Molecular Anatomy of a Light-sensitive Circadian Pacemaker in *Drosophila*

M.W. YOUNG, K. WAGER-SMITH, L. VOSSHALL,[1] L. SAEZ, AND M.P. MYERS
National Science Foundation Science and Technology Center for Biological Timing, Howard Hughes Medical Institute, and Laboratory of Genetics, The Rockefeller University, New York, New York 10021

Adult *Drosophila* produce locomotor activity rhythms with strain-specific periods close to 24 hours. These circadian behavioral rhythms resemble mammalian sleep/wake cycles; the phase of such a rhythm can be set by environmental cycles of light and dark or temperature, but the rhythm persists under constant environmental conditions. In sustained darkness, pulses of light advance or delay the rhythm in a fashion that is dependent on the time of light administration with respect to the phase of the behavioral cycle.

The generation of circadian rhythms in *Drosophila* requires the activity of two genes, *period* (*per*, Konopka and Benzer 1971) and *timeless* (*tim*, Sehgal et al. 1994; Vosshall et al. 1994). Loss of either gene abolishes circadian rhythmicity, and missense mutation of either gene can alter period length (Konopka and Benzer 1971; Baylies et al. 1987, 1992; Yu et al. 1987; Rutila et al. 1992, 1996; Sehgal et al. 1994; A. Rothenfluh-Hilfiker and M.W. Young, unpubl.). The period length of *Drosophila*'s circadian rhythms is also sensitive to changes in the dosage of these genes. Using transgenic flies, Baylies et al. (1987) showed a uniform relationship for *per* RNA titer and period. Lowering the dose of *per* lengthened circadian rhythms. For example, a 20-fold reduction in *per* RNA was associated with a lengthening of period to 37–38 hours.

Transgenic flies expressing *per* in restricted regions of the brain have identified pacemaker cells that promote circadian behavioral rhythms (Ewer et al. 1992; Frisch et al. 1994; Vosshall and Young 1995). In these and other central brain cells, and in photoreceptor cells composing the eyes, the amounts of PER and TIM proteins fluctuate with a circadian rhythm (Siwicki et al. 1988; Edery et al. 1994; Hunter-Ensor et al. 1996; Myers et al. 1996; Zeng et al. 1996). These oscillations occur with similar phases (Siwicki et al. 1988; Edery et al. 1994; Hunter-Ensor et al. 1996; Myers et al. 1996; Zeng et al. 1996) such that peak levels of the PER and TIM proteins are observed late at night. *per* and *tim* mRNAs also accumulate with the same circadian rhythm (Hardin et al. 1990; Sehgal et al. 1995), but the phase of RNA accumulation precedes that of protein accumulation by 4–6 hours. Highest RNA levels are observed late in the day and into the early evening.

A functional interaction between these genes was originally revealed by molecular studies of *per* RNA and PER protein in *tim* mutants (Sehgal et al. 1994; Vosshall et al. 1994). Circadian cycles of *per* mRNA accumulation were eliminated by arrhythmic (null) mutations of *tim* (Sehgal et al. 1994). However, a key observation was that the product of *tim* appeared to be a posttranslational regulator of PER; null mutations of *tim* (*tim*^{0}) blocked nuclear localization of the PER protein (Vosshall et al. 1994; see below). Evidently, nuclear localization of PER is a control point in the circadian system; without *tim*'s regulation of PER's subcellular distribution, oscillations in transcription are no longer possible (Vosshall et al. 1994). In addition to blocking PER's nuclear entry, *tim* null mutations also appear to destabilize PER. Very low levels of the PER protein accumulate in the cytoplasm of *tim*^{0} flies (Vosshall et al. 1994; Price et al. 1995).

Positional cloning of *tim* (Myers et al. 1995) allowed the mechanism of this *per*/*tim* interaction to be resolved. The PER and TIM proteins are physical partners, binding to each other, in part, through PER's PAS domain (Gekakis et al. 1995; Saez and Young 1996), a protein interaction sequence supporting dimerization of several previously characterized proteins (Huang et al. 1993). The proteins bind to each other in vitro, in yeast, and in flies (Gekakis et al. 1995; Lee et al. 1996; Saez and Young 1996; Zeng et al. 1996). Moreover, this binding is highly specific, allowing efficient selection of cDNAs encoding TIM from *Drosophila* total head cDNA libraries using PER protein baits in yeast two-hybrid screens (Gekakis et al. 1995). Immunocytochemical studies have shown that TIM proteins do not translocate to nuclei in *per* null mutants. Thus, PER and TIM must both be present in a cell to afford nuclear localization of either protein (Hunter-Ensor et al. 1996; Myers et al. 1996). However, unlike PER, TIM is stable in the cytoplasm when its partner protein is missing (Myers et al. 1996).

Figure 1 presents a model for generation of circadian rhythms in *Drosophila* in which cycles of *per* and *tim* transcription are regulated by alternating cycles of nuclear localization of a PER/TIM protein complex. Our model is based on the following observa-

[1]Present address: Center for Neurobiology and Behavior, Howard Hughes Medical Institute, Columbia University, 701 W. 168th Street, New York, New York 10032.

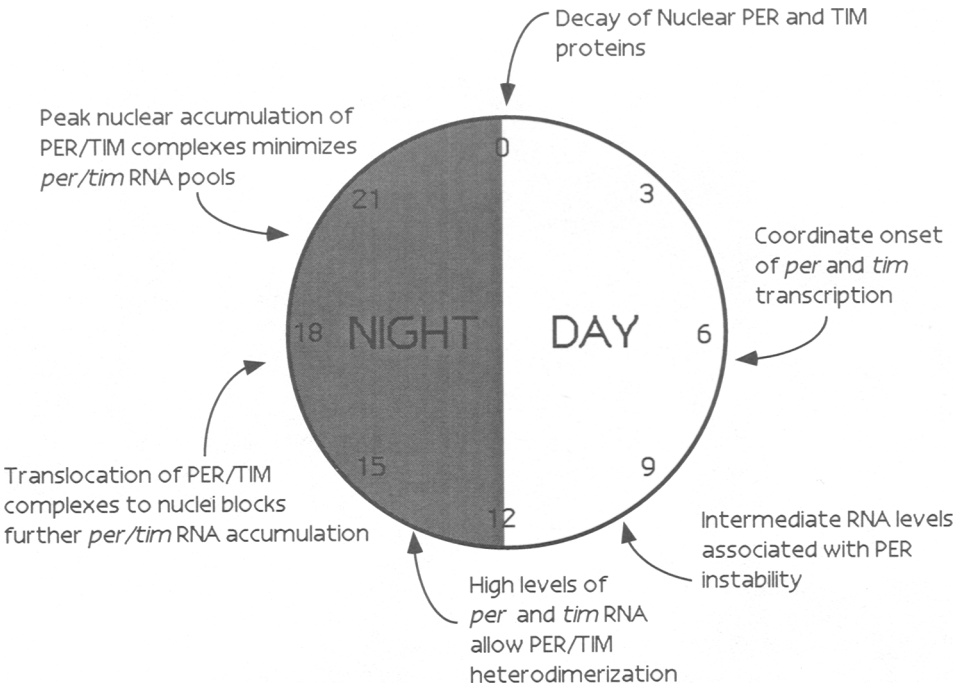

Figure 1. Physical interaction of PER and TIM produces circadian cycles in feedback regulation (see text).

tions and interpretations: (1) Several studies have suggested that, on assembly and nuclear localization, the PER/TIM complex suppresses *per* and *tim* transcription (discussed below). (2) As PER protein accumulation and nuclear localization are suppressed in tim^o mutants (Vosshall et al. 1994; Price et al. 1995), and the TIM and PER proteins bind to each other in vitro, in vivo, and in yeast, heterodimerization of the proteins is likely to be required for nuclear translocation and stabilization of PER. Because TIM accumulates in the cytoplasm of per^o mutants (Hunter-Ensor et al. 1996; Myers et al. 1996), heterodimerization is probably required for TIM nuclear localization. (3) *tim* RNA expression cycles, so PER stabilization should be restricted to only some times of day. (4) Given the instability of PER in the absence of TIM, PER proteins would accumulate in conjunction with *per* mRNA only if TIM proteins were amassed prior to *per* transcription. Because *per* and *tim* RNAs accumulate with the same phase, delays in PER accumulation and nuclear localization of PER/TIM complexes are expected, probably reflecting times of PER/TIM heterodimer formation. (5) If high levels of PER and TIM protein suppress *per* and *tim* expression, cycles in this regulation will result from separate temporal phases of *per* and *tim* RNA accumulation and PER/TIM nuclear localization. In the absence of a mechanism supporting such delays, feedback control would lead to constitutive gene and protein synthesis, albeit at intermediate levels, rather than to clock-like oscillations of RNA and protein as observed.

PER AND TIM MONOMERS ARE RETAINED IN THE CYTOPLASM

Vosshall et al. (1994) found that proteins composed of the amino-terminal half of PER (a.a. 1–636) fused to β-galactosidase translocated to brain and photoreceptor nuclei in wild-type, but not in tim^o flies. The fusion protein was expressed in the correct tissues of the head in tim^o flies but was retained in the cytoplasm (Fig. 2) (Vosshall et al. 1994). Western blots showed no effect of the tim^o mutation on the level of the fusion protein produced in these flies (Vosshall et al. 1994). In contrast, PER-β-gal fusion proteins lacking PER amino acids 95–529 were nuclear in tim^o flies (Vosshall et al. 1994). Two additional reporter genes have been constructed for analysis of expression patterns in wild-type and tim^o mutant backgrounds. The first of these reporters encoded PER amino acids 1–212 fused to β-gal and was transported to nuclei in both tim^+ and tim^o flies (data not shown). The second, a fusion protein, composed of PER 1-515 followed by β-gal, moved to nuclei in a tim^+ background but was cytoplasmic in tim^o flies (data not shown). Thus, a 303-amino-acid region (between amino acids 212 and 515, and including PAS) contains sequences promoting cytoplasmic accumulation. This segment of PER has been shown to support TIM binding in yeast and in vitro (Gekakis et al. 1995; Saez and Young 1996).

Consistent with the behavior of PER-β-gal fusion proteins in transgenic flies, cultured *Drosophila* cells (Schneider line 2, S2) show cytoplasmic accumulation

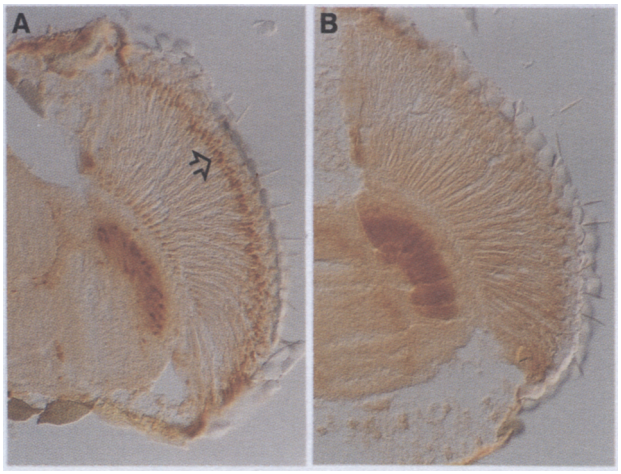

Figure 2. Nuclear localization of a PER reporter protein is blocked by the *tim*^{o} mutation. Transgenic (*A*) *tim*$^{+}$ or (*B*) *tim*^{o} mutants that express a PER-β-galactosidase fusion protein were stained with antibody to β-gal. Open arrowhead (*A*) marks PER accumulation in nuclei of photoreceptor cells in the wild-type eye. No reactivity is observed in photoreceptor nuclei in *tim*^{o} mutants, but intense cytoplasmic staining can be seen in the elongated photoreceptor cells (*B*). PER amino acids 1–636 are present at the amino terminus of the β-gal fusion protein (for methods, see Vosshall et al. 1994).

of full-length PER proteins when transfected with *per* genomic or cDNA (Saez and Young 1996). Full-length TIM proteins, which fail to translocate to nuclei in *per*^{o} flies, also accumulate in the cytoplasm of *tim*-transfected S2 cells (Saez and Young 1996). S2 cells produce no detectable PER or TIM prior to transfection (Saez and Young 1996). When *per* and *tim* are coexpressed in S2 cells, both proteins are translocated to nuclei. This suggests that in the cultured cells and in vivo, nuclear translocation depends on physical association of the PER and TIM proteins (Saez and Young 1996). The results also show that no other clock proteins are required for nuclear localization.

Deletion studies of PER proteins expressed in S2 cells show that, as predicted from work with transgenic flies, a sequence near PAS inhibits PER nuclear accumulation (Saez and Young 1996). A sequence inhibiting TIM nuclear localization was also revealed near the TIM carboxyl terminus. Protein-binding studies indicate that PER sequences that inhibit PER nuclear translocation form a site for physical interaction with TIM. In fact, at least two binding sites on each protein mediate PER/TIM dimerization (Saez and Young 1996). Thus, formation of PER/TIM complexes is restricted to the cytoplasm by binding and localization activities carried on both proteins.

A consequence of this requirement for cytoplasmic assembly of PER/TIM heterodimers is that factors affecting rates of PER and TIM synthesis and accumulation should affect timing of nuclear localization. Aspects of PER regulation in *tim*^{o} flies may be especially important in this regard: Monomeric PER proteins appear quite unstable when restricted to the cytoplasm of brain and photoreceptor cells in *tim*^{o} flies (Vosshall et al. 1994; Price et al. 1995). This suggests that PER is unstable when not complexed with TIM. In wild-type flies, as titers of *tim* and *per* RNA and TIM protein rise, rates of nuclear translocation may become largely dependent on the half-life of monomeric PER proteins. For flies maintained in a light/dark cycle (such as the solar day), daytime levels of PER and TIM will remain low due to TIM's light sensitivity (below) and PER's dependence on TIM. After sunset, dark-dependent stabilization of TIM should be followed by TIM-dependent stabilization of PER.

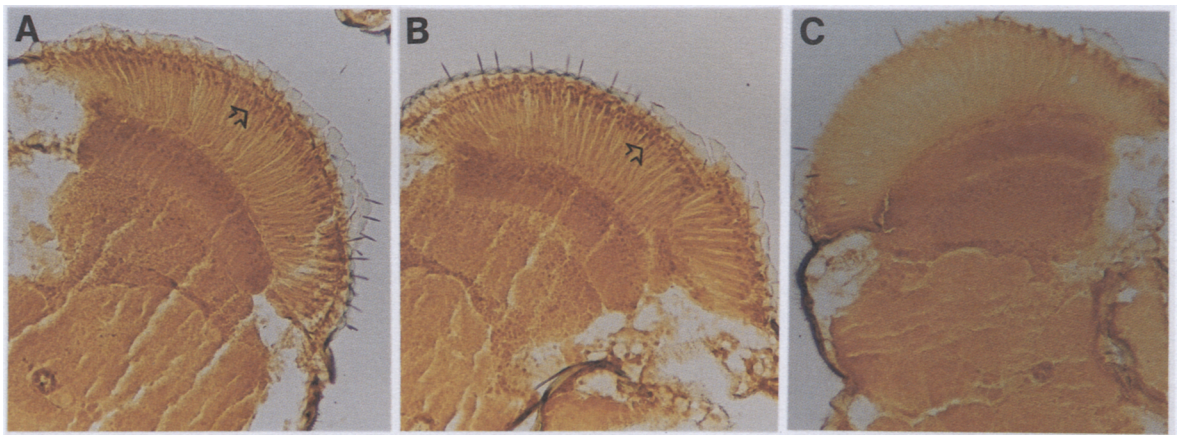

Figure 3. TIM proteins are degraded following a brief exposure to light. Immunocytochemical detection of TIM proteins in photoreceptor nuclei (*open arrowheads*) of control (non-light-pulsed) flies at CT 20.5 (*A*) and CT 22 (*B*). A 10-min light pulse (~8,000 lux) was administered to flies at CT 16. Flies were returned to constant darkness and collected for immunocytochemical detection of TIM at CT 21 (*C*). Note absence of TIM staining. The light pulse produces a phase delay in the behavioral rhythm of about 4 hr. For methods, see Myers et al. (1996).

LIGHT INDUCES RAPID LOSS OF TIM

The model presented in Figure 1 describes how a self-sustained molecular rhythm can be produced in constant darkness, but it does not provide an explanation for the rapid alignment of molecular and behavioral rhythms with the phase of environmental rhythms such as 24-hour cycles of light and dark. In wild-type flies, constant light induces arrhythmicity and low levels of PER as in *tim*^{0} mutants (Price et al. 1995). Because *tim*^{0} mutants produced indistinguishable levels of PER proteins in light/dark cycles (LD), constant darkness, and constant light, it was suggested that constant light might eliminate circadian rhythms in wild-type flies by reducing an activity dependent on *tim* (Price et al. 1995). The level of TIM protein was subsequently investigated in *per*^{0} fly heads (Myers et al. 1996; Zeng et al. 1996). TIM was expressed at fairly high levels in *per*^{0} flies reared in constant darkness, but the level of TIM was found to oscillate in *per*^{0} flies when they were exposed to light/dark cycles. Since earlier work had clearly shown that *tim* RNA levels do not oscillate in *per*^{0} mutants (Sehgal et al. 1995), the results suggested that the TIM protein is lost when flies are exposed to light.

This phenomenon was investigated more fully by exposing *per*^{0} flies to 1-hour pulses of light, followed by a recovery period in constant darkness. Western blot analysis showed that TIM levels dropped rapidly after the light treatment and began to rise within the first hour after return to darkness (Myers et al. 1996). The rapidity of this response was further indicated by measuring TIM protein amounts immediately following a 15-minute pulse of light. Substantial loss of TIM was again observed (Myers et al. 1996). As shown in Figure 3, this sensitivity to light is also readily detectable in immunocytochemical studies of TIM proteins in the eyes and brains of wild-type flies (data in Fig. 3C show response to a 10-min light pulse). The data derived from *per*^{0} mutants indicate that there exists a mechanism for the light-induced degradation of TIM protein that does not require PER, and thus, by definition, is clock-independent (Myers et al. 1996).

These and related observations (Hunter-Ensor et al. 1996; Lee et al. 1996) suggested a role for TIM in light-dependent entrainment of the *Drosophila* clock. Pulses of light given to wild-type flies during the night portion of their behavioral cycle can advance or delay the behavioral rhythm. Studies of *Drosophila melanogaster* show resetting of the locomotor activity rhythm by such light pulses within 24 hours (cf. Myers et al. 1996). It was found that light pulses in the early evening produce a delay in the phase of TIM diminution within the current cycle of the molecular rhythm, whereas light pulses delivered late at night (1–3 hr before dawn) produce a monotonic advance in the phase of TIM diminution within the current cycle of the molecular rhythm (Myers et al. 1996). Because TIM's light-dependent degradation is clock-independent (occurs in *per*^{0} flies), and the phase of the TIM protein rhythm is reset within hours of exposure to light, TIM's response to light appears to be at the core of the *Drosophila* entrainment mechanism (Myers et al. 1996; see also below).

LIGHT-DEPENDENT DEGRADATION OF TIM RESETS *per* AND *tim* MRNA RHYTHMS

What is the function of the PER/TIM complex once transported to the nucleus? Because null mutations of *per* and *tim* abolish rhythms of *per* and *tim* mRNA synthesis (Hardin et al. 1990; Sehgal et al. 1995), and constitutive expression of PER (from the rhodopsin promoter) suppresses transcriptional cycling of the endogenous gene in the adult eye (Zeng et al. 1994), it has been suggested that the PER/TIM complex may contribute to an autoregulatory loop affecting *per* and *tim* transcription (Sehgal et al. 1995; Hunter-Ensor et al. 1996; Lee et al. 1996; Myers et al. 1996; Zeng et al. 1996). One way to test this model would be to acutely perturb PER or TIM protein levels posttranscriptionally and to measure any derived effect on *per* and *tim* RNA levels. A tool for carrying out such an experiment emerged with the discovery that TIM protein is rapidly eliminated in vivo by exposure to light. Because *per* and *tim* RNA levels in the head undergo a rhythmic oscillation in flies entrained to a normal cycle of 12 hours of light and 12 hours of dark (LD 12:12), we asked how a pulse of light delivered either before the declining phase of the RNA curve, or before the rising phase, would affect subsequent RNA levels. Since TIM proteins are degraded in response to light, secondary changes in *per* and *tim* mRNA synthesis are expected following the light pulse if PER/TIM complexes regulate transcription.

Prior studies showed that *tim* RNA levels do not oscillate in *per*^{0} or *tim*^{0} flies, even when maintained in LD cycles (Sehgal et al. 1995; Hunter-Ensor et al. 1996). As predicted from those results, Figure 4 indicates no acute effect of light on either *per* or *tim* RNA levels in wild-type flies pulsed with light 2 hours after sunset (ZT14), which produces behavioral delays, or 1 hour prior to expected dawn (ZT23), which produces behavioral advances. Figure 4 does, however, show an elevation relative to control in *tim* and *per* RNA levels following times of TIM degradation. This elevation in RNA level is seen 4–5 hours after the light pulse and is therefore distinct from the timing of TIM degradation, which was previously shown to be completed within 1 hour, whether TIM loss was induced by an advancing or delaying light pulse (Myers et al. 1996). A similar elevation in *per* RNA level following exposure to light was recently reported by Lee et al. (1996). Together, the results suggest that TIM/PER complexes repress both *per* and *tim* transcription. Whether the PER/TIM complex regulates transcription by directly binding DNA or by indirect means is unknown.

Figure 4 also shows that as a consequence of the elevated *per* and *tim* RNA levels, the phases of *tim* and

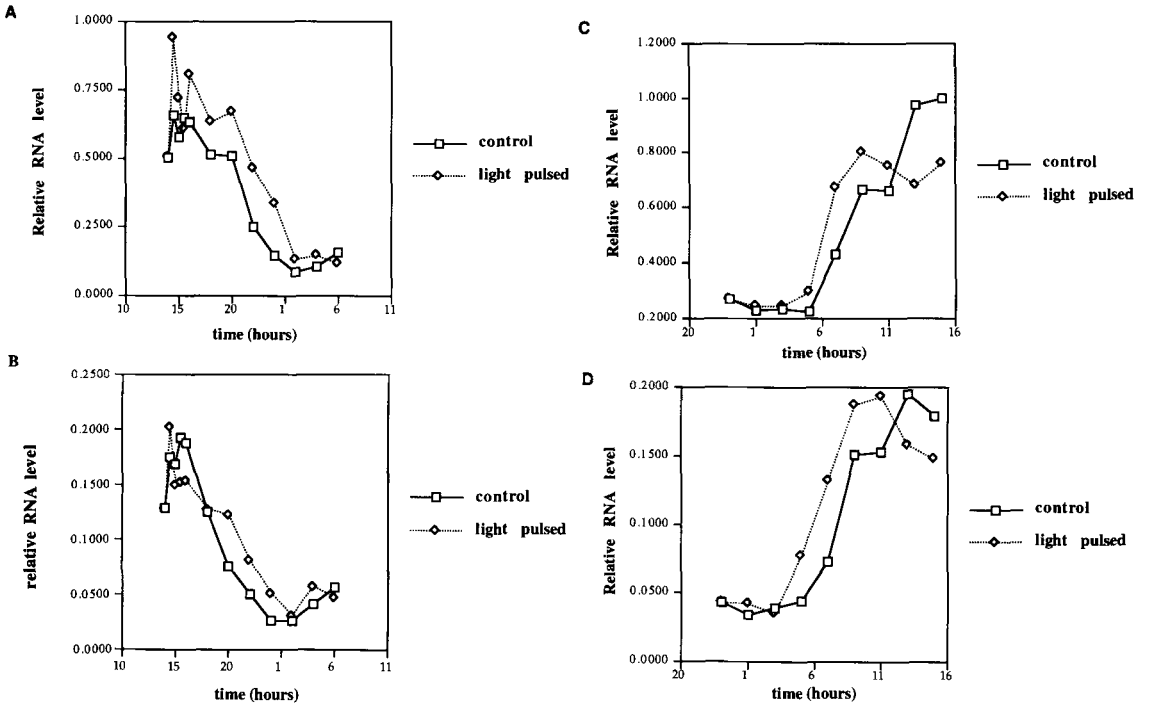

Figure 4. Light pulses lead to elevated *per* and *tim* RNA levels. Relative RNA levels for control flies (*solid line*) or flies which received a 1-hr light pulse (*dotted line*). (*A*) *tim* RNA, CT 14 light pulse. (*B*) *per* RNA, CT 14 light pulse. (*C*) *tim* RNA, CT 23 light pulse. (*D*) *per* RNA, CT 23 light pulse. Numbers on the X axis indicate circadian time (CT0= subjective lights on; CT12= subjective lights off in constant darkness). Canton S flies were entrained for several days to cycles of 12 hr light and 12 hr dark (LD 12:12). Records begin at CT 14 (*A*, *B*) or CT 23 (*C*, *D*) in the final LD cycle. At CT 12 in the last LD cycle, the lights were turned off for the remainder of the experiment. Each point is the mean of six RNase protection assays representing three separate experiments with all protection assays performed twice. RNA was derived from adult heads. *per* and *tim* values were normalized to tubulin (for methods, see Sehgal et al. 1995).

per RNA diminution are delayed by light pulses given at ZT14, and the phase of RNA accumulation is advanced by pulses supplied at ZT23. Such phase shifts are consistent with a previously suggested mechanism for the resetting of the TIM protein rhythm by light (Myers et al. 1996). For light pulses in the early evening (e.g., ZT14), rapid elimination of TIM is followed by resynthesis of the protein from an existing *tim* RNA pool that is high at that time of day (Fig. 1) (Myers et al. 1996). Because the decline in the RNA pool is delayed by extended RNA synthesis that resulted from transient loss of TIM, resynthesis of TIM protein continues with a new and delayed phase. For light pulses prior to dawn (ZT23), TIM is again rapidly degraded, but the existing *tim* RNA pool is low (Fig. 1) (Myers et al. 1996). TIM cannot be replaced in the current molecular cycle, but advanced protein synthesis occurs in the following day's cycle due to prematurely rising *tim* and *per* RNA pools (Fig. 4C,D).

Our results suggest that light-induced degradation of TIM protein causes an elevation of *per* and *tim* RNA levels. Figure 4 demonstrates that stimulation of *tim* and *per* RNA levels accompanies both advancing and delaying light pulses, and conforms to studies that indicated complete resetting of *D. melanogaster* behavioral rhythms within a single circadian cycle (cf. Myers et al. 1996). An important feature of this mechanism is that phases of PER and TIM protein synthesis appear to be coordinately reset by all light pulses. This would move both central components of the pacemaker mechanism to the new phase. The findings support a model in which PER and TIM function as negative transcriptional regulators, probably as a PER/TIM molecular complex.

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REFERENCES

Baylies, M. K., T.A. Bargiello, F.R. Jackson, and M.W. Young. 1987. Changes in abundance of the *per* gene product can affect periodicity of the *Drosophila* clock. *Nature* **326:** 390.

Baylies, M.K., L.B. Vosshall, A. Sehgal, and M.W. Young.

1992. New short period mutations of the *Drosophila* clock gene *per*. *Neuron* **9:** 575.

Edery, I., L.J. Zwiebel, M.E. Dembinska, and M. Rosbash. 1994. Temporal phosphorylation of the *Drosophila* period protein. *Proc. Natl. Acad. Sci.* **91:** 2260.

Ewer, J., B. Frisch, M.J. Hamblen-Coyle, M. Rosbash, and J.C. Hall. 1992. Expression of the *period* clock gene within different cell types in the brain of *Drosophila* adults and mosaic analysis of these cells' influence on circadian behavioral rhythms. *J. Neurosci.* **12:** 3321.

Frisch, B., P.E. Hardin, M.J. Hamblen-Coyle, M. Rosbash, and J.C. Hall. 1994. A promoterless *period* gene mediates behavioral rhythmicity and cyclical per expression in a restricted subset of the *Drosophila* nervous system. *Neuron* **12:** 555.

Gekakis, N., L. Saez, A.-M. Delahaye-Brown, M.P. Myers, A. Sehgal, M.W. Young, and C.J. Weitz. 1995. Isolation of *timeless* by PER protein interaction: Defective interaction between *timeless* protein and long-period mutant Per^{L}. *Science* **270:** 811.

Hardin, P.E., J.C. Hall, and M. Rosbash. 1990. Feedback of the *Drosophila period* gene product on circadian cycling of its messenger RNA levels. *Nature* **343:** 536.

Huang, Z.J., I. Edery, and M. Rosbash. 1993. PAS is a dimerization domain common to *Drosophila* period and several transcription factors. *Nature* **364:** 259.

Hunter-Ensor, M., A. Ousley, and A. Sehgal. 1996. Regulation of *Drosophila* protein *timeless* suggests a mechanism for resetting the circadian clock by light. *Cell* **84:** 677.

Konopka, R.J. and S. Benzer. 1971. Clock mutants of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci.* **68:** 2112.

Lee, C., V. Parikh, T. Itsukaichi, K. Bae, and I. Edery. 1996. Resetting the *Drosophila* clock by photic regulation of PER and PER-TIM complex. *Science* **271:** 1740.

Myers, M.P., K. Wager-Smith, A. Rothenfluh-Hilfiker, and M.W. Young. 1996. Light-induced degradation of TIMELESS and entrainment of the *Drosophila* circadian clock. *Science* **271:** 1736.

Myers, M.P., K. Wager-Smith, C.S. Wesley, M.W. Young, and A. Sehgal. 1995. Positional cloning and sequence analysis of the *Drosophila* clock gene, *timeless*. *Science* **270:** 805.

Price, J.L., M.A. Dembinska, M.W. Young, and M. Rosbash. 1995. Suppression of PERIOD protein abundance and circadian cycling by the *Drosophila* clock mutation *timeless*. *EMBO J.* **14:** 4044.

Rutila, J.E., I. Edery, J.C. Hall, and M. Rosbash. 1992. The analysis of new short-period circadian rhythm mutants suggests features of *D. melanogaster period* gene function. *J. Neurogenet.* **8:** 101.

Rutila, J.E., H. Zeng, L. Myai, K.D. Curtin, J.C. Hall, and M. Rosbash. 1996. The tim^{SL} mutant of the *Drosophila* rhythm gene *timeless* manifests allele-specific interactions with period gene mutants. *Neuron* (in press).

Saez, L. and M.W. Young. 1996. Regulated nuclear localization of the *Drosophila* clock proteins PERIOD and TIMELESS. *Neuron* (in press).

Sehgal, A., J.L. Price, B. Man, and M.W. Young. 1994. Loss of circadian behavioral rhythms and *per* RNA oscillations in the *Drosophila* mutant timeless. *Science* **263:** 1603

Sehgal, A., A. Rothenfluh-Hilfiker, M. Hunter-Ensor, Y. Chen, M.P. Myers, and M.W. Young. 1995. Rhythmic expression of *timeless:* A basis for promoting circadian cycles in *period* gene autoregulation. *Science* **270:** 808.

Siwicki, K.K., C. Eastman, G. Petersen, M. Rosbash, and J.C. Hall. 1988. Antibodies to the *period* gene product of *Drosophila* reveal diverse tissue distribution and rhythmic changes in the visual system. *Neuron* **1:** 141.

Vosshall, L.B. and M.W. Young. 1995. Circadian rhythms in *Drosophila* can be driven by *period* expression in a restricted group of central brain cells. *Neuron* **15:** 345.

Vosshall, L.B., J. Price, A. Sehgal, L. Saez, and M. Young. 1994. Block in nuclear localization of *period* protein by a second clock mutation, *timeless*. *Science* **263:** 1606.

Yu, Q., Y. Jacquier, Y. Citri, M. Hamblen, J.C. Hall, and M. Rosbash. 1987. Molecular mapping of point mutations in the *period* gene that stop or speed up biological clocks in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci.* **84:** 784.

Zeng , H., P.E. Hardin, and M. Rosbash. 1994. Constitutive overexpression of the *Drosophila period* protein inhibits *period* mRNA cycling. *EMBO J.* **13:** 3590.

Zeng, H., Z. Qian, M.P. Myers, and M. Rosbash. 1996. A light-entrainment mechanism for the *Drosophila* circadian clock. *Nature* **380:** 129.