(layers) and the tangential (areas) domains of neocortex. Whereas transplantation experiments indicate a lack of stable positional markers for neocortical areas (*13*), similar transplants reveal that the identity of premigratory neurons destined for a particular cortical layer is retained after transplantation (*14*), unless the cells are transplanted very early—before the time of cell division in the ventricular germinal zone, when their laminar fates can in fact be altered. Although no experiments have revealed clear molecular differences between neocortical areas, the expression of POU genes is layer-specific (*15*). Even the sequence of steps in the formation of specific sets of axonal connections differs in the radial and tangential dimensions of cortex. The formation of radial connections between the layers appears to be highly specific at the outset, with axons apparently avoiding growth into inappropriate cortical layers (*16*). In vitro, too, cocultures of pieces of cortex and thalamus form axonal connections that obey the laminar-specific rules present in vivo, with the thalamus connecting to layer 4 and layer 6 of cortex connecting back to the thalamus (*17*).

The rules governing the formation of horizontal connections within neocortex may be very different. The establishment of axonal connections between thalamus and cortex requires the presence of ongoing interactions between the ingrowing axons and subplate neurons; in the absence of subplate neurons, appropriate thalamic axons cannot select and invade their cortical target areas even though the cells of the cortical plate are still present directly above (*18*). Later in development, the formation of ocular dominance columns in visual cortex or of the long distance patchy horizontal connections within cortical layers 3 and 5 is accomplished by extensive axonal remodeling and pruning, which is thought to require neural activity (*19*). Indeed, the results of these experiments serve as a reminder that very precise and discrete boundaries can form from initially diffuse sets of axonal inputs, and they emphasize the point that dynamic competitive interactions, rather than initially highly restricted spatial cues, can also produce sharp boundaries as a developmental outcome.

This comparison between the tangential and radial development of neocortical connections suggests that perhaps it is in the radial domain of cortex—in the formation of cortical layers—that the underlying developmental mechanisms will turn out to be similar to those that operate to form segmental boundaries and compartments in the nervous systems of other species, or in the hindbrain of vertebrates—as if each layer functions as a separate region, segment, or domain. On the other hand, just how the neocortex is subdivided into discrete cytoarchitectonic areas, and how the appropriate subsets of axons from the thalamus form the correct sets of connections with cortex, is still a major unsolved puzzle in developmental neurobiology.

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Circadian Clock Genes Are Ticking

Joseph S. Takahashi

The nature and function of *per*, a gene that controls circadian rhythms in *Drosophila*, has remained a wonder and an enigma ever since its discovery. Now, 20 years later, the *per* gene remains a wonder but its biochemical function is finally being defined.

In 1971, R. Konopka and S. Benzer (*1*) isolated three mutations that affected circadian rhythms in *Drosophila*. The three mutations were, unexpectedly, allelic and defined a new gene, the *period* (*per*) locus. Normal per^+ flies expressed 24-hour rhythms; whereas per^S mutants had 19-hour rhythms, and per^L mutants had 29-hour rhythms. The arrythmic mutant per^0 behaved phenotypically as a null mutant.

From 1984 to 1987, two groups led by M. Young at Rockefeller and J. Hall and M. Rosbash at Brandeis independently cloned *per* in a series of groundbreaking experiments that were the first to show that germline transformation with DNA could rescue a complex behavioral program (*2*). At the time, the primary sequence of Per and the three mutant alleles did not reveal any clues to its function. Early work focused on an unusual threonine-glycine (TG) repeat region that gave Per the ignominious label as a proteoglycan. Collectively we wondered what an extracellular matrix-like protein could be doing in the circadian clock? Disgust was the most frequent response to the notion of Per as a proteoglycan. Most were relieved to learn later that the TG repeat was not necessary for circadian rhythms (*3*). A second hypothesis that proposed Per as a modulator of gap junction coupling has also apparently not passed the test of time (*4*).

New clues to the nature of the *per* gene product have appeared with the recent discovery of three genes that share sequence homology with Per (see figure). The *Drosophila single-minded* protein (Sim) (*5*), the human aryl hydrocarbon receptor nuclear transporter (ARNT) (*6*), and the aryl hydrocarbon receptor (AHR) (*7*) all share with Per a domain called PAS (for Per, ARNT, Sim). The PAS domain encompasses approximately 270 amino acids of similar sequence that contain two 51–amino acid direct repeats (A and B in the figure) (*5*). In Sim, ARNT, and AHR, the PAS domain lies adjacent to a basic region (BR) helix-loop-helix (HLH) domain, which functions as a DNA-binding and dimerization region in transcriptional regulator proteins (*8*). ARNT and AHR are thought to dimerize with each other and together regulate transcription after ligand-binding (*6*, *7*). It is interesting that the ligand-binding domain of AHR and the per^L mutation both map to the PAS domain (*2*, *7*). Burbach and colleagues (*7*) speculate that the BR/HLH-PAS domain may function as a dimerization domain analogous to BR/HLH–leucine zipper motif proteins such as Myc and Max, and MyoD and E2A (*9*). Per, however, does not possess a BR/HLH domain or any other

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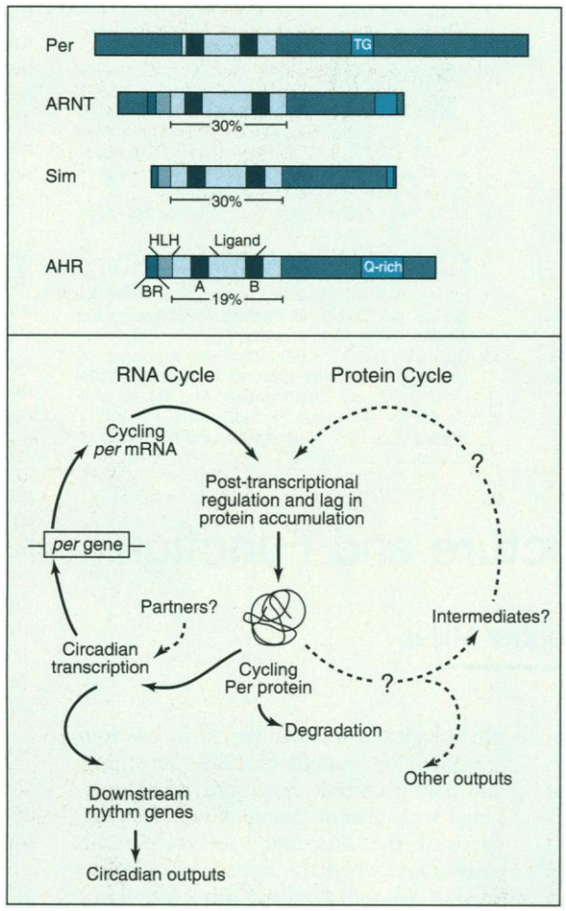
known DNA-binding motif. Thus, Per may use the PAS domain to dimerize and could interact with a partner that contains the DNA-binding region (*10*). This notion has led to the idea that Per could function as a transcriptional regulator (see below) either by working in concert with a partner to become activated or, alternatively, by acting as a dominant negative regulator by competing with a transcriptional regulator for dimerization or DNA binding.

Consistent with its possible role as a transcriptional regulator, electron microscopic analysis shows that Per is a nuclear protein in the adult *Drosophila* central nervous system (*11*), in particular in a cluster of lateral neurons and a set of smaller glial-like cells that are required for circadian rhythmicity (*12*). Thus, Per appears to be a member of a family of proteins that are transcriptional regulators, and Per is a nuclear protein in cells required for circadian rhythmicity.

Per itself exhibits a circadian rhythm (*13*). The rhythm in the amount of Per protein depends on *per*, because per^0 flies do not have a protein rhythm and because *per* mutants alter the Per rhythm (*14*). In an important paper, Hardin and colleagues (*15*) showed that amounts of *per* mRNA also show a striking circadian oscillation. The *per* RNA rhythm persists in constant darkness, and the period of the RNA rhythm is about 24 hours in per^+ flies and about 20 hours in per^S flies. The RNA of per^0 flies is present in amounts about 50% of those of normal flies but does not oscillate. In per^0 flies that have been rescued by germline transformation with wild-type per^+ DNA, both circadian behavior and *per* RNA cycles are restored. In these transformed flies both the exogenous per^+ RNA and the endogenous per^0 RNA levels oscillate. Therefore, the circadian expression of *per* mRNA and protein levels both depend on an active *per* gene. Because per^S shortens the period of the RNA cycle and because per^+ DNA transformation rescues per^0 RNA cycling, Per protein expression clearly regulates *per* cycling (*15*). Hardin and co-workers (*15*) propose that feedback by the *per* gene product regulates its own mRNA levels (see figure, bottom).

The Per protein rhythm appears to be regulated by two mechanisms: transcriptional and post-transcriptional (see figure). The 5′ noncoding sequences from *per* are sufficient to drive RNA cycles from a β-galactosidase fusion gene (*16*), and amounts of *per* precursor RNA also cycle in concert with mature *per* transcripts (*17*). In addition, *per* 5′ noncoding sequences are sufficient to drive heterologous RNA cycles. These results suggest that circadian fluctuations in *per* mRNA abundance are controlled by transcriptional regulation. However, an RNA rhythm is not sufficient to generate a protein rhythm. Per coding sequences in the amino-terminal half of the protein are necessary for expression of the Per protein rhythm (*16*). Thus, a post-transcriptional mechanism, under circadian clock control, is required for the Per protein oscillation.



How Per might control circadian rhythms. (**Top**) A family of proteins containing PAS domains. (**Bottom**) *Drosophila* Per molecular cycles. [Adapted from (*17*) and I. Edery and M. Rosbash, personal communication]

The peak of the *per* RNA cycle precedes the peak of the Per protein cycle by 6 to 8 hours. The lag in Per accumulation seems to be post-transcriptionally regulated. As shown in the figure, Per participates in two molecular loops. First, there is an RNA loop that involves transcriptional control of *per* RNA abundance followed by a post-transcriptional mechanism and lag in Per accumulation. Per then appears to act as a negative regulator of its own transcription (*10*), probably with a partner to confer DNA binding. In addition to inducing its own circadian transcription, Per could also control other downstream genes and subsequent circadian outputs (*17*).

The second molecular cycle is a Per protein loop. Although there is a *per* RNA cycle, this is not sufficient to drive the protein cycle. The nature of the post-transcriptional mechanism for protein cycling is not known. It is also not clear whether an RNA cycle is necessary for the protein cycle. Finally, it appears that a new gene is necessary for expression of the *per* RNA and behaviorial rhythms (*18*).

The discovery and analysis of *per* molecular cycles in the context of the overall regulation of circadian behavior in *Drosophila* are landmark contributions. Concurrently, diverse approaches in other systems suggest that the field of circadian rhythms is converging upon the identification of clock components and the elucidation of their function (*19*). How far can we extend the apparent convergence of clock mechanisms in different systems? Is the core mechanism of the circadian clock fundamentally similar or different across species? At this time, we can only speculate, but I believe that, as in the case of the cell cycle, a unified mechanism for the circadian clock is likely. Thus far the salient features of the clock are a requirement for macromolecular synthesis during a critical period for progression of the clock through its cycle (*19*), and the involvement of translational and transcriptional mechanisms in input pathways, the clock itself, and output pathways (*20*). It is intriguing that the model of Per function (see figure) can explain both the critical period for macromolecular synthesis and the involvement of transcriptional and translational control at a number of points in the circadian system. In mammals, the isolation of circadian clock mutants (*21*), and the circadian involvement and regulation of immediate-early genes (*22*) suggests that molecular models of vertebrate circadian clocks will also be ticking soon.

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23. Research in J.S.T.'s laboratory was supported by grants from the NIH, National Institute of Mental Health, National Eye Institute, and by an NSF Center for Biological Timing award. I thank C. Bradfield, J. Hall, M. Rosbash, and M. Young for advice and comments.

Ion Channel Structure and Function

Christopher Miller

The business of nerve cells is to generate, propagate, and integrate electrical signals, and ion channels are the proteins that support this cellular task. They directly catalyze the ion fluxes that bring about voltage changes across neuronal membranes and simultaneously act as sensors of those physiological signals—changes in ligand concentrations and in transmembrane voltage itself—that orchestrate the neuron's electrical symphony. Since their discovery nearly half a century ago, ion channels have been studied by exceptionally sophisticated assays of their electrical function, but until recently they have been molecular black boxes. Now, however, we are faced with a large number of ion channels for which the genes have been cloned and expressed in electrophysiologically accessible systems; consequently, the question "how do ion channels work?"—a question posed for decades but answered only in the formal language of state diagrams and kinetic models—is taking on a more palpable life. The field is beginning to smell blood as it approaches the molecular bases of channel gating and ion selectivity.

This Perspective is entitled "Ion channel structure and function" rather too hopefully, I think, since answers to these questions ultimately depend on structural information at the atomic level and since we have never seen a neuronal ion channel structure at high resolution. But the field's optimists—and there are many of them—argue that the unique strengths of electrophysiological assays manifested in fast voltage-clamping, single-channel recording, and gating current detection, when combined with genetic manipulation, can circumvent the structural vacuum. At this early stage, when the molecular questions are not yet too detailed, this enthusiastic attitude has produced important, novel conclusions about ion channel structure and its relation to function: pictures of the gross molecular architecture of these proteins, views of membrane topology of their constituent subunits, and identification of local regions of the protein sequence that participate in specific channel functions.

We now know that the two broad classes of channels involved in neuronal excitability—the ligand-gated and the voltage-activated channels—are formed as more or less cylindrically symmetric aggregates of multiple subunits (or of tandem-repeated homologous domains within a single polypeptide), with the ion conduction pore located on the axis of symmetry. Although esthetically pleasing and strongly implied by earlier low-resolution structural work (*1*), this symmetrical-pore picture was not a foregone conclusion; in the days of channel biophysics before cloning, when the pore's existence was clear but its shape was not, there were many unpublished arguments about what its "tortuosity" might be. Now we know that residues at equivalent positions on the five nicotinic acetylcholine receptor channel subunits have roughly equal electrostatic influence on ion conductance (*2*); similarly, in voltage-gated K^+ channels, a pore-blocking site for tetraethylammonium may be constructed out of four aromatic residues, one from each subunit, such that all four interact simultaneously with this hydrophobic cation with precisely equal energetic contributions (*3*). These mechanistic results nail down the rotational symmetry of the specific pores under study, and they argue for similar architectures for channels related to these by molecular homology—fivefold symmetry for the multitude of channels activated by the neurotransmitters acetylcholine, glutamate, glycine, and γ-aminobutyric acid (GABA), and fourfold symmetry for the voltage-gated K^+, Na^+, and Ca^{2+} channels, and perhaps for the distantly related cyclic nucleotide–activated channels as well.

Another broad result emerging from the recent work is that ion channels are constructed like other proteins, along modular lines, in which local regions of sequence can sometimes be identified with specific functions. Point mutation leading to alterations of ion selectivity, conductance, and affinity for blocking agents has led to wide, although happily not unanimous, acceptance of the idea that the linings of the conduction pores of both ligand-gated and voltage-dependent channels are formed by localized stretches of sequence of only 20 to 25 residues repeated in each subunit. It is surprising that even channel gating appears to be due to movements of local domains, at least in the case of ball-and-chain inactivation of A-type K^+ channels (*4*). Likewise, the ability of voltage-gated channels to sense the intramembrane electric field has been widely laid at the feet of a 20-residue positively charged transmembrane stretch of sequence (the infamous S4 region), although to be fair to any remaining skeptics, I must admit that no experimental evidence supports this notion in a rigorous (as opposed to a merely suggestive) fashion. In the nicotinic acetylcholine receptor, careful measurements of ligand-binding curves in cell lines expressing receptor complexes missing single subunits argue that the two nonequivalent ligand-binding sites located at the α subunit interfaces with γ and δ (*5*); the involvement of critical aromatic residues in forming this binding pocket is becoming clear (*6*), but the long-sought prize, the anionic group that attracts the choline headgroup, is still hiding in the bushes. However, not all channel functions are neatly modular; in K^+ channels, voltage-dependent activation and C-type inactivation are influenced by residues widely separated along the linear sequence (*7*), just as the quaternary interaction surfaces of hemoglobin are formed out of residues similarly dispersed.

Most of these early results have served mainly to locate hot spots in channel sequences, to list the cast of characters, but

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