CIRCADIAN RHYTHMS

PAS, Present, and Future: Clues to the Origins of Circadian Clocks

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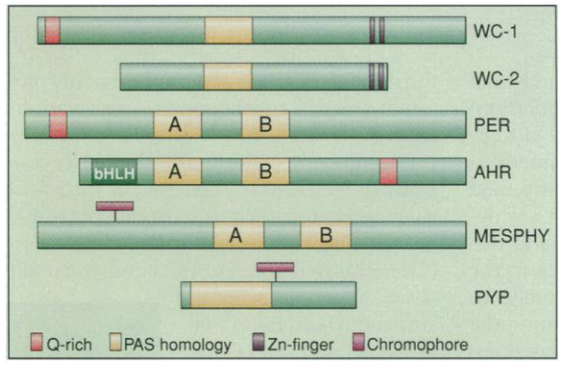
Last year, sales of melatonin in the United States surpassed those of vitamin C. Some of these pills were consumed in an attempt to overcome the recalcitrance of our internal circadian clock and force it to adapt more quickly to new day-night cycles—in a foreign time zone or in night shift work. The clock's self-sustained expression in such circumstances is a hallmark of its ubiquitous, and critical, role in all organisms from vertebrates to plants to single-cell life forms. The molecular circuitry of this internal timing system has become an intense field of study. The latest payoff for this intensity is the identification on page 763 of this issue of a known transcription factor, WC-2, as a clock protein in the fungus *Neurospora* (*1*). This news yields a two-for-one reward: It sheds light on how clocks whirl away inside us but also generates intriguing ideas about their origins.

Why do almost all organisms have circadian clocks? There is a potential adaptive advantage of timing behavioral, metabolic, and developmental processes to the appropriate phase of night or day. A simple biochemical sand timer might suffice for this task, but it could not cope with seasonal changes in day length. In contrast, a circadian clock could accommodate these conditions, by being sensitive to external signals. Thus the hands of the clock can be bumped forward or backward each day by light, maintaining the clock's relevance to the environment. Clock regulation and phototransduction are inextricably tied together.

Genetic analyses, notably in *Neurospora* and the fruit fly *Drosophila* (*2*), have led to the discovery of some of the clock's molecular cogs and to a general picture that clocks are constructed of transcription factors that feed back and inhibit their own transcription. *Neurospora* provides easy access to the inside of the clock. When inoculated into one end of a glass tube, the mycelium of this fungus will propagate along the tube and produce pigmented spores on top of aerial hyphae about every 24 hours. By using this phenotype, researchers have isolated arrhythmic mutants and mutants with altered period lengths, most notably in the *frequency* gene (*frq*), a bona fide clock component. To belong to this small and prestigious club, clock molecules must pass the admission criteria of molecular horology: A clock component must itself cycle; when it is held at a constant level, the clock should stop; and it must respond rapidly to signals that bump the phase of the clock, such as light (*3*). The sophisticated molecular and genetic tools available for *Neurospora* have shown that the *frq* gene passes on each of these criteria (*2*, *4*). In addition, the FRQ protein feeds back and inhibits its own transcription, as has also been found for *per* and *tim*—also bona fide clock genes—in *Drosophila* (*2*, *5*).



PAS domains line up. AHR, the mammalian dioxin receptor; MESPHY, an algal phytochrome.

While the clock itself was being "brought to its knees" (*5*), other groups have studied how light affects its function. Macino and his colleagues, and others, have isolated *Neurospora* mutants that are blind to light, and they have now cloned two of the responsible genes (*6*, *7*). These are *white collar* (*wc*)*-1* and *-2*, named not because of their social position, but because they lack carotenoid pigments at the fringes of colonies. Both genes are transcription factors containing zinc finger and transcriptional activation domain motifs. WC-1 and WC-2 thus appeared to be transcriptional components of the blue-light photosensory pathways in *Neurospora*. Furthermore, these proteins both have PAS domains. PAS was initially identified as two direct repeats (PAS-A and PAS-B) in the *Drosophila* clock protein PER, in the basic helix-loop-helix (bHLH)–containing transcription factors ARNT and AHR in mammals, and in SIM in flies (*8*) (see figure). Other PAS-containing proteins have now been identified (*9*), and PAS domains have been shown to mediate protein-protein interactions (*8*) and, in one case, to bind small ligands.

The identities of WC-1 and WC-2 as phototransducers have now been extended in a surprising way by Crosthwaite *et al.* (*1*), who have shown that WC-2 is also a clock component, required for the maintenance of expression of *frq*. Thus, WC-2 appears to be intimately involved in both phototransduction and clock function and represents the first positively acting clock component. Indeed, WC-2 could well be the positive factor regulating FRQ that is negated by the negative feedback of FRQ itself and therefore may provide the first example of "closing" the clock loop in any organism. Although the *wc-1* gene appears not to encode a clock component, it is essential for light responses, and the clock eventually sputters to a halt in its absence; therefore, WC-1 appears to be required generally for clock progression but is not directly involved in the feedback loop. One would expect several such factors to be found.

The significance of these findings is likely to extend well beyond *Neurospora*, because PAS is now recognized as a signature motif found in clock proteins from both *Neurospora* and *Drosophila*. This provides the first real evidence that clock proteins may have a common evolutionary origin. But where did the exons that constitute PAS domains arise? A wonderful twist in this tale can be found in clues from photoreceptor proteins. Lagarias and colleagues (*10*) pointed out that the plant photosensory photoreceptors, the phytochromes, contain evidence of homology to the PAS-A and -B domains, and also to almost half of the bacterial blue-light receptor, photoactive yellow protein (PYP). PYP binds its 4-hydroxycinnamyl chromophore in a loop, half of which is contained in the PAS/photoreceptor homologous regions (*10*, *11*). Did some clock genes, bearing PAS domains, arise from ancient photoreceptor proteins? The idea is attractive, given that light perception and clock function are so closely related.

How can this question be resolved? First and foremost, we will need to see sequences of clock genes from a range of organisms. The recent identification of clock mutants in the prokaryotic cyanobacteria (*12*), and also in

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higher plants (*13*), should lead to the cloning of more clock components. Will some of these contain PAS domains, making the use of this motif universal? Second, are proteins such as WC-1 and WC-2 (or even PER) also photoreceptor molecules themselves? Macino and his colleagues have speculated this could be so, on the basis of the homology of the PAS region to part of the chromophore-binding region of PYP (*7*). Modeling of PAS domains based on the PYP structure may indicate whether the domain is at least a structurally, if not evolutionarily, conserved motif. Whatever the answers one thing is clear: The study of photosensory processes and the the circadian clock must now proceed hand-in-hand.

References and Notes

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ANTHROPOLOGY

Monte Verde and the Pleistocene Peopling of the Americas

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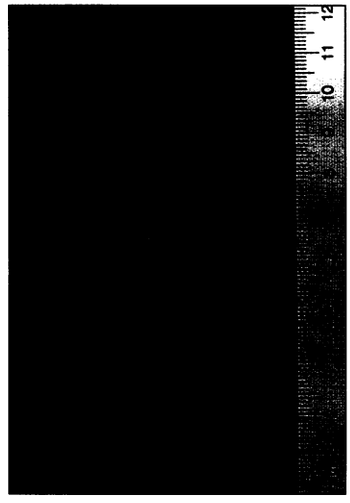
The publication of the second and final volume on the Monte Verde site in southern Chile by Dillehay of the University of Kentucky (*1*) marks a milestone in American archaeology. For over half a century, and with increasing rancor over the last few decades, archaeologists have sought and disputed evidence of a human presence in the Americas that predates the Clovis archaeological culture (~11,500 years before the present). Scores of pre-Clovis contenders have come forward, only to wither under critical scrutiny. So many have failed that the archaeological community has grown highly skeptical of any and all pre-Clovis claims (*2*, *3*). Few archaeologists would exclude the possibility that earlier evidence might be found, but most were unwilling to take such claims at face value. In the face of that accumulated skepticism, it was clear that the first site to break the Clovis barrier would have to effortlessly hurdle the traditional criteria by which early sites are judged (*4*): unambiguous artifacts or human skeletal remains in unimpeachable geological and stratigraphic context, chronologically anchored by secure and reliable radiometric dates.

The Monte Verde site was excavated from 1977 to 1985 and subsequently analyzed by Dillehay and an international and interdisciplinary team of nearly 80 collaborators. The remains they recovered are extraordinary. The Pleistocene occupants of Monte Verde camped on the sandy banks of Chinchihuapi Creek. Soon after their departure, water and fibrous peat spread over the site, blanketing the living surface, slowing the normal processes of decay and richly preserving many organic remains. Excavations recovered parts of nearly 70 species of plants (most unusually, in the form of chewed leaves), many of which have economic or medicinal value and were gathered from sources up to 400 km distant. Other remains included mastodon (Gomphothere) meat and bone with soft tissue adhering; wooden lances and mortars, as well as planks and stakes that formed the foundation of a tentlike structure evidently draped with mastodon hide; and hundreds of stone artifacts, including distinctive projectile points, spherical stones interpreted as bolas, and cutting and scraping tools that lack inherent attributes marking them as obviously the work of human hands but occur in a context bespeaking a cultural origin (*1*).



Signs of life. Stone implements found at the Monte Verde site dating to 12,500 years ago.

This material was found on a complex occupational surface representing the activities of a group living on site for what Dillehay estimates was roughly 1 year. Nearly 30 radiocarbon ages were obtained from charcoal, wood, and ivory materials on the occupational surface and from the strata bracketing that layer. These securely place the age of the occupation at ~12,500 before present (*5*).

Since finishing the excavations, Dillehay has directed a painstaking analysis of the site materials and spatial patterning, reported in a volume of over a thousand pages (*1*). The effort was analytical overkill. Yet, overkill was necessary, given the great skepticism facing this (or any) potentially early site and the doubts about Monte Verde's antiquity that have been expressed since the site's discovery was announced over a decade ago. The first volume (*6*) on the site resolved some of those initial concerns; the second volume puts the remainder to rest. These volumes, and an examination of the site and its collections in January of 1997, convinced a group of Paleoindian specialists—staunch skeptics among them—that the Monte Verde site is indeed archaeological and ~12,500 years old.

As such, its implications are profound. Although only slightly more than a thousand years older than Clovis, the site's great distance from the Bering Land Bridge (the entry route from Siberia) indicates initial arrival in the Americas must have occurred much earlier than 12,500 years ago. How much earlier depends partly on obstacles encountered along the way: Interior and coastal routes south from Alaska, for example, were impassable for long periods (~20,000 to after ~13,000 years before present on current evidence), as continental glaciers formed a physical and, for several millennia after their retreat, an ecological barrier to migration (*7*). It also depends on how quickly these groups adapted to the diverse and (as they moved south) increasingly exotic and unfamiliar New World, how easily they coped with novel pathogens and diseases (*8*), and how they maintained their population size and reproductive viability, contended with the potential genetic costs of inbreeding, all while living in relatively small numbers spread thinly over large and apparently unpopulated continents (*3*). On the basis of

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