some, but is its conclusion correct? The authors, recognizing that conflicting studies have recently deduced both pentameric (*2*) and tetrameric (*3*) architecture for glutamate receptors, are careful to point out that their conclusion follows only under an extremely simple picture of ligand binding. This is a problem because multisubunit ligand-binding proteins typically show lots of cross-talk between subunits; this is ugly and messy, but it is real, as if Rube Goldberg rather than Isaac Newton were the Great Designer of proteins. So at this point we are left with a devil's choice between a tetrameric glutamate receptor channel with a mechanism pristine—and unprecedented—in its simplicity, or a pentameric channel that behaves in a kinetically lumpy but wholly familiar way.

References

1. C. Rosenmund, Y. Stern-Bach, C. F. Stevens, *Science* **280**, 1596 (1998).
2. L. S. Premkumar and A. Auerbach, *J. Gen. Physiol.* **110**, 485 (1997).
3. I. Mano and V. I. Teichberg, *Neuroreport* **9**, 237 (1998).

CIRCADIAN RHYTHMS

An End in the Beginning

Jay Dunlap

Circadian rhythms and the cellular oscillators that underlie them are ubiquitous—and for good reason. For most organisms, dawn means food (either fixing carbon or hunting prey), predation, and changes in all the geophysical variables that accompany the sun—warmth, winds, and so on. It's a big deal when the sun comes up, and most living things time their days with an internal clock that is synchronized by external cues. Given this common and ancient evolutionary pressure, circadian clocks probably evolved early, and common elements are likely to be present up and down the evolutionary tree. A series of papers appearing in this week's *Science* (*1*, *2*) on pages 1564 and 1599, *Cell* (*3*, *4*), and the *Proceedings of the National Academy of Sciences* (*5*) reveals an appealingly similar pattern in the assembly of circadian oscillators ranging from fungi to mammals and gives us a close-up view of the way the gears within a clock drive its circadian feedback loop.

For some years evidence has been building in support of a model for a core circadian oscillator comprising, at least in part, a transcription/translation-based negative feedback loop wherein clock genes are rhythmically expressed, giving rise to cycling levels of clock RNAs and proteins (negative elements). The proteins then feed back, after a lag, to depress the level of their own transcripts, perhaps by interfering with positive elements that increase transcription of the clock genes. Although individual negative elements (canonical clock genes like *Drosophila per* and *Neurospora frq*) and positive elements (*CLOCK* in mammals, *white collar–1* and *white collar–2* in *Neurospora*) had been identified, yielding clues as to the general layout of the loop, a clear picture had not yet emerged. Another clue appeared last spring, when the the mouse gene *CLOCK* and the *Neurospora* genes *wc-1* and *wc-2* were found to contain PAS domains (*6*, *7*), regions also found in PER that interact with other PAS domain–containing proteins (*8*). Now several groups working independently have brought us to the next chapter in this story.

It is a truth universally acknowledged, that a single protein in possession of a good PAS dimerization domain, must be in want of a partner (with apologies to Jane Austen). Applying this maxim, Weitz and colleagues (*2*) used the recently identified mouse *CLOCK* gene sequence (*7*) in a yeast two-hybrid screen of hamster hypothalamic cDNAs and pulled up several likely candidates. Decades of careful analyses had pinpointed the mammalian pacemakers in the suprachiasmatic nuclei of the brain (where indeed *CLOCK* and mammalian *per* gene are expressed) and in the eye (*9*), so candidates were sifted by virtue of where they were expressed and all were found wanting except one, an orphan, BMAL1 (*10*). A similar screen, executed independently by Bradfield and his colleagues to catalog interactions among bHLH-PAS proteins, also turned up a strong interaction between a BMAL1 isoform (MOP3) and CLOCK. A third independent, but simultaneous, investigation in *Drosophila* began in Kay's lab with the careful application of low-stringency hybridizations with the mouse *CLOCK* gene to identify the *Drosophila* homolog, d*CLOCK* (d*CLK*). A collaboration between the Weitz and Kay labs ported the analysis of the CLOCK partner to the tractable fly genetic system. And finally (good news for those of us who still find comfort in informative phenotypes) classical forward genetic screens for rhythms mutations in the laboratories of Rosbash and Hall had identified and mapped two new *Drosophila* clock genes, *cyc* and *Jrk*, mutations in both of which eliminate expression of *per* and *tim*. This phenotype is enticingly similar to that accompanying mutations in *wc-1* and *wc-2* in *Neurospora*, which eliminate expression of the clock gene *frq* and are required for sustained rhythmicity, and to some extent to that of the phenotype of mice with a mutation in *CLOCK* (*6*). As expressed sequence tags corresponding to bHLH-PAS proteins, including dCLK (identified genetically as *Jrk*) and dBMAL1 (*cyc*) were deposited in the databases, it became clear that these mutations had yielded the phenotypes that were essential to anchor the emerging molecular biology to the organism's overt rhythms. The next step was to obtain a concrete description of what these proteins really do—and happily that is just what materialized, in a satisfying tale of great science flawlessly executed.

If the oscillator includes a transcription/translation-based negative feedback loop in which PAS protein partners are positive regulators, the right experiment is to show that the proteins bind to the pertinent clock gene promoters to activate their transcription and that the proposed negative regulators block this activation. This is just what was done. Weitz and colleagues, Kåy and colleagues, and Bradfield and colleagues all showed that the CLOCK-BMAL1 dimer binds DNA via a promoter sequence termed an E-box and activates transcription in vivo (*1*, *2*, *5*). Careful work by Hardin and colleagues (*11*) had already shown that a small enhancer region of the *per* promoter containing an E-box was sufficient to confer circadian regulation on *per* transcription in whole flies; the transcription part of the clock loop closed at least in part through an E-box. These studies were sufficient to suggest a model that has now been elegantly tested in intact cells. Weitz and colleagues in a collaboration with Takahashi have found the E-boxes in the mammalian *per1* promoter, showed them sufficient to activate *per1* transcription, and confirmed that the dominant negative phenotype of the original *CLOCK* allele (*7*) is due to the mutant protein's inability to activate transcription, although it retained the ability to form heterodimers with BMAL1. Kay and colleagues have used the E-box element in the promoter of the other *Drosophila* negative clock element *tim*, shown it sufficient to confer dCLK responsiveness to a reporter in a naïve cell line and, in the *coup de grace*,

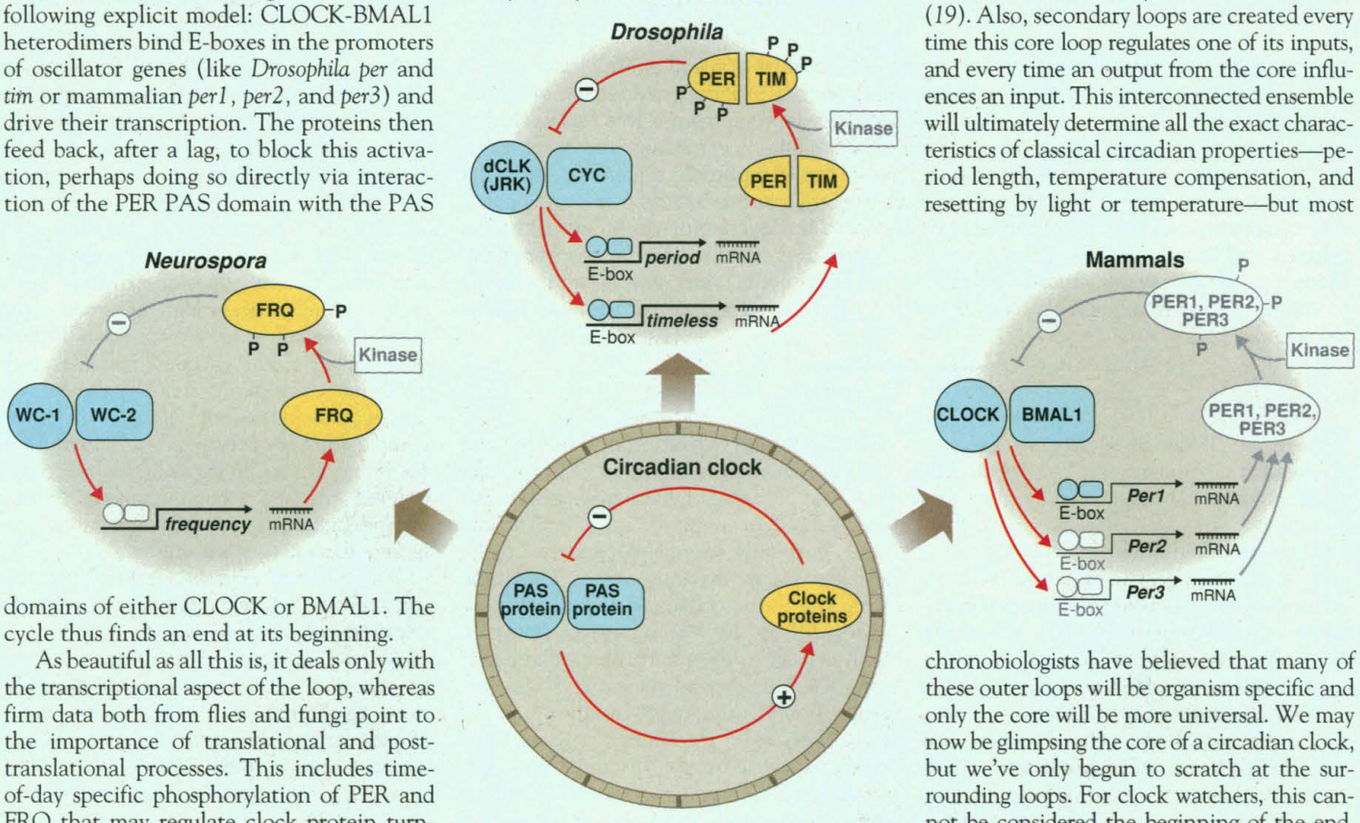
The author is in the Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03755, USA. E-mail: jay.c.dunlap@dartmouth.edu

shown that coexpression of PER and TIM blocked dCLK's ability to transactivate via the E-box (*2*). As they note, this closes the circadian feedback loop and the elements of the cycle are finally in view.

The new information gives rise to the following explicit model: CLOCK-BMAL1 heterodimers bind E-boxes in the promoters of oscillator genes (like *Drosophila per* and *tim* or mammalian *per1*, *per2*, and *per3*) and drive their transcription. The proteins then feed back, after a lag, to block this activation, perhaps doing so directly via interaction of the PER PAS domain with the PAS domains of either CLOCK or BMAL1. The cycle thus finds an end at its beginning.

As beautiful as all this is, it deals only with the transcriptional aspect of the loop, whereas firm data both from flies and fungi point to the importance of translational and post-translational processes. This includes time-of-day specific phosphorylation of PER and FRQ that may regulate clock protein turnover and thereby contribute to the long time constant of the cycle [for example (*12*, *13*)].

The figure attempts to draw together what is known about the three best-studied cellular circadian oscillators. Bold type indicates a known element, and shaded type denotes what seemed to me to be a reasonable extrapolation from sequence to function. Several common threads emerge. First, in all cases, there is a feedback loop that involves both positive and negative elements and that is centered on the transcription and translation of clock genes and clock proteins. The positive element in the loop is the transcriptional activation of clock genes through binding of paired transcriptional activators on the clock gene promoter; they heterodimerize by virtue of interaction via PAS domains. Transcription of the clock gene gives rise to a message whose translation (subject to additional regulation) generates clock proteins that provide the negative element in the feedback loop. After a lag, the negative element feeds back to negate the heterodimer's activation so the amount of clock gene mRNA declines, and eventually the level of clock protein also declines. Although not all of the details have been described in all systems and some aspects are missing (the kinase, proof of physical association between negative and positive elements), the overwhelming consistency among systems strongly suggests that these interactions reflect a common mechanistic core contributing to many eukaryotic circadian oscillators.



Circadian systems. Cycles within the circadian systems of the fruit fly *Drosophila*, mammals, and the fungus, *Neurospora*. Elements in gray are educated guesses.

So is this the whole enchilada; is this, simply, how all circadian systems work? Not a chance. To name a few discomfiting facts: In apparent violation of the model, PER cycling persists in the fly eye, albeit weakly, in the absence of *per* mRNA cycling (*14*), suggesting an additional exclusively posttranscriptional loop. In PER-expressing presumptive clock neurons in the moth brain, PER appears always non-nuclear (*15*). Antisense clock gene transcripts have been detected in the same moth (*15*) and in *Neurospora* (*16*), suggesting additional regulation. Regulated translational control gives rise to multiple forms of FRQ (*17*), a process that may also occur with TIM (*18*). The *frq* and mammalian *per* transcripts peak in the day, whereas the fly has a night phase clock. Mutant genes with strong effects on period length exist in flies and fungi that are not yet cloned and placed in the scheme. Finally, of course it isn't at all clear yet that cyanobacterial or plant clocks will follow this scheme. At best, the model that the new work allows us to build is a pleasing caricature of reality.

Circadian systems will almost certainly be made up of more than one interconnected feedback loop. Of these, one may be dominant and take the lead in determining phase (the time of day indicated by the clock) and others may be more like slaves (*19*). Also, secondary loops are created every time this core loop regulates one of its inputs, and every time an output from the core influences an input. This interconnected ensemble will ultimately determine all the exact characteristics of classical circadian properties—period length, temperature compensation, and resetting by light or temperature—but most chronobiologists have believed that many of these outer loops will be organism specific and only the core will be more universal. We may now be glimpsing the core of a circadian clock, but we've only begun to scratch at the surrounding loops. For clock watchers, this cannot be considered the beginning of the end, but it might be the end of the beginning.

References

1. N. Gekakis *et al.*, *Science* **280**, 1564 (1998).
2. T. K. Darlington *et al.*, *Science* **280**, 1599 (1998).
3. R. Allada, J. Hall, M. Rosbash, *Cell* **93**, 791 (1998).
4. J. Rutila, M. Rosbash, J. Hall, *ibid.*, p. 805.
5. J. B. Hogenesch, Y.-Z. Gu, S. Jain, C. A. Bradfield, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 5474 (1998).
6. S. C. Crosthwaite, J. C. Dunlap, J. J. Loros, *Science* **276**, 763 (1997).
7. D. King *et al.*, *Cell* **89**, 641 (1997); M. Antoch *et al.*, *ibid.*, p. 655.
8. J. Huang, I. Edery, M. Rosbash, *Nature* **364**, 269 (1993).
9. G. Tosini and M. Menaker, *Science* **272**, 419 (1996).
10. M. Ikeda and M. Nomura, *Biochem. Biophys. Res. Commun.* **233**, 258 (1997).
11. H. Hao *et al.*, *Mol. Cell. Biol.* **17**, 3687 (1997).
12. I. Edery *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 2260 (1994); M. W. Young *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **61**, 279 (1996).
13. N. Garceau, Y. Liu, J. J. Loros, J. C. Dunlap, *Cell* **89**, 469 (1997).
14. Y. Cheng and P. E. Hardin, *J. Neurosci.* **18**, 741 (1998).
15. I. Sauman and S. Reppert, *Neuron* **17**, 889 (1996).
16. J. C. Dunlap *et al.*, *Ciba Found. Symp.* **183**, 3 (1995).
17. Y. Liu, N. Garceau, J. J. Loros, J. C. Dunlap, *Cell* **89**, 477 (1997).
18. E. Rosato *et al.*, *Nucleic. Acids Res.* **25**, 455 (1997).
19. C. Heintzen, M. Nater, K. Apel, D. Staiger, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 8515 (1997).