Why no discussion? (A) MG

Circadian Rhythms of PER and TIM Proteins in *Drosophila*

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Molecular and Cell Biology 137
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Introduction:

Living organisms must adapt to changes in their environment. Animals, for example, change seasonally by shedding fur, acquiring fat, hibernating, or migrating. Such seasonal adaptations repeat every year.

In addition to annual adaptations, animals also exhibit daily behavioral patterns. Humans, for example, generally tend to feel fatigued when their surroundings are dark and energetic when their surroundings are light. Humans therefore tend to be active during the day and sleep at night. The fundamental molecular cause of these daily behavioral patterns is repetitive fluctuations of proteins.

Protein levels often oscillate because of feedback inhibition. To illustrate how feedback works, consider the hypothetical reaction involving proteins A, B, and C:

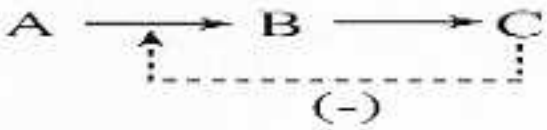


Figure 1: A hypothetical reaction of proteins A, B, and C.

Negative feedback occurs when protein C inhibits the transformation of A to B. An increase in the amount of A will first lead to an increase in B, then to an increase in C. But high levels of C will prevent the formation of B, thus leading eventually to a decrease in C. With less C, feedback inhibition decreases, allowing more B to form. Increased B levels then lead to more C, causing the cycle the repeat again. The end result is that these proteins oscillate rhythmically. If the period of these oscillations is 24 hours, the organism containing these proteins is said to experience *circadian rhythms*.

The proteins involved in circadian rhythms are not yet understood in all animals. In *Drosophila*, however, scientists have known for several decades that these rhythms involve the *period protein* ("PER"). It was once believed that PER was this system's only oscillatory protein but recent discoveries have revealed that another protein, *timeless* ("TIM"), behaves similarly.

It is now believed that daily fluctuations of PER and TIM are the primary cause of *Drosophila's* circadian rhythms. Though the exact mechanism of their interaction has yet to be deciphered, a theoretical model that agrees with experimental evidence has been constructed (figure 1):

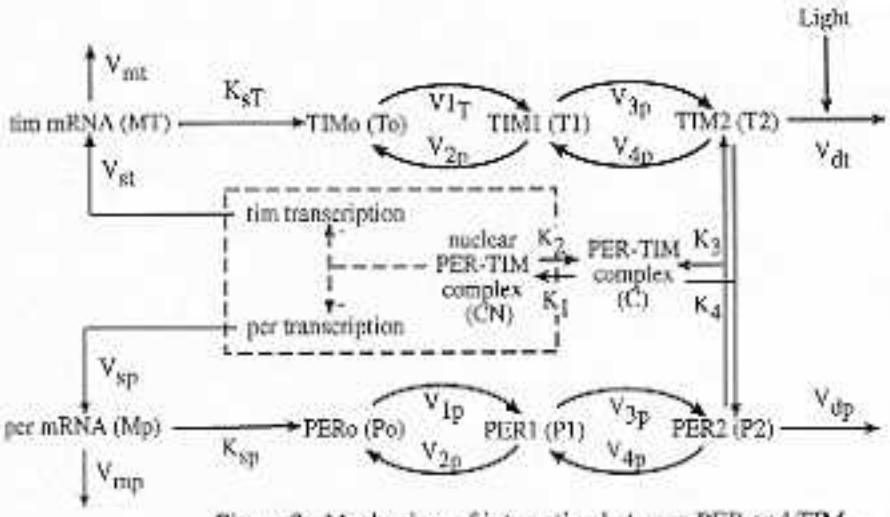


Figure 2: Mechanism of interaction between PER and TIM.

In this diagram, the PER and TIM genes are first transcribed in the nucleus. The resulting mRNA (M_P & M_T) is then transported into the cytosol where it is translated into PER and TIM protein (P_o & T_o). PER and TIM are then each phosphorylated two times, first forming monophosphorylated PER and TIM (P_1 & T_1), then hiphosphorylated PER and TIM (P_2 & T_2). These proteins then interact to form a PER-TIM complex (C) that is transported into the nucleus (C_N). This nucleic complex then behaves as a transcription factor, directly inhibiting mRNA formation through negative feedback.

It should be noted that the PER-TIM complex might not directly inhibit transcription. It is possible that the complex activates an intermediate protein that acts as an inhibitor. Also, it is possible that PER and TIM are phosphorylated more than twice. It has even been proposed that phosphorylation does not occur until after the PER-TIM complex forms. Numerous other arguments exist that are contrary to the mechanism proposed in figure 2.

This project's purpose is not to debate the mechanism of PER-TIM interaction. While it is true that intermediates might form, additional phosphorylation steps might occur, and phosphorylation might happen later than expected, these facts are unimportant to this project. They unnecessarily complicate this theoretical model without adding any substantial insight. This project will therefore adhere to the mechanism proposed in figure 2 because it is the simplest arrangement that is capable of producing values that agree with experimental observations.

Equations:

The first equation describes the change in the amount of PER mRNA (M_P):

$$dM_P/dt = v_{sp}K_{IP}^n/(K_{IP}^n+C_N^n) - v_{mp}M_P/(K_{mP}+M_P) - k_dM_P \qquad (1)$$

The first term refers to feedback inhibition by the nucleic PER-TIM complex (C_N) on M_P synthesis. If C_N increases, the model predicts that feedback inhibition will increase, resulting in decreased M_P formation. This term confirms this prediction because if C_N

becomes infinite, M_P formation approaches zero in this term. Similarly, the model predicts that a decrease in C_N causes a decrease in feedback inhibition, resulting in increased M_P synthesis. This condition is also satisfied because if C_N approaches zero, this term approaches its maximum rate v_{sp}. The second term describes a Michaelis-Menten relationship between a degradation enzyme and M_P. Notice that as Mp becomes infinite, enzymatic degradation approaches its maximum rate v_{mP}. When M_P approaches zero, the rate does, too. The third term refers to non-enzymatic M_P degradation that follows first-order kinetics, increasing linearly in magnitude with M_P.

The second equation describes the change in the amount of unphosphorylated PER protein (P_0):

$$dP_0/dt = k_{sP}M_P - v_{1P}P_0/(K_{1P}+P_0) + v_{2P}P_1/(K_{2P}+P_1) - k_dP_0 \quad (2)$$

The first term describes the formation of P_0 from PER mRNA (M_P). Notice that there is a linear (first-order) relationship between M_P and P_0 synthesis. The second term describes PER phosphorylation. This Michaelis Menten expression is negative because P_0 is consumed in this transformation. The third term is analogous to the second but it describes the formation of P_0 (from monophosphorylated PER protein P_1) and is therefore positive. The final term is analogous to the final term in equation 1.

The third equation describes the change in the amount of monophosphorylated PER protein (P_1):

$$dP_1/dt = v_{1P}P_0/(K_{1P}+P_0) - v_{2P}P_1/(K_{2P}+P_1) - v_{3P}P_1/(K_{3P}+P_1) + v_{4P}P_2/(K_{4P}+P_2) - k_dP_1 \quad (3)$$

The first term is the same as the second term in equation 2 but is positive because it describes an increase in P_1. The second term is the same as the third term in equation 2 but is negative because it requires P_1 consumption. The third and fourth Michaelis Menten expressions are analogous to those found in previous equations; the former is negative because it describes P_1 consumption while the latter is positive because it describes P_1 production. The fifth term is analogous to previously described first-order decay expression.

The fourth equation describes the change in the amount of biphosphorylated PER protein (P_2):

$$dP_2/dt = v_{3P}P_1/(K_{3P}+P_1) - v_{4P}P_2/(K_{4P}+P_2) - k_3P_2T_2 + k_4C - v_{dP}P_2/(K_{dP}+P_2) - k_dP_2 \quad (4)$$

The first and second terms are the same as the third and fourth terms in equation 3 but opposite in sign. The third term describes second-order cytosolic PER-TIM complex (C) formation. It is negative because it requires P_2 consumption. The fourth term is positive because it describes first-order dissociation of C, a process that releases P_2. The fifth and sixth terms represent decay; the former is a Michaelis Menten expression because it involves enzymatic degradation while the latter follows first-order kinetics.

The fifth equation:

$$dM_T/dt = v_{sT}K_{IT}^n/(K_{IT}^n+C_N^n) - v_{mT}M_T/(K_{mT}+M_T) - k_dM_T \quad (5)$$

Is analogous to equation 1 but describes the change in the amount of TIM mRNA (M_T) instead of PER mRNA (M_P).

The sixth equation:

$$dT_o/dt = k_{sT}M_T - v_{1T}T_o/(K_{1T}+T_o) + v_{2T}T_1/(K_{2T}+T_1) - k_dT_o \quad (6)$$

Is analogous to equation 2 but describes the change in the amount of TIM protein (T_o) instead of PER protein (P_o).

The seventh equation:

$$dT_1/dt = v_{1T}T_o/(K_{1T}+T_o) - v_{2T}T_1/(K_{2T}+T_1) - v_{3T}T_1/(K_{3T}+T_1) + v_{4T}T_2/(K_{4T}+T_2) - k_dT_1 \quad (7)$$

Is analogous to equation 3 but describes the change in the amount of monophosphorylated TIM protein (T_1) instead of monophosphorylated PER protein (P_1).

The eighth equation:

$$dT_2/dt = v_{3T}T_1/(K_{3T}+T_1) - v_{4T}T_2/(K_{4T}+T_2) - k_3P_2T_2 + k_4C - v_{dT}T_2/(K_{dT}+T_2) - k_dT_2 \quad (8)$$

Is analogous to equation 4 but describes the change in the amount of biphosphorylated TIM protein (T_2) instead of biphosphorylated PER protein (P_2).

The ninth equation describes the change in the amount of cytosolic PER-TIM complex (C):

$$dC/dt = k_3P_2T_2 - k_4C - k_1C + k_2C_N - k_{dC}C \quad (9)$$

The first term, which also appears in equations 4 and 8, describes the second-order formation of C from biphosphorylated PER protein (P_2) and biphosphorylated TIM protein (T_2). The second term, also appearing in equations 4 and 8, describes the first-order dissociation of C. The third term describes first-order transport of PER-TIM complex from the cytosol to the nucleus, while the fourth term describes transport from the nucleus to the cytosol. Notice that these two expressions have different signs because they have opposing directionalities. The final term is analogous to previous first-order decay expressions.

The tenth equation describes the change in the amount of nucleic PER-TIM complex (C_N):

$$dC_N/dt = k_1C - k_2C_N - k_{dN}C_N \quad (10)$$

The first and second terms are the same as the third and fourth terms in equation 9 but their signs are reversed. The final term is analogous to previous expressions of first-order decay.

The eleventh equation is the sum of all proteins containing PER. It therefore represents the total amount of PER protein in the system:

$$P_t = P_o + P_1 + P_2 + C + C_N \quad (11)$$

The twelfth equation represents the total amount of TIM protein in the system:

$$T_t = T_o + T_1 + T_2 + C + C_N \quad (12)$$

List of Equations:

$$dM_P/dt = v_{sP}K_{IP}^n/(K_{IP}^n + C_N^n) - v_{mP}M_P/(K_{mP}+M_P) - k_dM_P \quad (1)$$

$$dP_o/dt = k_{sP}M_P - v_{1P}P_o/(K_{1P}+P_o) + v_{2P}P_1/(K_{2P}+P_1) - k_dP_o \quad (2)$$

$$dP_1/dt = v_{1P}P_o/(K_{1P}+P_o) - v_{2P}P_1/(K_{2P}+P_1) - v_{3P}P_1/(K_{3P}+P_1) + v_{4P}P_2/(K_{4P}+P_2) - k_dP_1 \quad (3)$$

$$dP_2/dt = v_{3P}P_1/(K_{3P}+P_1) - v_{4P}P_2/(K_{4P}+P_2) - k_3P_2T_2 + k_4C - v_{dP}P_2/(K_{dP}+P_2) - k_dP_2 \quad (4)$$

$$dM_T/dt = v_{sT}K_{IT}^n/(K_{IT}^n + C_N^n) - v_{mT}M_T/(K_{mT}+M_T) - k_dM_T \quad (5)$$

$$dT_o/dt = k_{sT}M_T - v_{1T}T_o/(K_{1T}+T_o) + v_{2T}T_1/(K_{2T}+T_1) - k_dT_o \quad (6)$$

$$dT_1/dt = v_{1T}T_o/(K_{1T}+T_o) - v_{2T}T_1/(K_{2T}+T_1) - v_{3T}T_1/(K_{3T}+T_1) + v_{4T}T_2/(K_{4T}+T_2) - k_dT_1 \quad (7)$$

$$dT_2/dt = v_{3T}T_1/(K_{3T}+T_1) - v_{4T}T_2/(K_{4T}+T_2) - k_3P_2T_2 + k_4C - v_{dT}T_2/(K_{dT}+T_2) - k_dT_2 \quad (8)$$

$$dC/dt = k_3P_2T_2 - k_4C - k_1C + k_2C_N - k_{dC}C \quad (9)$$

$$dC_N/dt = k_1C - k_2C_N - k_{dN}C_N \quad (10)$$

$$P_t = P_o + P_1 + P_2 + C + C_N \quad (11)$$

$$T_t = T_o + T_1 + T_2 + C + C_N \quad (12)$$

Results:

When these twelve equations are run on Madonna the resulting graph is convoluted and difficult to interpret. An understanding of the results is facilitated if the curves are compared two at a time. For example, the model predicts that PER and TIM mRNA (M_P & M_T) must be present before unphosphorylated PER and TIM protein (P_o & T_o) because mRNA is required for protein synthesis. The graph in figure 3 confirms this prediction:

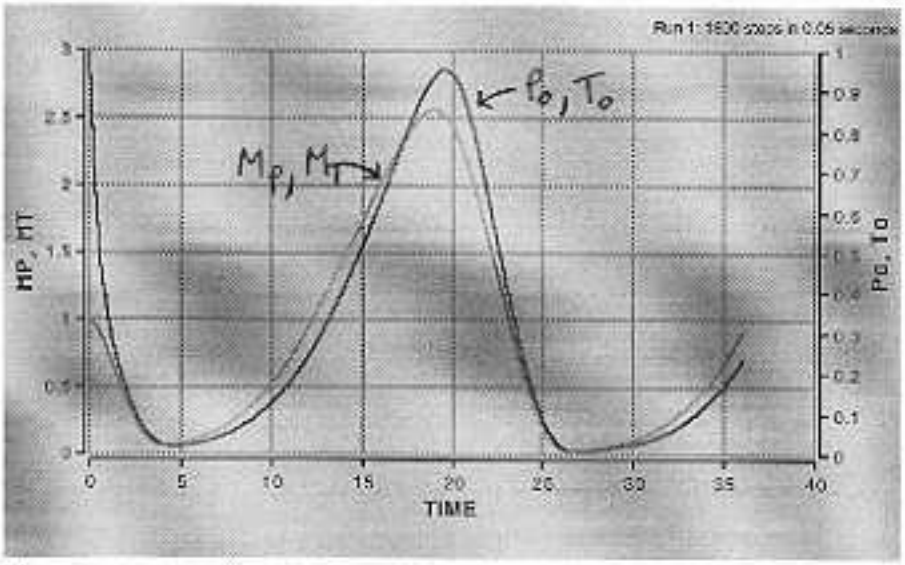


Figure 3: A comparison of PER and TIM mRNA (M_P & M_T) and unphosphorylated PER and TIM protein (P_o & T_o). Notice that protein changes lag slightly behind changes in mRNA.

The model also predicts that unphosphorylated PER and TIM (P_o & T_o) must be present before phosphorylated PER and TIM (P_1 & T_1) because protein must first be present before a phosphate can be added. This prediction is supported by figure 4:

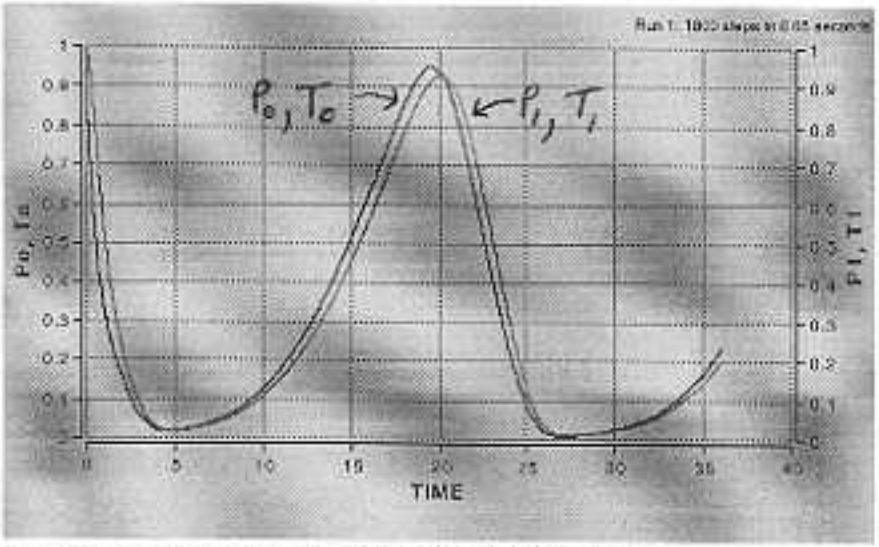


Figure 4: A comparison of unphosphorylated (P_o & T_o) and monophosphorylated (P_1 & T_1) proteins. Notice that P_1 & T_1 lag slightly behind Po & To.

The model also predicts that the behavior of monophosphorylated PER and TIM (P_1 & T_1) precedes that of biphosphorylated PER and TIM (P_2 & T_2). This prediction is supported by figure 5:

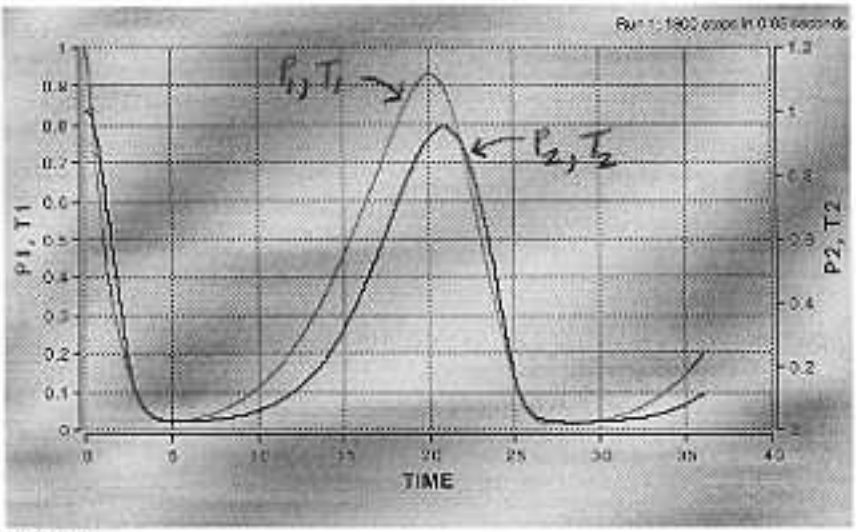


Figure 5

The prediction that the behavior of biphosphorylated protein (P_2 & T_2) precedes that of cytosolic PER-TIM complex (C) is supported by figure 6:

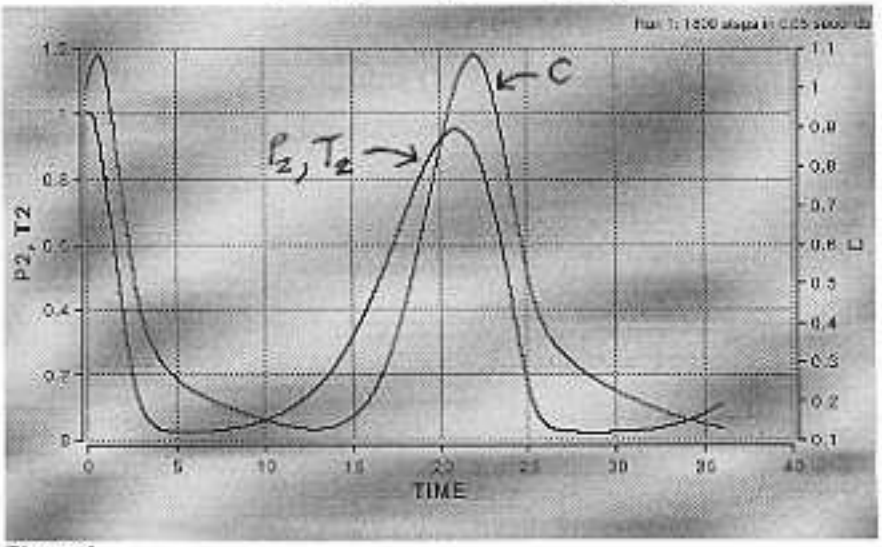


Figure 6

The prediction that the behavior of the cytosolic PER-TIM complex (C) precedes that of the nucleic PER-TIM complex (C_N) is supported by figure 7:

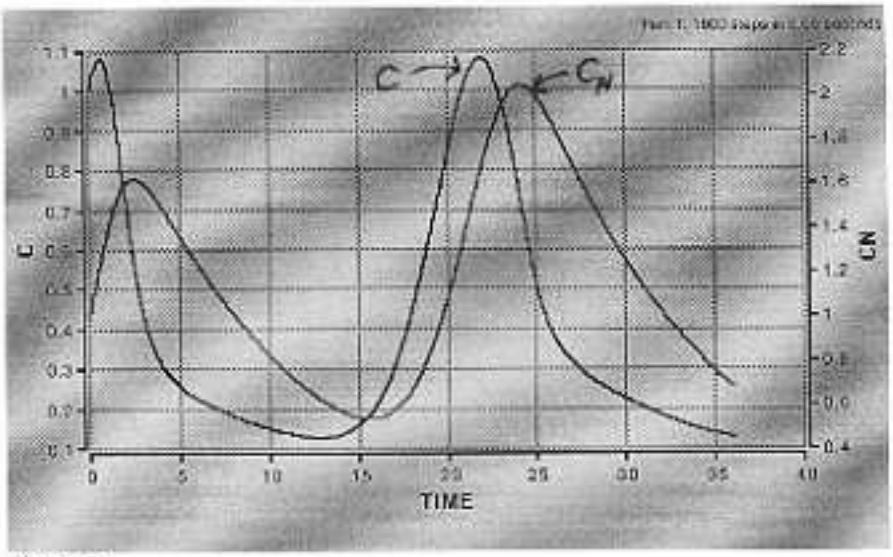


Figure 7

After examining each parameter separately, it is easier to understand a graph of these variables together (figure 8):

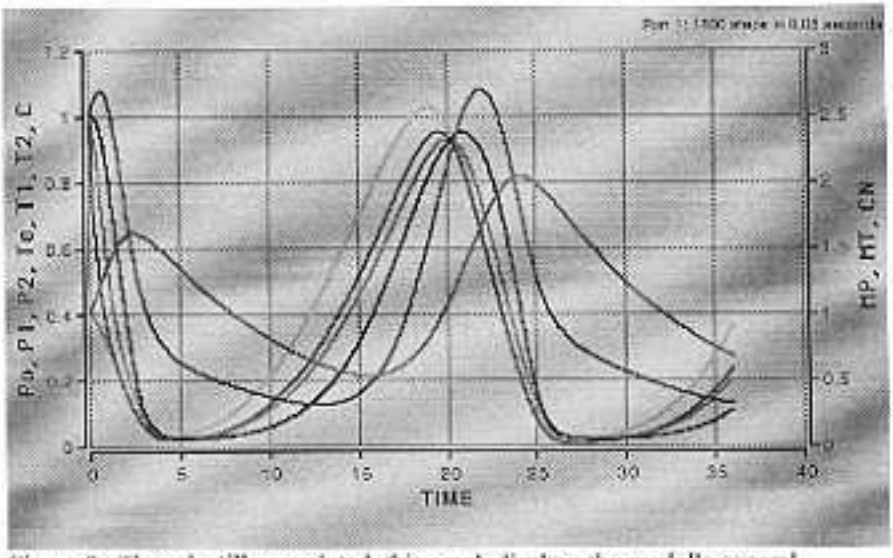
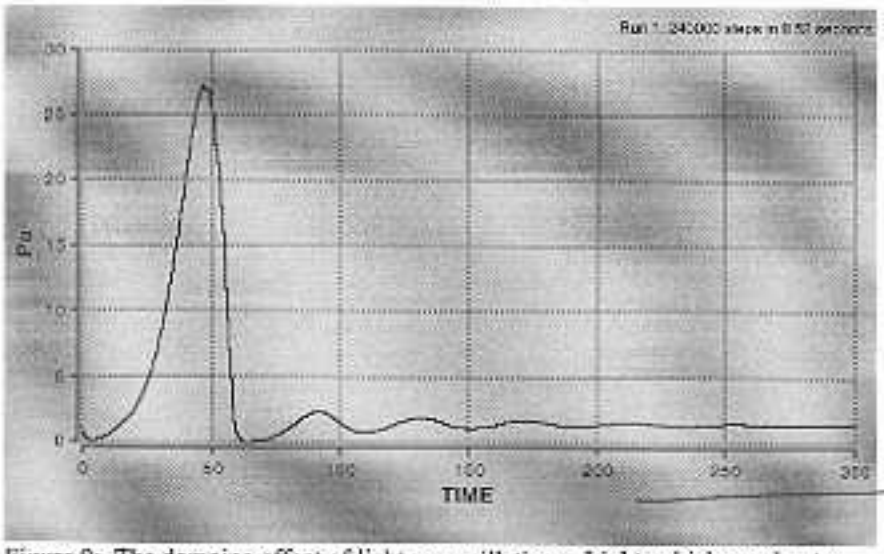


Figure 8: Though still convoluted, this graph displays the model's general sequence of events. First the PER and TIM genes are transcribed into mRNA (M_P & M_T) which is then translated into protein (P_0 & T_0). These proteins are then first phosphorylated to P_1 and T_1, then phosphorylated again to P_2 and T_2. The resulting biphosphorylated proteins form a cytosolic complex (C) that is then transported into the nucleus (C_N).

Graphs 1-8 are good checks but too obvious.

The previous graphs describe behavior at normal conditions. When environmental factors are adjusted, however, these values shift to compensate. If, for example, the system is exposed to light, biphosphorylated TIM (T_2) decay accelerates.

The resulting shortage of TIM decreases the amount of PER-TIM complex, weakening negative feedback. Without substantial feedback inhibition (the fundamental cause of periodic behavior in this system), oscillations stop.



units

units?

Figure 9: The damping effect of light on oscillations. Light, which accelerates TIM decay, is mimicked by increasing v_{dT} to 300 nMh^{-1}.

Oscillations will also stop in constant darkness. The reason is that decreased TIM decay increases the amount of biphosphorylated TIM (T_2), thus increasing the amount of PER-TIM complex (C). Negative feedback becomes so strong that transcription eventually approaches zero. Without new mRNA, protein is not produced, so oscillations are unable to continue.

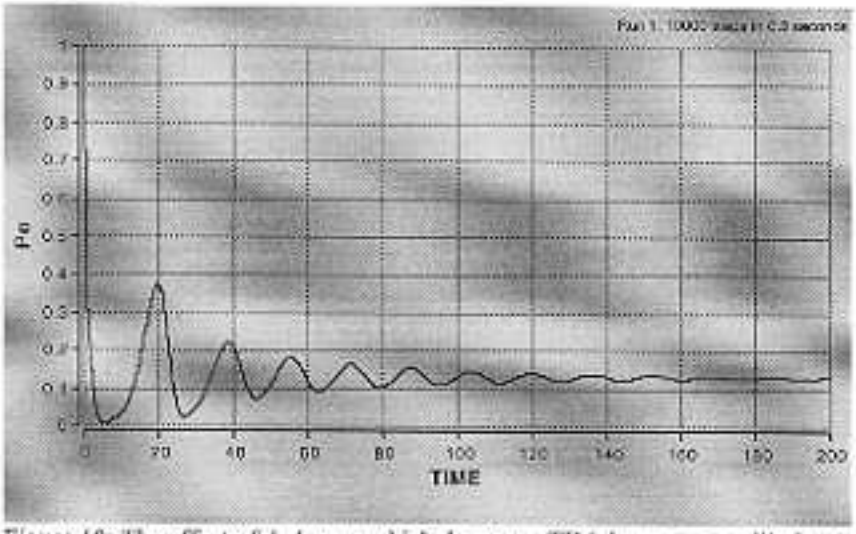


Figure 10: The effect of darkness, which decreases TIM decay, on oscillations. Darkness is mimicked by decreasing v_{dT} to .4 nMh^{-1}.

Altering the rate constants for PER-TIM complex formation (k_3) and dissociation (k_4) also affects oscillatory behavior. If, for example, k_3 decreases to zero, PER-TIM complex (C) will not form. In the absence of C, there is no negative feedback so

oscillations are impossible. Similarly, if k_4 decreases to zero, C will be unable to dissociate. The hyper-stable C will exert such strong negative feedback that mRNA will not form. Without mRNA, protein cannot be translated, thus preventing oscillatory behavior.

Altering k_3 and k_4 does not always have such profound consequences. A moderate decrease in k_4 causes C to become "sticky" (more time is required for it to dissociate). The complex's increased life-span causes it to perform feedback inhibition for a longer duration, thus slowing the nucleus' production of mRNA. This delays protein synthesis, ultimately causing the period of each cycle to increase. This phenomenon is illustrated through a comparison of figures 11 and 12:

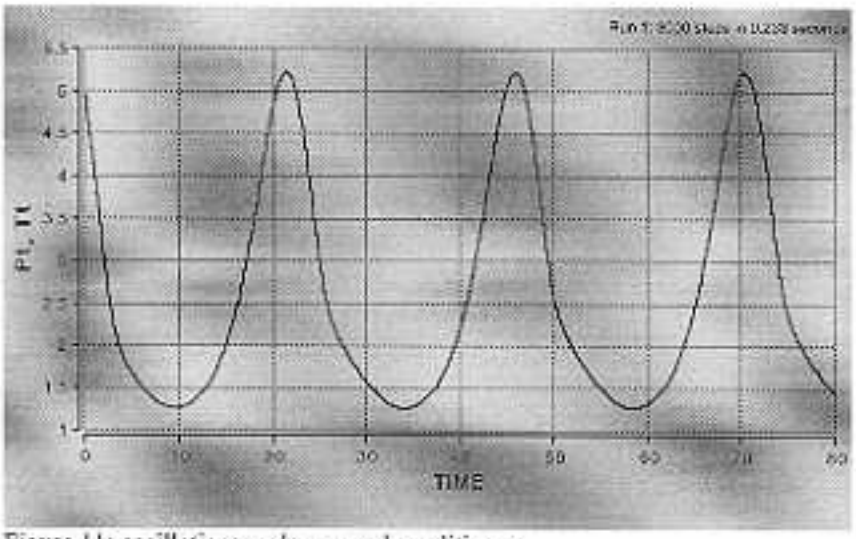


Figure 11: oscillations under normal conditions.

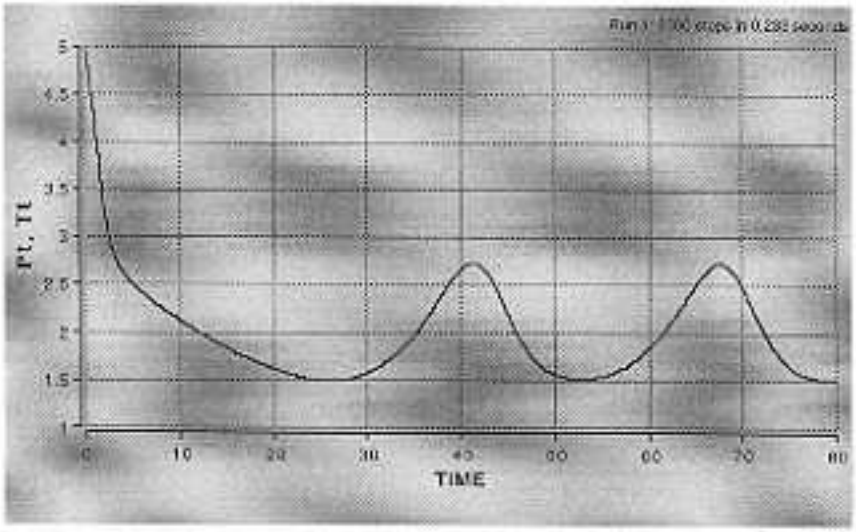
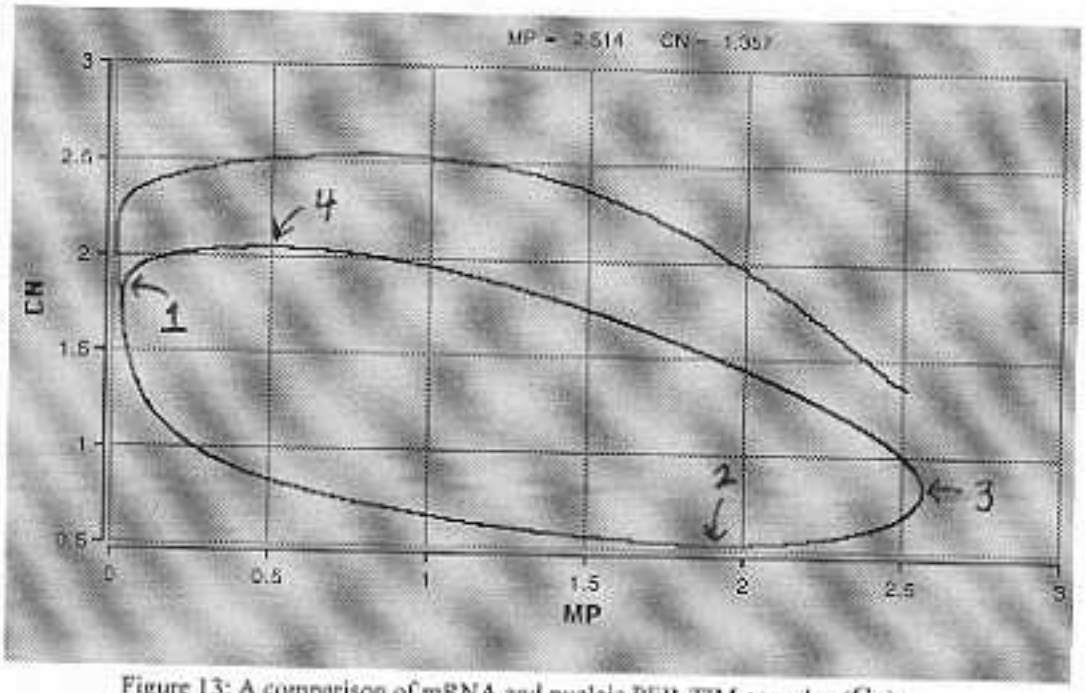


Figure 12: The effect of decreasing the dissociation constant k_4 to $.1h^{-1}$. Notice that oscillations are slower (the period is longer) than in figure 11.

A final graph worth noting compares mRNA (M_P & M_T) to nucleic PER-TIM complex (C_N):



Nice Graph.

Figure 13: A comparison of mRNA and nucleic PER-TIM complex (C_N). Notice that the graph proceeds counterclockwise.

It is important to understand the meaning of this graph. Starting at point 1 and moving counterclockwise, notice that the amount of nucleic PER-TIM complex (C_N) decreases. This decrease means that there is less negative feedback, enabling transcription to occur. The amount of mRNA thus increases, but not for long. For each mRNA formed, a certain amount of protein is produced that later becomes C_N (that exerts negative feedback on transcription). Notice that the amount of C_N begins to increase at point 2. A short time later, at point 3, feedback inhibition (from C_N) causes the amount of mRNA to start to decrease. C_N then increases and mRNA decreases until point 4 when the lack of mRNA for translation causes the amount of protein (including C_N) to start to decline. The cycle then begins again, oscillating indefinitely with rhythmically fluctuating levels of PER/TIM mRNA and protein.

Parameters and Values:

Parameter	Value	Units	Description
v_{sP}	1	nMh^{-1}	maximum rate of PER mRNA formation
v_{sT}	1	nMh^{-1}	maximum rate of TIM mRNA formation
v_{mP}	.7	nMh^{-1}	maximum rate of PER mRNA enzymatic degradation
v_{mT}	.7	nMh^{-1}	maximum rate of TIM mRNA enzymatic degradation
K_{mP}	.2	nM	Michaelis constant for PER mRNA enzymatic degradation
K_{mT}	.2	nM	Michaelis constant for TIM mRNA enzymatic degradation
k_{sP}	.9	h^{-1}	rate constant for PER protein formation
k_{sT}	.9	h^{-1}	rate constant for TIM protein formation
v_{dP}	2	nMh^{-1}	maximum rate of biphosphorylated PER enzymatic degradation
v_{dT}	2	nMh^{-1}	maximum rate of biphosphorylated TIM enzymatic degradation
K_{dP}	.2	nM	Michaelis constant for biphosphorylated PER enzymatic degradation
K_{dT}	.2	nM	Michaelis constant for biphosphorylated TIM enzymatic degradation
k_1	.6	h^{-1}	rate constant for transport of PER-TIM complex from cytosol to nucleus
k_2	.2	h^{-1}	rate constant for transport of PER-TIM complex from nucleus to cytosol
k_3	1.2	$nM^{-1}h^{-1}$	rate constant for PER-TIM complex formation
k_4	.6	h^{-1}	rate constant for PER-TIM complex dissociation
K_{IP}	1	nM	threshold constant for repression of PER mRNA transcription
K_{IT}	1	nM	threshold constant for repression of TIM mRNA transcription
n	4	---	cooperativity constant for feedback inhibition
k_d	.01	h^{-1}	general rate constant for decay
k_{dC}	.01	h^{-1}	rate constant for cytosolic PER-TIM complex decay
k_{dN}	.01	h^{-1}	rate constant for nucleic PER-TIM complex decay
v_{1P}	8	nMh^{-1}	maximum rate of monophosphorylated PER formation
v_{1T}	8	nMh^{-1}	maximum rate of monophosphorylated TIM formation
v_{2P}	1	nMh^{-1}	maximum rate of monophosphorylated PER degradation
v_{2T}	1	nMh^{-1}	maximum rate of monophosphorylated TIM degradation
v_{3P}	8	nMh^{-1}	maximum rate of biphosphorylated PER formation
v_{3T}	8	nMh^{-1}	maximum rate of biphosphorylated TIM formation
v_{4P}	1	nMh^{-1}	maximum rate of biphosphorylated PER degradation
v_{4T}	1	nMh^{-1}	maximum rate of biphosphorylated TIM degradation
K_{1P}	2	nM	Michaelis constant for monophosphorylated PER formation

K_{1T}	2	nM	Michaelis constant for monophosphorylated TIM formation
K_{2P}	2	nM	Michaelis constant for monophosphorylated PER degradation
K_{2T}	2	nM	Michaelis constant for monophosphorylated TIM degradation
K_{3P}	2	nM	Michaelis constant for biphosphorylated PER formation
K_{3T}	2	nM	Michaelis constant for biphosphorylated TIM formation
K_{4P}	2	nM	Michaelis constant for biphosphorylated PER degradation
K_{4T}	2	nM	Michaelis constant for biphosphorylated TIM degradation

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