sality classes relate to the known ones in equilibrium systems? In addition, there are also likely to be other, yet to be formulated questions that are specific to the nonequilibrium nature of the systems.

What are the appropriate qualitative measures of chaotic behavior? Is there a compact way of defining spatiotemporal chaos? And what quantitative measures are useful? The conventional characterization techniques for chaos with a few degrees of freedom, such as Lyapunov exponents and fractal attractor dimensions (*1*), involve the geometry of the motion in the full phase space of the system. For a spatially distributed system, the phase space is enormous, and it is not clear whether the traditional diagnostics can be suitably modified. One would have to seek extensive quantities to define intensive densities, which may become independent of the system size for large enough systems. To be practical, there must be ways to calculate appropriate quantitative measures by studying small subsystems of the spatially extended system.

It is natural in our present stage of understanding to attempt to characterize the systems in terms of correlation times and lengths. The relation between correlation times or lengths defined through different properties (for example, from the two-point correlation function or from the attractor dimension) is unknown and could be quite complicated. However, if there is a divergence approaching some point in parameter space, a comparison between the divergences of different lengths or times becomes particularly interesting.

Are there simple reduced descriptions, emphasizing the collective behavior of many chaotic degrees of freedom, again analogous to the reduced long-wavelength descriptions provided by thermodynamics and hydrodynamics for equilibrium systems? Are there simple limits that can be studied? One possibly useful limit is weak coupling. An example is a set of mappings (such as the logistic map) placed on lattice sites, with the dynamics of each map weakly (through a coupling parameter g) dependent on the dynamics of its neighbors in some specified way. For $g = 0$ the behavior is completely understood as the sum of the individual map properties, and the system is clearly extensive. For example, if the dimension of the attractor of the single map is d_f, then the dimension of N maps on a lattice is $D_f = Nd_f$ in this limit. It is natural to assume that for weak coupling ($g << 1$), such results continue to apply approximately, so that we expect $D_f = N\rho$, where ρ is a *dimension density* of the form $\rho = d_f + O(g)$. For general values of the coupling, we still expect the dimension D_f to be extensive, but the dependence of the dimension density ρ on the coupling g is more complicated.

Are there analytically tractable theoretical models of spatiotemporal chaos? In this connection, the work of Hansel and Sompolinsky (*15*) should be mentioned, where a lattice model of coupled m-component elements is solved exactly in the limit as m goes to infinity and is reduced to a single degree of freedom in the presence of a Gaussian noise, which must then be determined self-consistently.

Weak spatiotemporal chaos is a ubiquitous phenomenon in large nonequilibrium systems near the threshold to pattern formation. Recent experimental and theoretical work has identified a number of well-characterized systems showing this behavior and yielding detailed data on the spatiotemporal evolution. What is lacking is a simple phenomenology of spatiotemporal chaos that would reveal the essential features buried in the wealth of available data. Developing such an understanding is a challenge to theorists and experimentalists in the field of nonequilibrium phenomena.

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Time *Is* the Essence: Molecular Analysis of the Biological Clock

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Biological clocks underlie rhythmic processes from daily variation in photosynthesis in the single-celled dinoflagellates to the annual breeding cycles of some mammals. By the 1970s, it was clear that such clocks were ubiquitous and that their formal properties were similar among phylogenetically diverse organisms. The stage was set for tackling one of the major questions: What is the mechanism responsible for the generation of circadian (24-hour) oscillations?

The early demonstration of circadian rhythms in unicellular organisms had pointed to intracellular, biochemical processes as the underlying mechanism. The paradigm illustrated in the upper part of the figure has formed the heuristic basis for the experimental attack on mechanism. The oscillator is a negative feedback *loop* in which individual elements function either as *state variables* (A–D) of the oscillator or as *parameters* (*a–d*), which mediate their interaction. Pathways for input to the loop and for output complete the model. The biochemical problem was to identify the molecular correlates of the input, loop, and output elements.

One approach to the problem was genetic. Could a mutational analysis lead to the identification of genetic loci and the products of their expression that were part of the central mechanism? The isolation of single gene mutations that had profound effects on the circadian phenotype initially generated much excitement. In particular, in the early 1970s Konopka (*1*) and Feldman (*2*) discovered the *per* locus in *Drosophila melanogaster* and the *frq* locus in *Neurospora crassa*, both in organisms in which the power of genetics was well established. These discoveries greatly raised the expectations of the field. Mutations in both *per* and *frq* either abolished rhythm expression or altered its period, suggesting that these loci were central to clock function. But in the ensuing decade little new information on the biochemical function of these loci was forthcoming, and the genetic analysis seemed less productive than had

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been hoped initially. These doubts have been put to rest in the last 10 years. As Dunlap put it, the "awesome power of molecular genetics" has finally been brought to bear on the problem (3).

This power has been used to tackle three fundamental questions about the *per* and *frq* genes and their role in the circadian system. Is the level of the transcript or its protein product a state variable? What is the functional identity of the protein? What are the other molecules needed to complete the oscillatory loop?

The State Variable Question

Although there is no definitive set of criteria that can be used to uniquely identify a molecule as a state variable of the oscillator, there are two criteria that are strongly suggestive. First, the amount of the putative clock molecule should oscillate with a circadian period and abolition of this oscillation through constitutive expression of the molecule must eliminate overt expression of circadian rhythms. Second, transient changes in the amount of the component should, at least at some phases, shift the phase of the oscillation and expressed rhythms.

That both the *per* messenger RNA and its protein product (PER) exhibit circadian oscillations is well documented (4, 5). The effect of abolition of the oscillation in *per* is less certain. One allele of the gene, per^0, is a null mutation in which there is a premature stop codon in the protein coding sequence. This mutation abolishes the *per* mRNA and protein rhythms and further abolishes overt behavioral rhythms. The caveat, however, is that constitutive expression of *per* should also eliminate rhythmicity, and here the results are not clear. A heat shock promotor–*per* gene construct (*hsp-per*) lacking the 5' flanking DNA that contains cis acting elements that are sufficient (and were thought to be necessary) for *per* mRNA cycling (4) is still capable of some restoration of behavioral rhythmicity in per^0 at permissive temperatures (6). However, the coding region of *per* may contain sequences that can direct a rhythm in the transcript (7). In addition, cycling of PER may be posttranscriptionally regulated (5). Thus, both the transcript and the protein may still cycle in these constructs.

With respect to the second criterion, a recent report in *Science* by Edery and coworkers (8) suggests that acute elevation of *per* mRNA causes the phase of the circadian clock to shift. They used a wild-type fly strain into which a different heat-inducible *per* construct (*hspcper*) had been inserted. Transient induction of *hspcper* expression with pulses of heat caused phase shifts in the locomotor activity rhythm at phases of the circadian cycle where temperature pulses had no effect on wild type. This experiment indicated that changes in the amount of *per* expression alone could shift the phase of the oscillation.

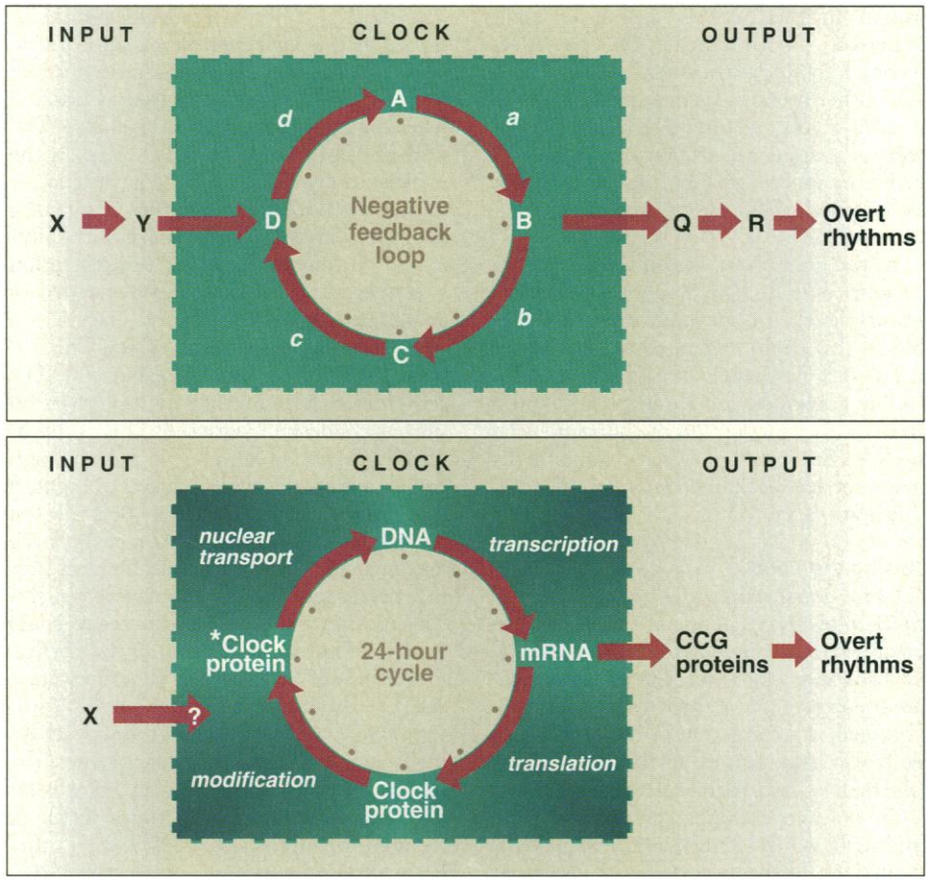

ILLUSTRATION: K. SUTLIFF

Translating a model into reality. (Upper) Heuristic model of a circadian (24-hour) biological clock. A, B, C, and D are state variables, and *a*, *b*, *c*, and *d* are parameters of an oscillating loop. X and Y are elements of the input pathway and Q and R are on the output pathway. **(Lower)** A biochemical circadian clock: A feedback loop consisting of transcription and translation of a clock protein. Both mRNA and protein are state variables. Transcriptional regulation leads to overt rhythms via expression of clock-controlled genes (CCG) [adapted from (*3*, *8*)]. Asterisk denotes posttranslational modifications.

These results suggested that the *per* protein or mRNA (or both) functions as a state variable of the oscillator. In this issue of *Science*, Aronson and co-workers (9) report parallel results for *frq*. They show that the *frq* mRNA levels, like those of *per* mRNA, are rhythmic. They further demonstrate the requirement for rhythmic expression of the *frq* gene for generation of the circadian rhythm of conidiation in *Neurospora* by using a construct in which an inducible promotor (qa-2) was used to drive expression of the open reading frame of *frq*. The construct, in which expression occurs only in the presence of quinic acid, was transformed into a frq^+ strain to test whether constitutive expression would abolish rhythmicity and whether transient expression would set the phase of the rhythm.

Although the transformed frq^+ strains exhibited normal rhythmicity in the absence of inducer, in the presence of quinic acid the overt expression of the conidiation rhythm was abolished. In addition, when quinic acid was removed, the phase of the conidiation rhythm was reset in the transformed strain but not in the wild-type strain. These results indicate that *frq* expression is integral to the circadian clock in *Neurospora* and that the *frq* mRNA or protein (or both) are state variables of the oscillator loop.

The Functional Identity Question

To really understand how *per* and *frq* underlie circadian rhythms, we need to elucidate their biochemical functions. Sequence analysis has not provided an unequivocal answer for either gene. To date, the most salient observation is that both *per* and *frq* directly or indirectly regulate their own expression. For *per*, the mutant allele (*perS*), which shortens the period of the overt rhythms, also shortens the period of the mRNA cycle (4). Further, transformation of the arrhythmic strain bearing the null mutation of *per* (per^0) with per^+ rescues per^0 mRNA cycling (4, 7). Clearly, PER regulates the rhythm of *per* expression. In this context, it is notable that PER is a nuclear

protein in the nervous system of adult *Drosophila* (*5*, *10*). Further, PER shares sequence homology (the PAS domain) with three other proteins known or suspected to be transcription factors (*11*). The PAS domain is a dimerization domain capable of mediating protein-protein interactions that could underlie PER's regulation of per transcription (*12*).

In the case of *frq*, the mRNA does not cycle in a null-allele mutant (*frq9*), and mRNA levels are elevated two- to threefold in this strain compared to peak levels in frq^+ (*9*). Further, in the quinic acid–inducible construct, induction of *frq* expression represses production of endogenous *frq* mRNA. So, *frq* too is self-regulating by means of feedback repression of its transcription (*9*).

Closing the Loop

The functional similarities between *per* and *frq* (despite structural dissimilarities) are intriguing. Both appear to be elements of feedback loops in which the proteins regulate the expression of their respective genes. This feedback regulation of transcription is likely a central feature of the clock's loop (see figure, lower part). However, neither of the proteins contains any known DNA-binding domains (*3*, *12*), so transcriptional regulation likely occurs by interactions with other elements. Two papers, also in this week's *Science*, have focused attention on a genetic locus that may code for just such an element for *per*. Sehgal *et al.* (*13*) have isolated a mutation, *timeless* (*tim*), that abolishes the circadian rhythms of behavior and, perhaps most notably, abolishes the rhythm of *per* mRNA cycling. This latter result suggests that there is an interaction between the *tim* and *per* loci.

In wild-type flies, immunocytochemical staining of PER in the nuclei of cells in the eye and brain shows a daily rhythm that peaks late in the night (*5*). In contrast, in *tim* mutants nuclear staining was not seen at any of the four time points during the day-night cycle that were examined (*14*). The effect of *tim* on PER was further investigated with recombinant flies bearing a construct containing the amino-terminal half of PER fused to β-galactosidase (PER-SG) in either wild-type (tim^+) or *tim* flies. The PER-SG fusion protein was located in the nucleus in tim^+ flies, whereas nuclear localization was not observed in flies bearing the *tim* mutation (*14*). Thus, transport of PER to the nucleus is crucial for the regulation of *per* expression, and a protein encoded by *tim* may facilitate this transport (*14*).

A second element with which PER interacts is a protein kinase (or kinases). The *per* protein has multiple phosphorylation sites, and phosphorylation of PER is rhythmically driven (*15*). The phosphorylation begins during the early subjective night, a time when the amounts of PER in the nucleus are increasing, and appears to be maximal just before the decline in PER that occurs during the late subjective night. This posttranslational modification could contribute to the generation of the rhythm in PER levels. For example, phosphorylation could be the trigger for proteolysis (*15*). Alternatively, a phosphorylation step, perhaps involving *tim*, may be necessary for the transport of PER to the nucleus, as it is for the *Drosophila* morphogen dorsal (*16*), or required for interaction of PER with other PAS-containing transcription factors (*15*).

The remarkable progress in the last few years on the molecular basis of the circadian clocks in both *Drosophila* and *Neurospora* has heightened enthusiasm for the genetic analysis of the clock problem and has also led to the search for new systems amenable to molecular analysis. In the past year, new clock mutations have been systematically induced in organisms as diverse as the cyanobacterium *Synechococcus* (*17*) and the mouse (*18*). Thus, there is the exciting prospect that we will soon be treated to the identification of new clock components in a variety of systems. The suggestion that transcription and translation may be integral to clock function is consistent with results from diverse organisms that show that inhibition of these two processes can abolish or reset the phase of circadian rhythms (*19*). Precisely what macromolecular synthesis does and whether it does the same thing across phylogenetic lines are questions of fundamental importance. The recent successes with *Drosophila* and *Neurospora* suggest that the answer to these questions may be forthcoming much sooner than might have been anticipated only a decade ago.

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