assessing heterogeneity in environmental conditions at finer spatial scales is key to characterizing the interplay between physical drivers and plant attributes. As a supplement to the space-for-time substitution approach, the limitation in time-series length has been overcome by the analyses of chronosequences (*4*) and paleorecords (*15*) to identify shifts, mechanisms, and transient behaviors for millennia time spans. Also, the combination of empirical and observational data and complex Earth-system models might help to extend the time-series length and to analyze ecosystem dynamics in a more comprehensive manner.

The expansion of knowledge about soil-plant-climate interactions from local and regional to global scales is fundamental to evaluating how local shifts in one place are likely to strengthen or dampen remote shifts occurring in other parts of Earth (*1*). These teleconnections join both the interacting elements of the Earth system and their varied spatial and temporal scales. The recent use of complex networks has served as a starting point to connect global climatic patterns. Such networks might prove to be promising tools for mapping mechanisms that lead to changes in local thresholds through cascading effects.

Interdisciplinary science, achieved through collaboration across multiple fields of research, should certainly include the provision of ecosystem services, particularly those related to food security and water availability. Beyond that, understanding the ecological mechanisms and mapping the trajectories of endangered ecosystems can provide a range of possibilities for managing and restoring damaged and degraded land. Collective actions can build stewardship over the Earth system and help to chart a stabilizing pathway for the planet (*1*). ■

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PHYSIOLOGY

Marching to another clock

Robust daily rhythms of RNA and protein expression occur in "clockless" cells

By **Steven A. Brown** *and* **Miho Sato**

For several decades, researchers have studied the molecular mechanisms underlying circadian rhythms, the daily oscillations ubiquitous in biology. This basic clockwork is well understood in animal cells: Conserved clock proteins form a transcription-translation feedback loop that drives circadian oscillations of gene expression and downstream processes. These cellular clocks in peripheral tissues are hierarchically synchronized by a "master clock" in the brain [the suprachiasmatic nucleus (SCN) in mammals] responding to daylight, and also by other physiological signals such as feeding. On page 800 of this issue, Ray *et al.* (*1*) demonstrate that many circadian oscillations—in transcription, translation, and protein phosphorylation—can continue in mouse cells in the absence of an essential circadian clock gene, *Bmal1* (brain and muscle ARNT-like 1). Thus, there might be other unknown clocks that also control circadian gene expression.

An overwhelmingly consistent literature confirms the known "canonical" clock mechanism, but some evidence suggests that it might not explain all circadian rhythms. In cyanobacteria, a posttranscriptional phosphorylation loop lies at the heart of the clock, driving circadian transcription and translation. In plants and fungi, circadian transcription-translation loops exist that have limited structural similarity to the animal canonical clockwork (*2*). Additionally, across all of these kingdoms, curious circadian oscillations in oxidation and reduction of peroxiredoxins, a family of antioxidant enzymes, continue through unknown mechanisms even in the absence of transcription and translation (*3*).

Within mammalian cells, *Bmal1* encodes a transcriptional activator that is essential to the canonical circadian feedback loop (*4*). Ray *et al.* noticed that when skin

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cells or liver slices lacking BMAL1 were cultivated ex vivo and treated with dexamethasone [a synthetic glucocorticoid that is known to modulate circadian timing (*5*)], robust rhythms in the expression of thousands of transcripts and hundreds of proteins and in protein phosphorylation continued for days. This phenomenon was not just some form of residual compensation for BMAL1 by other factors: the *Bmal1*-deficient cellular oscillations were as robust and numerous as those found in cells where *Bmal1* is expressed, and they occurred in an almost nonoverlapping set of transcripts. Together, the BMAL1-dependent and BMAL1-independent circadian transcripts measured in cells made up the set of circadian transcripts observed in vivo.

Certainly a fascinating finding, this study raises as many questions as it answers. How was this "noncanonical" clock overlooked for so long? Loss-of-function circadian clock mutants have become a gold standard to demonstrate circadian control. It is formally possible that numerous experiments using such *Bmal1* mutants missed this residual rhythmicity. More likely, BMAL1-independent noncanonical circadian rhythmicity might have been masked in previous experiments by top-down control from the SCN. In this scenario, both external signals from the SCN and local noncanonical clocks would control the same set of transcripts. In vivo, in the absence of a functional SCN [the neurons of which are predominantly γ-aminobutyric acid (GABA) releasing and inhibitory], an arrhythmic signal instead arrives to cells, essentially blocking underlying oscillations (see the figure). Only in the somewhat artificial case where tissues or cells are cultured in the absence of the SCN (as in Ray *et al.*) can these normally SCN-regulated BMAL1-independent oscillations be observed, dependent upon an unknown noncanonical molecular clockwork. SCN-driven transcriptional oscillations have been demonstrated previously (*6*), so this idea should be entirely testable in vivo. These tests, using a wider range of tissue-specific circadian mutants than those studied by Ray *et al.*, would also help define where and how this new clock mechanism might be physiologically important and whether it is self-sustained.

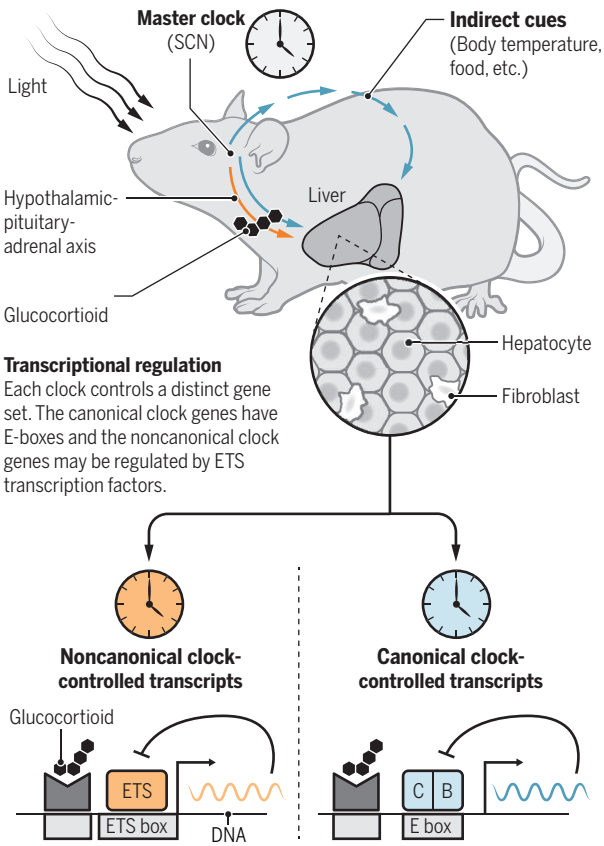
A broader take-home message from the study of Ray *et al.* is that the physiological circuits relating SCN to clocks in peripheral tissues might be more complex than currently appreciated. For example, in the circadian control of contextual memory (*7*), interference from a nonfunctional SCN might also disrupt local circadian function in a way that SCN deletion does not. Such complexity could be medically important, because SCN clocks likely do not always tell the same time as peripheral clocks (as might occur, for example, in shift workers). If multiple different clocks were responding to different signals in peripheral cells, cellular chaos might result.

What could be the mechanism of this new clock, if it exists? Ray *et al.* noticed the enrichment of E26 transformation-specific (ETS)–family transcription factor binding sites, called ETS boxes, among oscillating genes in *Bmal1* mutants, in much the same way that E-boxes are present at canonical circadian genes. They suggest that ETS transcription factors are part of their new clockwork mechanism (see the figure). As a large family of transcription factors (29 activators and repressors in humans) that act as convergent hubs of cellular signaling (*8*), ETS factors make good candidates for transcription feedback loops that might also be driven by external signals from the SCN. Indeed, one well-known systemic circadian signal is glucocorticoids (*5*), the same used by Ray *et al.* to synchronize their *Bmal1* mutant cells. ETS-family transcription factors are glucocorticoid receptor cofactors (*9*). Another systemic circadian signal is serum response factor (*10*), which responds to bloodborne growth signals and also physically modulates ETS factor activity (*11*).

More generally, do these noncanonical oscillations directly couple to cellular metabolism like canonical ones? Ray *et al.* also demonstrate that oscillations in peroxiredoxin oxidation continue in *Bmal1*-deficient cells. They suggest that this oxidation-reduction cycling might also be important for their new clock. However, evidence for this is lacking in the current study. All six mammalian peroxiredoxins are coupled to a sulfiredoxin that is encoded by a single gene (*12*), so finding genetic evidence for their idea should be feasible. Moreover, unlike peroxiredoxin oscillations that are biochemically cumbersome to detect, these noncanonical circadian oscillations are a tractable target for mechanistic discovery, amenable to the same reporter technologies that have permitted rapid discoveries about the mechanism of the canonical circadian clock (*13*). ■

Two different clocks in mammals?

The suprachiasmatic nucleus (SCN) regulates circadian signals, including glucocorticoids from the hypothalamus-pituitary-adrenal axis. These might synchronize transcriptional oscillations in two different clocks: a canonical one driven by feedback regulation of clock proteins [such as CLOCK (C) and brain and muscle ARNT-like 1 (BMAL1, B)]; and a noncanonical one, perhaps involving E26 transformation-specific (ETS) factors.



GRAPHIC: C. BICKEL/*SCIENCE*

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