Supporting information for Gonze *et al.* (January 15, 2002) *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.022628299.

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):

$$\begin{aligned}
\frac{dM_P}{dt} &= v_s \frac{K_I^n}{K_I^n + P_N^n} - v_m \frac{M_P}{K_m + M_P} \\
\frac{dP_0}{dt} &= k_s M_P - v_1 \frac{P_0}{K_1 + P_0} + v_2 \frac{P_1}{K_2 + P_1} \\
\frac{dP_1}{dt} &= v_1 \frac{P_0}{K_1 + P_0} - v_2 \frac{P_1}{K_2 + P_1} - v_3 \frac{P_1}{K_3 + P_1} + v_4 \frac{P_2}{K_4 + P_2} \\
\frac{dP_2}{dt} &= v_3 \frac{P_1}{K_3 + P_1} - v_4 \frac{P_2}{K_4 + P_2} - v_d \frac{P_2}{K_d + P_2} - k_1 P_2 + k_2 P_N \\
\frac{dP_N}{dt} &= k_1 P_2 - k_2 P_N
\end{aligned} \qquad \textbf{[1]}$$

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

$K_I = 2$ 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,... 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 unphosphorylatyed 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 Ω (3, 4). When varying Ω 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 Ω 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 5×10^4 molecule^{-1} h^{-1} for Ω 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 1.5×10^{10} to 7.5×10^{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, or 4) are chosen such that the dissociation constant $K_i = d_i/a_i$ (with $K_I^n = \prod_{j=1}^{n} K_j$, 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.

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