, pp. 496–501, 1989

0006-3509/89 $10.00 + .00

HOMEOSTASIS OF THE PERIOD OF AUTO-OSCILLATIONS IN A MODEL OF THE CIRCADIAN CELL CLOCK*

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(*Received* 26 *March* 1987)

A mathematical model describing the auto-oscillatory temporal organization of futile cycles of the carbohydrate branch of cell energy metabolism has been investigated numerically. Optimization in the space of the parameters of the model gave a region in which the circadian period of the oscillations is nearly constant despite variation in eight basic parameters within wide limits. The homeostasis of the period detected is ensured by the synergic action of four mechanisms of negative feedback controlling the activity of cell enzymes. The result obtained reinforces the metabolic theory of the circadian cell clock.

THE nature of circadian rhythms detected even at the cellular level has for several decades been the subject of intense theoretical discussion [1–9]. Of special concern to investigators is the pronounced stability of the period of the circadian cell clock and, in particular, the very weak dependence of the period on the ambient temperature found even in the simplest eukaryotes [1, 3–5, 7–9]. To many investigators it appears doubtful that the biochemical reactions that are very sensitive to change in temperature and many other factors determining the activities of the enzymes might be a suitable elemental base for the stable cell clock [1–7, 9]. In this connexion numerous attempts have been made to construct a cell clock theoretically in which the time setting element would be any physical process weakly depending on temperature. In the last three decades a large number of models has been proposed in which diffusion of substances across the nuclear or plasma membrane [1–5, 7, 9] or lateral diffusion of substances within the membrane itself [6] have been used in one way or another as the physical process.

In work undertaken in our laboratory [10–14] the metabolic theory or cell clocks has been developed. According to this theory cell clocks constitute an auto-oscillatory regime of work of cell energy metabolism (c.e.m.) necessary for suppressing parasitic recirculation of substrates in the futile cycles of amphibolic pathways. Because of the deposition effect [10, 12, 13] this regime may have a circadian period. The assumption has been advanced [10–13] that thanks to the combined action of the mechanisms of negative feedback (n.f.) the c.e.m. is in a position to maintain high stability of the period of oscillations. Recently this assumption was backed by the results of numerical analysis of a mathematical model of the carbohydrate section of the c.e.m. [14]. In this model the

* Biofizika **34**: No. 3, 459–463, 1989.

n.f. mechanisms ensured the stability of the circadian period in relation to disturbance of only one, though the main, parameter of the c.e.m. – the ATPase load.

Below, we give the results of computational experiments with a model [14] demonstrating the possibility of the existence in the carbohydrate branch of the c.e.m. of very low sensitivity of the period to disturbance of all the most important parameters.

MODEL

Let us use the mathematical model proposed earlier [14] for describing the auto-oscillatory time organization of the carbohydrate section of the c.e.m. (Fig. 1). This model represents a system of differential equations:

$$\frac{d\sigma_1}{d\tau} = -v, \quad \varepsilon_1 \frac{d\sigma_2}{d\tau} = v - 0{\cdot}5v_2, \quad \varepsilon_2 \frac{d\sigma_3}{d\tau} = v_2 - v_3 - v_7, \tag{1}$$

$$\varepsilon_3 \frac{d\alpha_1}{d\tau} = v_4, \quad \varepsilon_4 \frac{d\alpha_2}{d\tau} = v_+ - v_2 - v_{3+} + 2v_{3-} + v_5 - v_6 - 2v_4,$$

where $\alpha_3 = 1 - \alpha_1 - \alpha_2$; $v = v_+ - v_-$

$$v_+ = \frac{c_3 \sigma_1 \alpha_3}{\Delta_+^R} \frac{1}{1 + L_+ (\Delta_+^T / \Delta_+^R)^4}, \quad v_- = \frac{\beta_1 c_5 \sigma_2}{\Delta_-^R} \frac{1}{1 + L_-},$$

$$\Delta_+^R = (1 + c_1 \sigma_1)(1 + c_2 \alpha_3) + \alpha_2 + \sigma_2 (1 + \alpha_2), \quad \Delta_+^T = 1 + c_2 \alpha_3,$$

$$\Delta_-^R = 1 + c_4 \sigma_1 + c_5 \sigma_2, \quad L_+ = L_{0+} / ((1 + c_a \sigma_2)(1 + c_{a1} \alpha_1))^4,$$

$$L_- = L_{0-} ((1 + c_i \sigma_2)(1 + c_{i1} \sigma_1)(1 + c_{i2} \alpha_1))^4, \quad v = v_+ - v_-,$$

$$v_2 = \beta_2 \sigma_2 \alpha_2 - \beta_3 \alpha_3 \sigma_3, \quad v_{3+} = \beta_4 \sigma_3 \alpha_2 \sigma_2 / (C_{a2} + \sigma_2^4),$$

$$v_3 = v_{3+} - v_{3-}, \quad v_{3-} = \beta_5 \alpha_3 / (1 + C_{i3} \sigma_2), \quad v_4 = \alpha_2^2 - \alpha_1 \alpha_3,$$

$$v_5 = L \alpha_3, \quad v_6 = \beta_6 \alpha_2, \quad v_7 = \beta_7 \sigma_3.$$

The parameters have the following meaning:

$$C_1 = [\mathrm{F6P}]_{\max} / K_1, \quad C_2 = A_0 / K_2, \quad C_3 = [\mathrm{F6P}]_{\max} A_0 / (K_1 K_2),$$

$$C_4 = [\mathrm{F6P}]_{\max} / K_5, \quad C_5 = [\mathrm{FBP}]_{\max} / K_4, \quad C_a = [\mathrm{FBP}]_{\max} / K_{R1},$$

$$C_{a1} = A_0 / K_{R2}, \quad C_i = [\mathrm{FBP}]_{\max} / K_{T1}, \quad C_{i1} = [\mathrm{F6P}]_{\max} / K_{T2}, \quad C_{i2} = A_0 / K_{T3},$$

$$C_{a2} = K_a / [\mathrm{FBP}]_{\max}^4, \quad C_{i3} = [\mathrm{FBP}]_{\max} / K_i;$$

K_1, K_2, K_3 are Michaelis constants of the R form of PFK for F6P, ATP, FBR; K_4, K_5 are the Michaelis constants of the R form of FBPase for FBP and F6P; K_{R1}, K_{R2} are the allosteric constants of binding to the R form of PFK, FBP and AMP; K_{T1}, K_{T2}, K_{T3} are the allosteric constants of binding to the T form of FBPase, FBP, F6P and AMP; K_a, K_i are the constants of activation of PK and depression of PEPCK by FBP; β_5 is the relative rate of inflow of substrate into the system; L is the relative rate of the metabolic

load; the other parameters are the same as in [14]: $\beta_7 = 0{\cdot}6$; $c_1 = 40$; $c_2 = 10$; $c_3 = 400$; $c_4 = 0$; $c_5 = 100$; $C_a = 20$; $C_i = 600$; $C_{i1} = 40$; $\varepsilon_1 = \varepsilon_3 = \varepsilon_4 = 0{\cdot}01$. Columns *4* and *5* are the data of the optimized model.

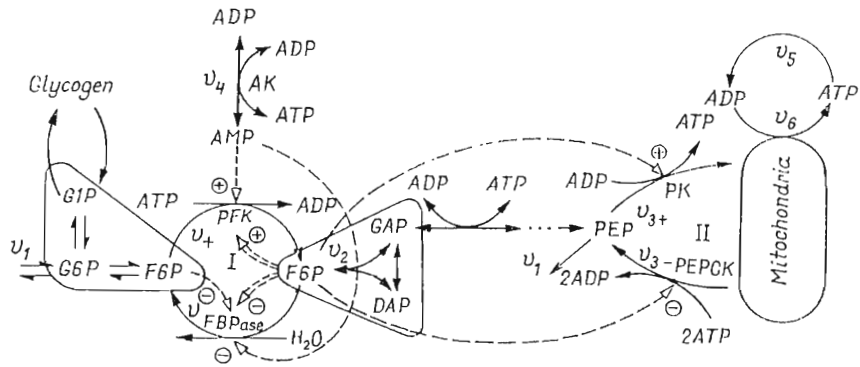

FIG. 1. Scheme of the carbohydrate branch of the cell energy metabolism where: the futile cycle I is formed by 6-phosphofructokinase (PFK; ν_{+1}) and fructose-1,6-bisphosphatase (FPBase, ν_{-}) reactions. The futile cycle II is formed by pyruvate kinase (PK, ν_{3+1}) and the sequence of reactions of phosphorylation of pyruvate which ends in the phosphoenol pyruvate carboxykinase reaction (PEPCK, ν_{3-}). ν_5 is the ATPase load. Double broken arrows point to allosteric positive feedbacks generating auto-oscillations in the c.e.m. Broken arrows show allosteric negative feedbacks.

Optimization of parameters. Model (I) was so optimized in [14] as to ensure the maximum interval of permissible variations in the relative load L corresponding to change in the period of auto-oscillators τ_0 by ± 1 per cent of the mean value. Such optimization, however, did not ensure stability in relation to disturbance of the other parameters as may be seen from the Table (column 3).

Designation of parameters in model (I)	Initial model [14]		Optimized model (I)	
p_i	$\bar{p}_i$	$\Delta p_i/\bar{p}_i$	$\bar{p}_i$	$\Delta p_i/\bar{p}_i$
1	*2*	*3*	*4*	*5*
C_{a2}	$0{\cdot}1924 \times 10^{-7}$	0·27	$0{\cdot}1616 \times 10^{-7}$	0·34
L_{0-}	$0{\cdot}1177 \times 10^{-15}$	0·27	$0{\cdot}1344 \times 10^{-15}$	0·32
C_{i2}	46·18	0·075	4·11·6	0·11
L_{0+}	$0{\cdot}6464 \times 10^{11}$	0·125	$0{\cdot}49 \times 10^{11}$	0·25
C_{a1}	27·708	0·035	260·9	0·13
C_{i1}	6·145	0·205	34·3	0·11
L	16·5	1·15	25·3	0·29
β_8	3·5	0·04	8·08	0·07

Note. The intervals (columns *3* and *5*) are the deviations from the values of the parameters $p_i (i = 1 \ldots 8)$ given in columns *2* and *4*. In these intervals the period τ_0 changes by not more than 1 per cent of τ_0 ($p_1 \ldots p_8$). Columns *2* and *3* give data for the initial model [14] corresponding to the following values of the other parameters: $\beta_1 = 1$; $\beta_2 = 200$; $\beta_3 = 18$; $\beta_4 = 6$; $\beta_6 = 30$.

To eliminate this shortcoming of the model we shall try to choose its parameters in such a way that the disturbance of all its most important parameters (L_{0+}, L_{0-}, c_{a1}, c_{i2}, c_{a2}, c_{i3}, L, β_5) has little impact on τ_0. For this we used the following algorithm.

1) Let us construct a graph (Fig. 2) of the dependence of the period on one of the parameters of the system $\tau=\tau(p_k)$ in a certain permissible interval (p_{k1}, p_{k2}) $(k=7)$, determining the region of the existence of auto-oscillations.

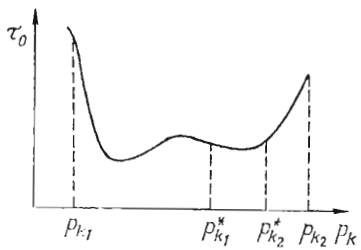

FIG. 2

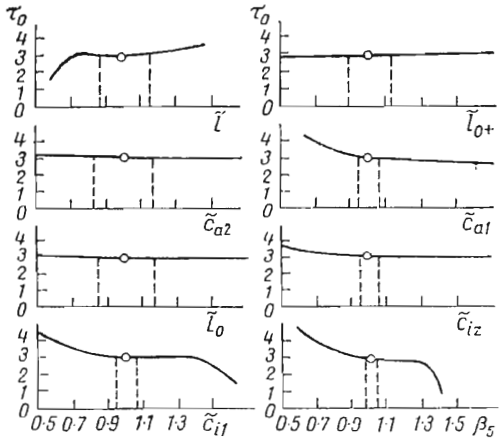

FIG. 3

FIG. 2. Dependence of the period of the auto-oscillations τ_0 on the kth parameter of the model p_k in the interval of permissible values (p^*_{k1}, p^*_{k2}). The interval (p^*_{k1}, p^*_{k2}) is determined by the deviation by ± 1 per cent. $\bar{p}_k=(p^*_{k1}, p^*_{k2})/2$ is the mean value of p_k from the interval of 1 per cent deviation (p^*_{k1}, p^*_{k2}).

FIG. 3. Dependence of the period of auto-oscillations τ_0 on the parameters of the model (1) after optimization. Abscissa gives the normalized values of the parameters $(\tilde{p}_i=p_i/\bar{p}_i)$, $\bar{p}_i$ is the value of the parameter p_i such that relative to τ_0 $(\bar{p}_1, \ldots, \bar{p}_8)$ the 1 per cent deviation is determined. Ordinate – value of τ_0. The intervals of the 1 per cent deviation of the period are denoted by a broken line. The points (○) in the curves denote the value of τ_0 $(\bar{p}_i)$. The values of the parameters are indicated in the text.

2) Let us determine the maximum interval (p^*_{k1}, p^*_{k2}) for which the value of the function $\tau(p_k)$ is almost constant, i.e. $\exists\, \tau_0$ such that $\forall p_k \in (p^*_{k1}, p^*_{k2})$:

$$\tau(p_k) \in (0{\cdot}99\tau_0;\ 1{\cdot}01\tau_0).$$

3) Let us fix the mean value $\bar{p}_k=(p^*_{k1}+p^*_{k2})/2$ and for all the other parameters p_i $(i=1, \ldots, 6)$ construct intervals of 1 per cent deviation (p^*_{i1}, p^*_{i2}) taking the value $\tau_0(p_1, \ldots, p_i, \ldots, \bar{p}_7, p_8)$ as 100 per cent. Thus (p^*_{i1}, p^*_{i2}) is the maximum interval for

which $p_i \in (p_{i1}^*, p_{i2}^*)$ and $\forall p_i^* \in (p_{i1}^*, p_{i2}^*)$:

$$0{\cdot}99 < \frac{\tau_0(p_1, \ldots, p_i^*, \ldots, p_7, p_8)}{\tau_0(p_1, \ldots, p_i, \ldots, p_7, p_8)} < 1{\cdot}01.$$

4) The parameters $p_1, \ldots, p_8$ are so chosen that the minimum of the intervals (p_{i1}^*, p_{i2}^*), $i = 1, \ldots, 7$ may be as long as possible (using the Hook and Jives method [17]). We would note that the ratio $\Delta p_8/(p_8 \equiv \beta_5)$ was not optimized but as may be seen from the above data and Fig. 3 model (1) has a region of β_5 values in which τ_0 changes insignificantly.

The application of the algorithm described to model (1) greatly weakened the sensitivity of τ_0 to disturbance of the eight basic parameters. This may be seen from the above data on the intervals of the permissible deviations of the parameters in the initial model [14] and in the optimized model (1). In these intervals (see Table and Fig. 3) the period τ_0 changes by not more than ± 1 per cent of the mean value. A criterion of the quality of stabilization (known as the Skoeffer criterion) representing the sum of the squares of the variations in the functions (τ_0) for all the disturbed parameters for both sets of parameters of the model (1) amounts to

$$\Phi = \sum_{i=1}^{7} \left(\frac{\Delta\tau_0}{\tau_0}\right)_i^2 = 7 \times 10^{-4}.$$

However, in the optimized model low sensitivity to disturbances persists over a considerably wider region of values of the parameters. As criterion of the permissible variations of the parameters we chose a fairly rigorous requirement—deviation of τ_0 by ± 1 per cent. As may be seen from Fig. 3 τ_0 changes very weakly and outside the limits of the 1 per cent deviation indicating the very high effectiveness of the four n.f. controlling the carbohydrate branch of the c.e.m. (Fig. 1).

DISCUSSION OF RESULTS

The low sensitivity of τ_0 to disturbance of the parameters of the model (1) automatically entails low sensitivity of τ_0 to temperature changes which may disturb the values of all the parameters. The "temperature compensation" phenomenon very often used to discuss the temperature stability of circadian rhythms [1, 3, 4, 7] proves unnecessary. In this connexion our results confirm the assumption once advanced by Pittendrig [7, p. 38] that the temperature stability of the circadian period is only a special manifestation of the general homeostasis of the period.

The model (1) studied does not allow for the fact that the true autogenerator has a far more complex structure. According to current ideas [15, 16] the futile cycle (I) in the scheme (Fig. 1) is controlled not by fructose-1,6-bisphosphate but by fructose-2,6-bisphosphate. However, preliminary investigations of modification of model (1) taking into account these new findings and also the expenditure of ATP on recirculation of substrates in the glycogen cycle show that the expansion of the model (1) does not disturb the stability of τ_0.

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Biophysics Vol. 34, No. 3, pp. 501–505, 1989
Printed in Poland

0006-3509/89 $10.00 + .00

EFFECT OF DIMETHYLSULPHOXIDE AND THIOUREA ON THE DIFFUSIONAL WATER PERMEABILITY OF *E. COLI* COATS*

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(*Received* 2 *April* 1987)

The permeability of *E. coli* cell coats for the diffusion of water has been measured by the n.m.r. relaxation method. It is shown that in the 4–24°C interval it is 16·6–35·0 $\mu m \cdot sec^{-1}$ which is close to the water permeability of erythrocyte membranes and considerably higher than that of lipid bilayers. It has been established that cryoprotectors (DMSO and thiourea) at a concentration of 1·0 M reduce the water permeability of bacterial membranes 1·5 and 2 fold respectively. Two routes of water transport are postulated through pores of a protein nature and defects in the lipid bilayer.

* Biofizika **34**: No. 3, 464–467, 1989.