Appendix

**Kinetic Equations of the Deterministic Model.** In the model schematized in Fig. 6, the temporal variation of the concentrations of mRNA ($M_P$) and the various forms of clock protein, cytosolic ($P_0, P_1, P_2$) or nuclear ($P_N$), is governed by the following system of kinetic equations (1, 2):

\[
\frac{dM_P}{dt} = v_s \frac{K_l^n}{K_l^n + P_N^m} - v_m \frac{M_P}{K_m + M_P}
\]

\[
\frac{dP_0}{dt} = k_s M_P - v_1 \frac{P_0}{K_1 + P_0} + v_2 \frac{P_1}{K_2 + P_1}
\]

\[
\frac{dP_1}{dt} = v_1 \frac{P_0}{K_1 + P_0} - v_2 \frac{P_1}{K_2 + P_1} - v_3 \frac{P_1}{K_3 + P_1} + v_4 \frac{P_2}{K_4 + P_2}
\]

\[
\frac{dP_2}{dt} = v_3 \frac{P_1}{K_3 + P_1} - v_4 \frac{P_2}{K_4 + P_2} - v_d \frac{P_2}{K_d + P_2} - k_1 P_2 + k_2 P_N
\]

The results shown in Fig. 2A (see article) have been obtained by numerical integration of Eqs. 1 for the following parameter values:

- $K_l = 2$ nM, $n = 4$, $v_s = 0.5$ nMh$^{-1}$, $v_m = 0.3$ nMh$^{-1}$, $K_m = 0.2$ nM, $k_s = 2.0$ h$^{-1}$,
- $v_1 = 6.0$ nMh$^{-1}$, $K_1 = 1.5$ nM, $v_2 = 3.0$ nMh$^{-1}$, $K_2 = 2.0$ nM, $v_3 = 6.0$ nMh$^{-1}$, $K_3 = 1.5$ nM,
- $v_4 = 3.0$ nMh$^{-1}$, $K_4 = 2.0$ nM, $v_d = 1.5$ nMh$^{-1}$, $K_d = 0.1$ nM, $k_1 = 2.0$ h$^{-1}$, $k_2 = 1.0$ h$^{-1}$.

**Decomposition of the Deterministic Model into Elementary Reaction Steps.** To perform stochastic simulations of the circadian clock mechanism, the deterministic model schematized in Fig. 6, governed by the five kinetic equations (Eqs. 1), is decomposed into a detailed reaction system consisting of 30 elementary steps. These steps are listed in Table 1 with the probability of their occurrence, denoted $w_i$ ($i = 1, \ldots, 30$). Each $w_i$ is the product of a rate constant times the number(s) of molecules involved in the reaction step. Because each enzymatic reaction is decomposed fully into elementary steps, enzyme-substrate complexes are considered explicitly. The detailed reaction system thus contains 22 variables instead of 5 in the deterministic model. In Table 1, the central column shows the reaction steps involving the indicated molecular species, with the rate constant indicated above the arrow. In the right column, showing the probability of occurrence of the various reaction steps, italicized capitals denote the numbers of molecules of the corresponding species involved in the particular reaction step.

Steps 1-8 pertain to the formation and dissociation of the various complexes between the gene promoter and nuclear protein $P_N$. $G$ denotes the unliganded promoter of the gene, and $GP_N$, $GP_{N2}$, $GP_{N3}$, and $GP_{N4}$ denote the complexes formed by the gene promoter with 1, 2, 3, or 4 $P_N$ molecules. Step 9 relates to the active state of the promoter leading to expression of the gene and synthesis of mRNA ($M_P$). In the case considered we assume that only the complex
between the promoter and four molecules of $P_N$ is inactive. Steps 10-12 pertain to the degradation of $M_P$ by enzyme $E_m$ through formation of the complex $C_m$. Step 13 relates to synthesis of unphosphorylated clock protein ($P_0$) at a rate proportional to the number of mRNA molecules. Steps 14-16 refer to the phosphorylation of $P_0$ into $P_1$ by kinase $E_1$ through formation of complex $C_1$. Steps 17-19 refer to the dephosphorylation of $P_1$ into $P_0$ by phosphatase $E_2$ through formation of complex $C_2$. Steps 20-25 pertain to the corresponding phosphorylation of $P_1$ into $P_2$ and dephosphorylation of $P_2$ into $P_1$. Steps 26-28 relate to the degradation of the phosphorylated form $P_2$ by enzyme $E_d$ through formation of complex $C_d$. Steps 29 and 30 refer to entry of $P_2$ into and exit of $P_N$ from the nucleus, respectively.

**Parameter Values for Stochastic Simulations.** Stochastic simulations of the detailed reaction system consisting of the 30 reaction steps listed in Table 1 have been carried out by means of the algorithm proposed by Gillespie (3, 4), in which in a random, infinitesimal time interval computed by the method, one of the $i$ reactions occurs with a probability proportional to $w_i$ ($i = 1,..., 30$). Parameter values used for stochastic simulations are listed in Table 2.

**Remarks.** In the column listing the probability of occurrence of the various reaction steps in Table 1, kinetic constants related to bimolecular reactions are scaled by $\Omega$ (3, 4). When varying $\Omega$ to modify the numbers of molecules involved in the circadian oscillatory mechanism, we wish to keep the number of gene promoters ($G$) equal to unity without altering the relative weights of the different probabilities $w_i$ so as to keep dynamic behavior consistent with that predicted by the corresponding deterministic model governed by Eqs. 1. The numbers of enzyme molecules and the kinetic constants related to the steps involving $G$ therefore are multiplied by $\Omega$ in Table 2 listing the parameter values.

The maximum value of $a_i$ ($i = 1, . . . , 4$) considered in Figs. 2 and 3 ranges from $10^3$ to $5 \times 10^4$ molecule$^{-1}$ h$^{-1}$ for $\Omega$ ranging from 10 to 500 (see Table 2). For a nuclear volume of $10^{-13}$ liters, for which a concentration of 1nM corresponds to 60 molecules per nucleus, these values of $a_i$ correspond to values of the bimolecular rate constant ranging from $1.5 \times 10^{10}$ to $7.5 \times 10^{11}$ M$^{-1}$ s$^{-1}$. Such values are larger than the diffusion limit of $10^8$–$10^9$ M$^{-1}$ s$^{-1}$ usually considered for bimolecular rate constants. However, values of up to $10^{10}$ M$^{-1}$ s$^{-1}$ (5, 6) or even higher values (7) characterize the binding of a repressor to the gene promoter because of a “facilitated diffusion” process mediated by encounter of the protein with the DNA molecule followed either by sliding (6-9) or direct intersegment transfer of the protein on DNA (6). The values of bimolecular rate constants $a_i$ considered in a previous report (10) were bounded by the “classical” diffusion limit; this may explain the lack of robustness reported by the authors, because at lower values of $a_i$ the oscillations are more affected by molecular noise (see article).

In steps 1-8 in Table 1, parameters $a_j$ and $d_j$ ($j = 1, ..., n$; with $n = 1, 2, 3, \text{or } 4$) are chosen such that the dissociation constant $K_i = d_j/a_i$ (with $K_i^n = \prod_j K_j$, where $K_i$ denotes the inhibition constant in the nondeveloped, deterministic model governed by Eqs. 1) decreases as the number of molecules of $P_N$ bound to the promoter increases (see Table 2); these conditions enhance the cooperativity of the repression process.


