# A molecular explanation for the long-term suppression of circadian rhythms by a single light pulse

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**Leloup, Jean-Christophe, and Albert Goldbeter.** A molecular explanation for the long-term suppression of circadian rhythms by a single light pulse. *Am J Physiol Regulatory Integrative Comp Physiol* 280: R1206–R1212, 2001.—With the use of a molecular model for circadian rhythms in *Drosophila* based on transcriptional regulation, we show how a single, critical pulse of light can permanently suppress circadian rhythmicity, whereas a second light pulse can restore the abolished rhythm. The phenomena occur via the pulsatile induction of either protein degradation or gene expression in conditions in which a stable steady state coexists with stable circadian oscillations of the limit cycle type. The model indicates that suppression by a light pulse can only be accounted for by assuming that the biochemical effects of such a pulse much outlast its actual duration. We determine the characteristics of critical pulses suppressing the oscillations as a function of the phase at which the rhythm is perturbed. The model predicts how the amplitude and duration of the biochemical changes induced by critical pulses vary with this phase. The results provide a molecular, dynamic explanation for the long-term suppression of circadian rhythms observed in a variety of organisms in response to a single light pulse and for the subsequent restoration of the rhythms by a second light pulse.

circadian clock; rhythm suppression; singularity; *Drosophila*; model

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ONE OF THE MOST INTRIGUING observations on circadian rhythms is that they can be suppressed in a prolonged manner by a single pulse of light. Long-term suppression has been reported for a variety of organisms including insects (33), plants (7), and mammals (12, 16, 19). The abolished rhythm can often be restored by a second light pulse (7, 12). These puzzling observations bear on the very nature of the circadian clock mechanism. Physical models of a nonmolecular nature have been used to account for suppression in terms of return of a limit cycle oscillator to its singularity (7, 17, 18, 34). To get deeper insight into the molecular bases of the phenomenon, it is crucial to account for prolonged suppression in a realistic biochemical model in which the effect of light is incorporated explicitly. We report here that a molecular model for the circadian clock, taking into account the triggering by light of either protein degradation or gene expression, can explain the long-term suppression of circadian rhythms by a single, critical light pulse and the restoration of rhythmicity by another such pulse. The model predicts how the duration and amplitude of the biochemical changes induced by critical pulses vary with the phase at which the rhythm is perturbed.

The most detailed model available for the circadian clock (23–25) is based on experimental observations collected for *Drosophila*; this model (schematized in Fig. 1) relies on negative autoregulation of gene expression (10). A similar feedback mechanism underlies circadian rhythms in other organisms (5) such as *Neurospora* (2), mammals (21, 30), plants (11), and cyanobacteria (14). For definiteness, we will focus on the *Drosophila* clock model, but we shall primarily take it as a tool to assess how a single light pulse can trigger long-term suppression of circadian rhythmicity. Thus we shall consider the cases in which light acts by inducing protein degradation, as in *Drosophila*, or gene expression, as in *Neurospora* and mammals.

## RESULTS

*Molecular model for the circadian clock.* Extending a previous version based on the regulation of *per* alone (8), the clock model (Fig. 1) takes into account nuclear transcription of the *per* and *tim* genes and transport of *per* and *tim* mRNAs into the cytosol, where they are translated into the PER and TIM proteins; the latter are multiply phosphorylated (6, 36) and form a complex that enters the nucleus and represses *per* and *tim* transcription (13, 22, 27, 35, 36). The model incorporates degradation of the PER and TIM proteins and their mRNAs. Negative regulation by the PER-TIM complex involves interaction with the CYC (28) and CLOCK (1) proteins, which are not considered explicitly in this model; incorporation of these two proteins in an extended model preserves oscillatory behavior. Light controls the *Drosophila* clock by triggering TIM degradation (13, 22, 27, 35, 36); the maximum rate of TIM degradation ($v_{dT}$) increases with light, accordingly. In mammals, where *per* and *tim* genes are also found (20, 32), light acts by enhancing the rate of *per* expression ($v_{sP}$) (31). The model is described by a set of
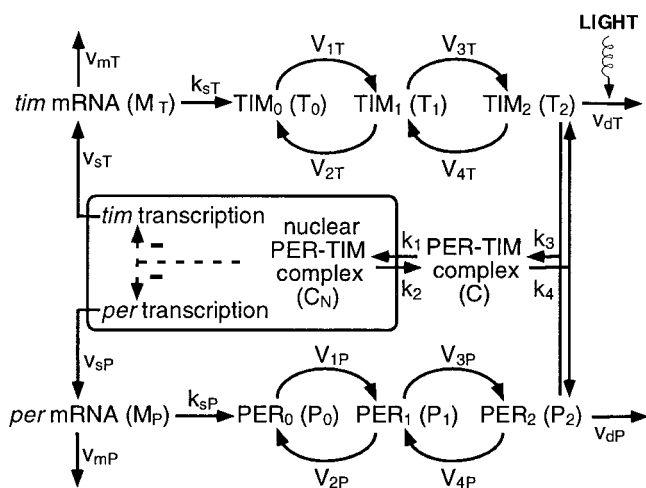
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Fig. 1. Scheme of the model for circadian oscillations in *Drosophila* involving negative regulation of gene expression by PER and TIM (23). *per* ($M_P$) and *tim* ($M_T$) mRNAs are synthesized in the nucleus and transferred into the cytosol, where they accumulate at the maximum rates $v_{sP}$ and $v_{sT}$, respectively; there they are degraded enzymatically at the maximum rates $v_{mP}$ and $v_{mT}$. The rates of synthesis of the PER and TIM proteins, proportional to $M_P$ and $M_T$, are characterized by the apparent first-order rate constants $k_{sP}$ and $k_{sT}$. Parameters $V_{iP}$ ($V_{iT}$) ($i = 1, \ldots 4$) denote the maximum rate of the kinase(s) and phosphatase(s) involved in the reversible phosphorylation of $P_0$ ($T_0$) into $P_1$ ($T_1$) and $P_1$ ($T_1$) into $P_2$ ($T_2$), respectively. The fully phosphorylated forms ($P_2$ and $T_2$) are degraded by enzymes of maximum rate $v_{dP}$, $v_{dT}$, and reversibly form a complex $C$ with association and dissociation rate constants $k_3$, $k_4$. Complex $C$ is transported into the nucleus at a rate characterized by the apparent first-order rate constant $k_1$. Transport of the nuclear form of the PER–TIM complex ($C_N$) into the cytosol is characterized by the apparent first-order rate constant $k_2$. The nuclear PER–TIM complex exerts a negative feedback on *per* and *tim* transcription (see Ref. 23 for further details and for a list of the kinetic equations). In *Drosophila*, light controls the rhythm by enhancing the rate of TIM degradation ($v_{dT}$). In mammals, in which homologous clock genes are at work and in which a similar model might apply, light acts by enhancing the rate of *per* expression ($v_{sP}$).

10 differential equations that govern the time evolution of the concentrations of *per* and *tim* mRNAs and of the various forms of PER and TIM proteins and PER-TIM complex (23, 25). This model accounts for circadian oscillations in continuous darkness, entrainment by light-dark cycles, and phase shifting by light pulses (23–25). Phase shifting by a brief light pulse can only be accounted for by assuming that the biochemical effects triggered by the pulse much outlast its actual duration.

*Coexistence of a stable rhythm with a stable steady state.* Here, we focus on the long-term suppression of circadian rhythmicity by critical light pulses. We will not consider explicitly the light pulse itself. Instead, we will investigate the effect of the pulsatile increase in the light-controlled parameters $v_{dT}$ or $v_{sP}$ that is triggered by the light pulse. Of key importance for suppression is the bifurcation diagram showing the dynamic behavior of the circadian regulatory system as a function of parameters $v_{dT}$ (Fig. 2A) or $v_{sP}$ (Fig. 2B). The diagram of Fig. 2A pertains to the case in which light acts by triggering protein degradation. It represents the dynamic behavior of the oscillatory system by a single state variable, the fully phosphorylated form of

the TIM protein ($T_2$), as a function of $v_{dT}$. At low values of $v_{dT}$, a stable steady state is obtained. As $v_{dT}$ increases, the steady state becomes unstable, and sustained oscillations of the limit cycle type occur. Shown in Fig. 2A is the envelope of oscillations giving the minimum and maximum levels of $T_2$ at different values of $v_{dT}$. Beyond a second bifurcation value, the steady state recovers its stability. For the set of parameter values considered, over a sizeable range of $v_{dT}$ values both to the left and to the right of the steady-state instability domain in Fig. 2A, a stable steady state coexists with a stable limit cycle. These two stable regimes are separated by an unstable limit cycle. Such
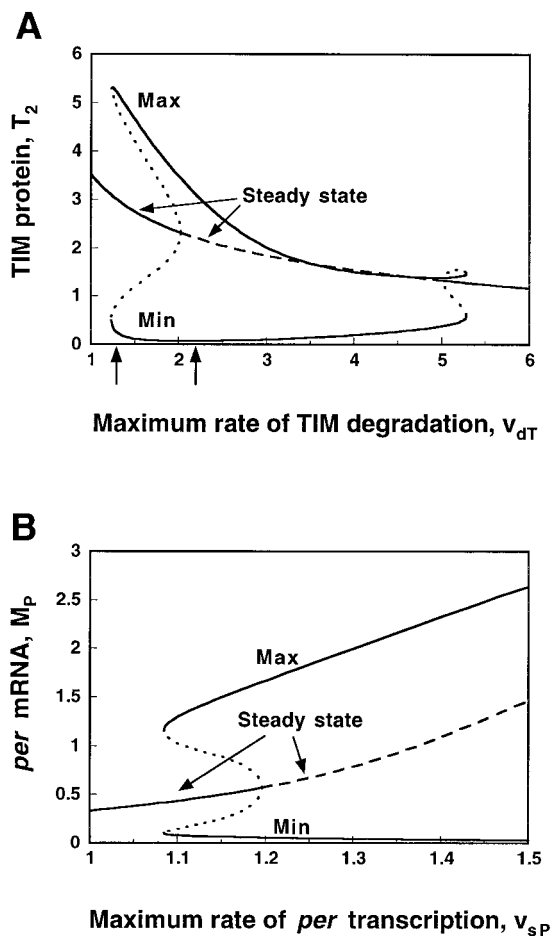


Fig. 2. Bifurcation diagram showing the domain of sustained oscillations as a function of the light-controlled parameter in the molecular model for the circadian clock. The diagrams represent the stable (solid line) or unstable (dashed line) steady-state value of a state variable [the concentration of the phosphorylated TIM form (*A*) or of *per* mRNA (*B*), in nM], as well as its envelope [maximum (Max) and minimum (Min)] in the course of stable (solid line) or unstable (dotted line) sustained oscillations, as a function of $v_{dT}$ (*A*) or $v_{sP}$ (*B*). In *A*, the 1st ($v_{dT} = 1.3$ nM/h) and 2nd arrow ($v_{dT} = 2.2$ nM/h) correspond to conditions exemplifying hard and soft excitation, respectively. In the domain of hard excitation, a stable limit cycle (see Fig. 4A) coexists with a stable steady state; these 2 attractors are separated by an unstable limit cycle. The diagrams are established by determining, by means of the AUTO program (4), the steady state and periodic solutions of the model listed as *equations 1a–1j* in Ref. 23. Parameter values are as in Ref. 23, except $v_{sP} = 1.1$ (for *A*), $v_{dT} = 1.3$ (for *B*), $v_{mP} = 1.0$, $v_{dP} = 2.2$ (all in nM/h), and $k_1 = 0.8$/h.

a situation is referred to as hard excitation (26), because the system in the stable steady state has to be excited by a finite perturbation to evolve to the stable limit cycle. In contrast, when the steady state is unstable, an infinitesimal perturbation suffices to drive the system away from steady state toward the limit cycle (soft excitation). A similar type of bifurcation diagram is obtained as a function of parameter $v_{sP}$ (Fig. 2$B$), for the case in which light acts by triggering gene expression.

*Circadian rhythm suppression by a single light pulse via light-induced protein degradation.* In the simulations, we first consider that a pulse of light results in a pulsatile increase in parameter $v_{dT}$ during a time that generally outlasts the duration of the light pulse itself. The light pulse may indeed trigger the pulsatile synthesis or activation of an enzyme involved in the proteolytic pathway. This enzyme may remain active long after the light stimulus has ended. In the range of $v_{dT}$ values, in which a stable limit cycle surrounds an unstable steady state (for example, for the value indicated by the second arrow in Fig. 2$A$), a pulse of light applied at the appropriate phase with the appropriate duration and magnitude can only suppress the rhythm transiently. Then, indeed, if the finely tuned pulse succeeds in bringing the oscillator in the close vicinity of the singularity (i.e., the steady state), the system will skip a variable number of peaks before returning spontaneously to the limit cycle; the closer the system approaches the steady state, the more delayed is this return.

In contrast, suppression of the rhythm becomes permanent in conditions of hard excitation. Then, as shown in Fig. 3, which corresponds to the $v_{dT}$ value indicated by the first arrow in Fig. 2$A$, when applied at the appropriate phase of the oscillations with appropriate duration and magnitude, a critical light pulse can permanently abolish circadian rhythmicity (Fig. 3, first arrow). Suppression of the rhythm results from the light-induced degradation of TIM that causes a decrease in the protein below a critical level, which

drives the oscillator into the basin of attraction of the stable steady state. An identical pulse (Fig. 3, second arrow) restores the suppressed rhythm; here, the decrease in TIM beyond a critical level brings the system back into the basin of attraction of the stable limit cycle.

To permanently suppress the rhythm by a single light pulse, neither the phase at which it is applied nor the characteristics of the critical perturbation are uniquely defined. As indicated in Fig. 4$A$, the model predicts that permanent suppression by a light pulse can be observed over a large portion of the limit cycle, corresponding roughly to the rising phase of the TIM protein (Fig. 4$B$), between the two black dots. At each of the phases in this portion of the limit cycle, several combinations of the duration and amplitude of the light-induced rise in parameter $v_{dT}$ are capable of permanent suppression. Thus, at a given phase of the cycle, in the amplitude-duration plane for the effect of the light pulse, there exists a domain (rather than a point corresponding to a unique pair of values) in which single critical pulses can abolish the rhythm. The shape of this domain changes as a function of phase: the model predicts (Fig. 4$C$) that as the oscillations progress in the permissive range from the minimum to the maximum level of TIM, the pulses capable of permanently suppressing the rhythm are, at first, those that produce a relatively long but small-amplitude increase in TIM degradation, whereas near the maximum of TIM, successful pulses are those that cause a large-amplitude but briefer increase in this parameter. Relating these predictions to experiments in *Drosophila* will require the quantitative determination of the effect of light pulses of varying intensity and duration on the rate of TIM degradation.

The reason why the suppressive range corresponds roughly to the rise in TIM becomes clearer when comparing the effect of the same pulse given either in the suppressive range (e.g., starting from *point 4* in Fig. 4$A$) or outside this range, after the maximum in TIM (see trajectory starting from the black square in Fig. 4$A$). In the former case, the effect of the pulse goes against the flow as it tends to reduce TIM at a time when the protein level is increasing. The system is thus pulled back and, as a result, evolves toward the inside of the limit cycle where it is captured by the attracting stable steady state. The level of *tim* mRNA, although high at the beginning of the pulse, cannot counteract the light-induced decrease in TIM and is also pulled to its steady-state value (see Fig. 4$A$). When the same pulse is given after the maximum in TIM, when the protein level has started to decrease, its effect accompanies the flow so that the decrease in TIM is larger than in the previous case; the system moves out of the limit cycle but returns to it asymptotically. In such a case, the perturbation does not suppress the rhythm but merely causes a phase shift.

*Circadian rhythm suppression via light-induced gene expression.* In view of experimental observations (31), circadian rhythm suppression by light pulses in mammals probably involves light-induced transcription rather than protein degradation. To address such a
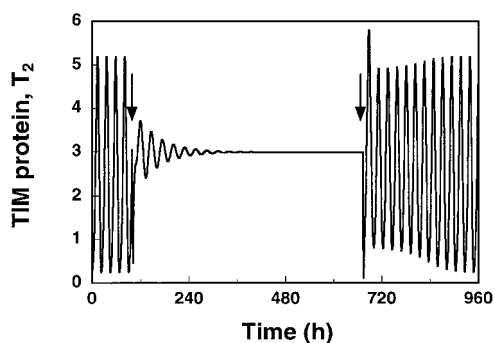


Fig. 3. Permanent rhythm suppression by a single, critical pulse of light in the circadian clock model and restoration of the rhythm by a similar pulse. At the time indicated by the 1st arrow, to mimic the effect of a light pulse, parameter $v_{dT}$ is increased during 2 h from the basal value of 1.3 nM/h up to 4.0 nM/h. Initial conditions correspond to *point 4* in Fig. 4, $A$ and $B$. At the time indicated by a 2nd arrow, a similar change in $v_{dT}$, mimicking a 2nd light pulse, is initiated. The curve is obtained by numerical integration of the model equations (23) for the parameter values of Fig. 2.

possibility in the present model, assuming that it holds for mammals, in which the same clock genes are present (20, 32), we have checked whether permanent suppression can occur in conditions of hard excitation in Fig. 2B, solely as a result of a transient increase in *per* transcription in response to a light pulse (31), in the absence of light-induced TIM degradation. Here, in the simulations, we implement the effect of a light pulse by increasing parameter $v_{sP}$ in a pulsatile man-
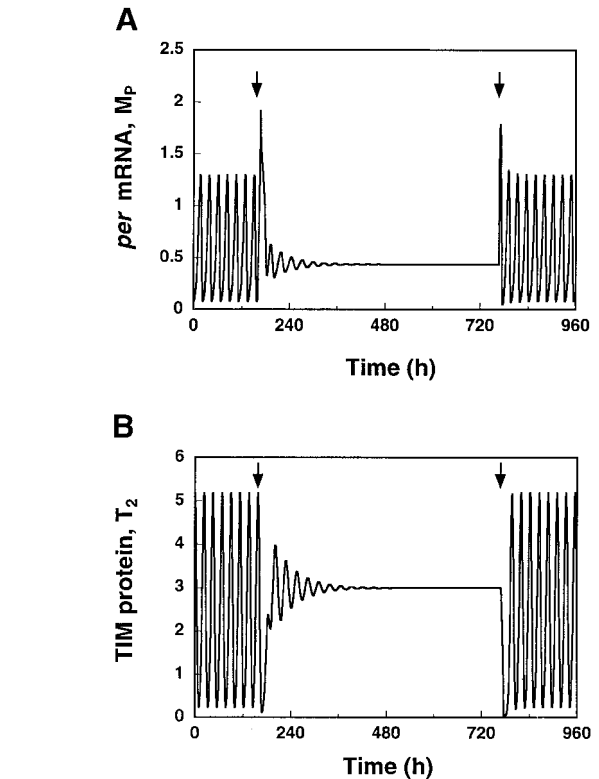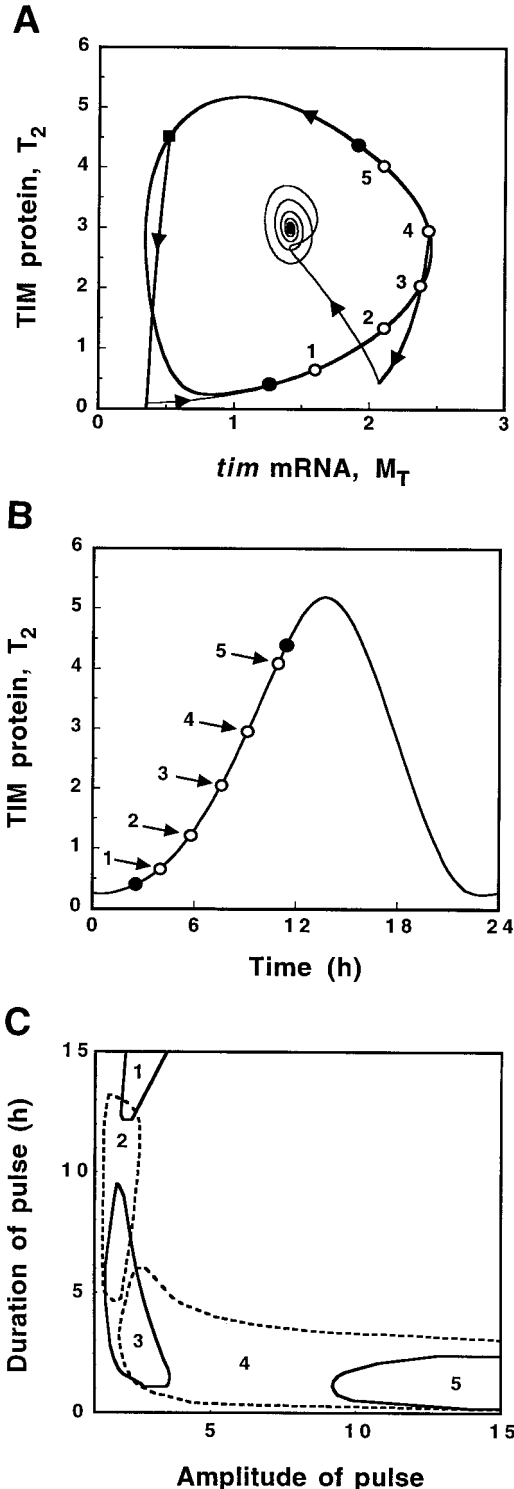
**A**



**B**



**C**



Fig. 5. Long-term suppression of circadian rhythmicity by pulsatile gene expression. In *A*, the oscillations in *per* mRNA are suppressed by a critical pulse in *per* expression (1st arrow) effected by a pulsatile increase in parameter $v_{sP}$, which is increased from a basal value of 1.1 nM/h up to 2.2 nM/h during 7.5 h. The initial conditions correspond to *point 3* in the 3 panels of Fig. 6. A second, similar pulse (2nd arrow) restores the oscillations. The associated time variation in TIM protein ($T_2$) is shown in *B*. Parameter values are as in Fig. 3 with $v_{dP}$ = 1.3 nM/h.

ner during a time that may exceed the duration of the triggering light pulse. The suppression of circadian oscillations of *per* mRNA by a critical pulse of *per* expression is demonstrated in Fig. 5*A* (1st arrow); restoration of the rhythm by a second such pulse can also occur (Fig. 5*A*, 2nd arrow). The changes in $T_2$

Fig. 4. Effects of phase on rhythm suppression through light-induced TIM degradation. *A*: single critical pulses can permanently suppress the rhythm when applied over the portion of the limit cycle (closed arrowed curve shown as projection onto the plane formed by the concentrations of phosphorylated TIM and *tim* mRNA) bounded by the 2 black dots. As shown in *B*, this portion corresponds to the rising phase of TIM. The trajectory starting from *point 4* corresponds to the rhythm suppression by a critical light pulse, shown in Fig. 3. The other trajectory starts at a point (■) located after the TIM maximum; the same stimulus used in Fig. 3 fails to suppress the rhythm, and the system returns to the limit cycle. Shown in *A* and *B* are 5 points of the limit cycle, marked 1–5, for which characteristics of suppressing pulses were determined. The 5 domains in *C*, within which permanent suppression is observed, correspond to these points. The domains, determined by numerical simulations using the parameter values of Fig. 2*A* (1st arrow), show the amplitude and duration of the light-induced increase in $v_{dT}$, which cause permanent suppression of the rhythm. Qualitatively similar results were obtained for other basal values of $v_{dT}$ in the domain of hard excitation in Fig. 2*A*. Amplitude is defined as the ratio of the light-induced value of $v_{dT}$ divided by the basal value (i.e., the value before the pulse). Concentrations in *A* and *B* are in nM.

associated with the suppression and subsequent restoration of circadian rhythmicity by critical pulses of *per* expression are shown in Fig. 5*B* (1st and 2nd arrow, respectively).

Permanent suppression of the rhythm resulting from the pulsatile expression of *per* can occur over a wide range of phases, corresponding to the portion of the limit cycle bounded by the two empty circles in Fig. 6*A*, that extends from the maximum of *per* mRNA to a
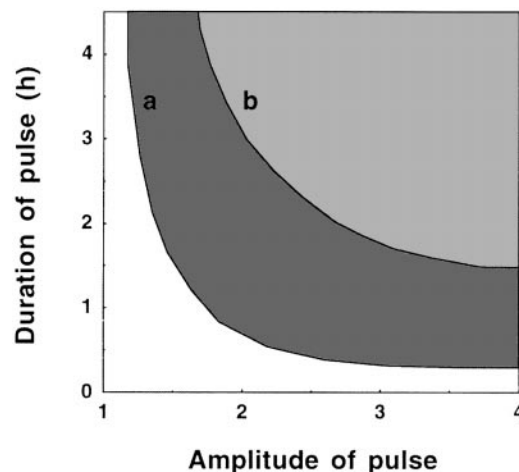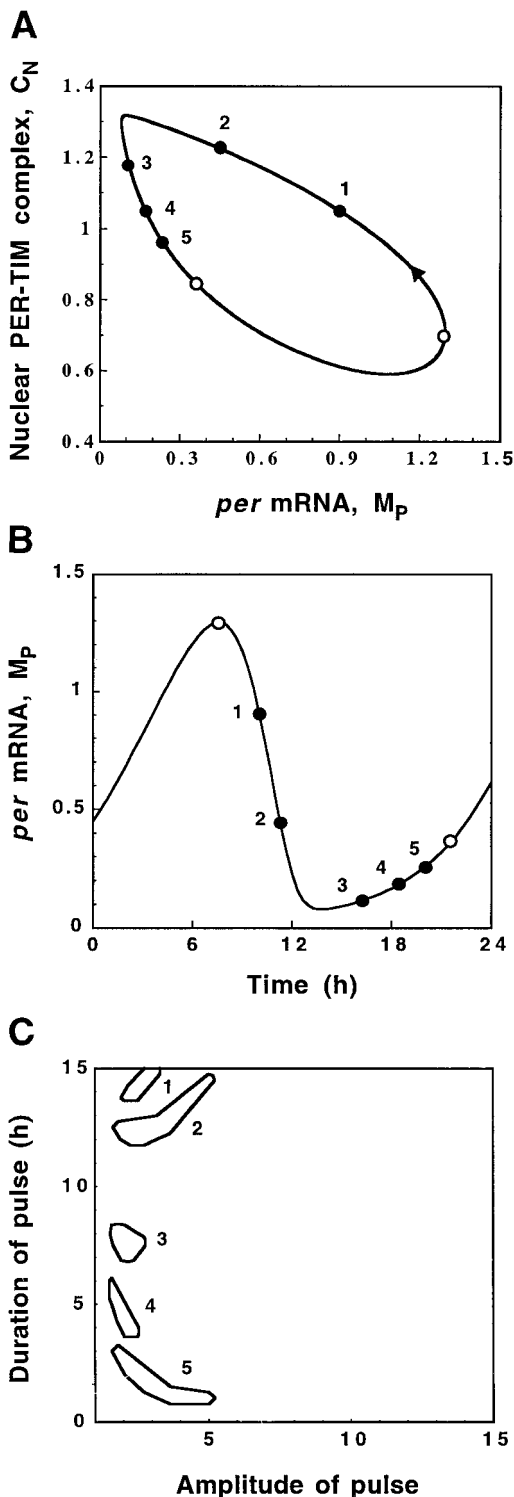
**A**



**B**



**C**





**Fig. 7.** Duration and amplitude of pulses restoring circadian oscillations. When the oscillations are suppressed and the system is at steady state, return to the limit cycle via a light-induced rise in parameter $v_{sP}$ measuring *per* expression occurs when the pulse characteristics correspond to a point above the lower boundary (*curve a*). Restoration of rhythmicity via a light-induced rise in parameter $v_{dT}$ occurs when the pulse characteristics correspond to a point above the upper boundary (*curve b*). Parameter values are as in Figs. 3–6; the amplitude of the pulse in $v_{dT}$ or $v_{sP}$ is defined as in Figs. 4 and 6, respectively.

point located beyond the trough (Fig. 6*B*). The shape of the domain of suppressing pulses in the amplitude-duration plane again changes with phase (Fig. 6*C*). Compared with Fig. 4*C*, however, as the phase changes, the duration of these pulses varies more than their amplitude. The domains of suppressing pulses in Fig. 6*C* are also smaller than those observed in Fig. 4*C*. Permanent suppression by light-induced gene expression might thus require finer tuning than suppression by light-induced protein degradation.

In contrast, restoration of the abolished rhythm appears to be more easy to achieve when the light pulse induces gene expression rather than protein degradation. In the former case, the return to the limit cycle occurs for changes in biochemical parameters of relatively shorter duration and amplitude (compare *curves a* and *b* in Fig. 7). More generally, it is less arduous to restore the rhythm than to suppress it, because it is enough for the pulse to exceed a critical duration, at a given suprathreshold amplitude, for the rhythm to resume.

**Fig. 6.** Effect of phase on rhythm suppression by pulsatile light-induced transcription of *per*. *A*: single critical pulses can permanently suppress the rhythm when applied over the portion of the limit cycle bounded by the 2 empty circles. Shown in this portion are 5 points marked 1–5, for which characteristics of suppressing pulses were determined. The 5 points are within the range extending from the maximum in *per* mRNA to slightly beyond the trough in this variable (*B*). The 5 domains in *C*, within which permanent suppression is observed, correspond to these points. The domains, determined by numerical simulations using the parameter values of Fig. 2*B*, show the amplitude and duration of the light-induced increase in $v_{sP}$ that cause permanent suppression of the rhythm. Amplitude is defined as the ratio of the light-induced value of $v_{sP}$ divided by the value (1.1 nM/h) before the pulse. Concentrations in *A* and *B* are in nM.

## DISCUSSION

The present results account, in terms of a realistic molecular mechanism, for the long-term suppression of circadian rhythms by a single light pulse. The phenomenon has been observed for the *D. pseudoobscura* circadian rhythm of pupal eclosion (33), for the circadian rhythm of petal movement in *Kalanchoe* (7), and for circadian rhythms in hamster (19), chipmunk (12), and human (16). The results also account for the restoration of these rhythms by a similar light pulse (7, 12). The link between suppression and the situation in which stable oscillations coexist with a stable steady state was already made when circadian rhythms were first related to limit cycle behavior (17). The suppression phenomenon in *Kalanchoe* was later accounted for in terms of hard excitation by means of the Van der Pol oscillator model borrowed from the physical literature (7). The present report provides a first instance in which permanent rhythm suppression by a critical light pulse occurs in a realistic molecular model for a circadian clock. This phenomenon eludes sheer intuition and can only be explained by means of a theoretical model.

The present explanation of long-term suppression of circadian rhythmicity differs from the alternative explanation based on a putative desynchronization of circadian pacemakers following perturbation by the light pulse (12, 34). Here, suppression results from the pulse-induced transition occurring in all pacemaker cells from a stable oscillatory regime to a stable steady state. Ultradian illustrations of an analogous phenomenon have been reported for squid axon membranes (9) and cardiac tissue (15), in which repetitive firing was suppressed by a brief depolarizing or hyperpolarizing current pulse, respectively.

The analysis of the model suggests that long-term suppression of circadian rhythms by critical light pulses should not necessarily be observed in all organisms. Whether suppression possesses a permanent or only transient nature will depend on whether the regulatory network controlling the circadian clock operates in conditions in which a stable limit cycle coexists with a stable singularity. Transient and permanent suppression differ in two respects. In the former case, the rhythm resumes by itself without external intervention; in the latter, restoration of the rhythm requires a new pulse of light. Permanent suppression is also more robust, i.e., less difficult to achieve, because the pulse has to bring the oscillator anywhere into the basin of attraction of the steady state rather than in its close vicinity.

Until systematic experiments are done over a wide range of light-pulse amplitudes and durations, the question remains as to whether permanent suppression of the locomotor activity rhythm can occur in *D. melanogaster*. Suppression of circadian rhythms has been linked to type 0 phase-response curves (PRC) associated with strong resetting to a single phase (33, 34). Such a PRC has been obtained in *D. melanogaster* for 6 h light stimuli, whereas a low-amplitude type 1 PRC showing only moderate phase advances or delays was obtained for a 1-h light pulse of similar intensity (29). The model can account for both types of PRC depending on the duration and magnitude of the pulse of light-induced TIM degradation. The fact that the experiments on the type 0 PRC showed phase shifts rather than long-term suppression of the locomotor activity rhythm in *D. melanogaster* (29) would suggest that the latter phenomenon does not occur in this organism, but the pulses used may have missed the domains of permanent suppression shown in Fig. 4*C*.

Although they were obtained in a model based on the molecular mechanism of the *Drosophila* clock, the results bear on light-induced suppression of circadian rhythms in other organisms. The negative autoregulatory feedback loop that forms the core of the oscillatory mechanism in *Drosophila* is indeed observed in mammals, in which homologs of the *Drosophila* clock genes are found (20, 32), and in *Neurospora*, in which the *frq* gene is negatively regulated by its protein product FRQ (2, 5). Mechanistic differences between flies, fungi, and mammals exist: for example, the role of TIM in mammals may differ from that seen in *Drosophila* (5). Moreover, in *Neurospora* (3) and mammals (31), light triggers transcription instead of protein degradation. The general significance of our results is nevertheless supported by the fact that hard excitation is a robust phenomenon in models for this and other nonlinear regulatory systems and by the finding that the long-term suppression and subsequent restoration of circadian rhythms by a critical perturbation can occur via pulsatile protein degradation or pulsatile gene expression.

The present results also indicate that long-term suppression of circadian rhythms should also be observable in *Drosophila* and other organisms by directly triggering *per* or *tim* expression. The pulsatile perturbation silencing rhythmicity in Fig. 6 may indeed be obtained either with light, in organisms in which it triggers transcription, or by means of a promoter inducing gene expression.

### Perspectives

Few phenomena in physiology remain as puzzling as the long-term suppression of circadian rhythmicity by a single light pulse and the subsequent restoration of the rhythm by a second pulse. Explanation of these observations escapes sheer intuition and has therefore much to gain from a modeling approach. One commonly invoked scenario for suppression rests on the pulse-induced desynchronization of oscillators responsible for circadian rhythmicity. An alternative mechanism investigated here involves the pulse-triggered transition of pacemaker cells from a stable oscillatory regime into a stable steady state in conditions in which these two states coexist. Restoration of the suppressed rhythm by a second light pulse involves the reverse transition from the stable steady state to stable oscillations. Although the theoretical principle of such an explanation is not novel, this study provides its first implementation based on a detailed molecular mechanism for circadian rhythms. The effects of light pulses

are mediated through induction of either protein degradation or gene expression. By providing an explicit mechanism in terms of complex dynamical processes at the molecular level for a behavioral response that largely stands as a physiological enigma, the present results yield a striking application of concepts from nonlinear dynamics to biology. They also give a clear-cut example of how theoretical models closely related to experiments may contribute new, counterintuitive insights that could not have been reached without resorting to a modeling approach.

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