Instability and Self-Oscillations in the Cell Energy Metabolism

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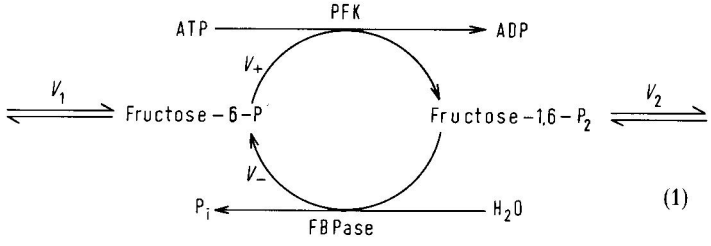
The temporal organization of the cell energy metabolism is the only way of maintaining a stable ATP concentration within the cell. This type of organization results directly from allosteric regulations of the key futile cycles of the cell energy metabolism such as substrate inhibition and product activation. These regulations switch the antagonist enzymes of the futile cycles on and off reciprocally to suppress recycling via self-oscillatory changes in the enzyme activities. The deposition effect (E. E. Sel'kov, in: 8th FEBS Meeting, Vol. 25, pp. 145–161, H. C. Hemker and B. Hess, eds., North-Holland, Amsterdam 1972) allows the oscillations on a circadian time scale.

The stabilization of ATP concentration over a wide range of total ATPase activity is an important function of cell energy metabolism (CEM), since it allows numerous ATP-consuming intracellular processes to proceed independently [1–3]. However, this stabilization (or even the simple export of ATP from CEM to ATP consumers) cannot be fulfilled if there is strong uncontrolled intermediate recycling within the so-called futile cycles [4] of CEM.

The effect of futile recycling upon CEM is clear from such an example as the key futile cycle of the carbohydrate branch of CEM catalyzed by phosphofructokinase (PFK) and fructose-1,6-bis-phosphatase (FBPase):



(1)

The PFK reaction forms fructose-1,6-P_2 (FBP) which is used for the glycolytic phosphorylation of ADP, whilst FBPase wastefully splits FBP back to fructose-6-P (FBP). On the other hand, the FBPase reaction supplies F6P for glucose and polysaccharide synthesis, but the PFK reaction channels F6P back to FBP and wastefully splits ATP to ADP.

From the stoichiometry of the glycolytic system it follows that the stationary rate v_{ph} of the glycolytic phosphorylation of ADP is

$$v_{ph} \leq 4v = 4(v_+ - v_-) \tag{2}$$

where v is net velocity of FBP formation by cycle (1); v_+ and v_- are velocities of the PFK and FBPase reactions. If there is no leakage of intermediates from the lower part of the glycolytic system (FBP → pyruvate) then $v_{ph} = 4v$. But $v_{ph} < 4v$ if there is such a leakage.

Since the hexokinase (HK) and PFK reactions take up ATP the stationary velocity, v_{out}, of ATP output from the whole of glycolysis is

$$v_{out} = v_{ph} - v_{HK} - v_+ \leq 3v_+ - 4v_- - v_{HK} . \tag{3}$$

When there is no exchange of hexomonophosphates with nonglycolytic pathways, $v_{HK} = v_+$ and

$$v_{out} \leq 2v_+ - 4v_- . \tag{4}$$

From this simple balance equation one can see a very strong negative influence of recycling on ATP production. Indeed, when PFK and FBPase reactions have comparable velocities ($v_+ \sim v_-$) then the glycolytic system not only cannot export ATP but rather consumes it with velocity $v_{out} \leq -2v_-$.

Thus, it is clear that to keep glycolysis in the active ATP-exporting state it is necessary to maintain more than twofold excess of the PFK over the FBPase activity:

$$v_+ > 2v_- . \tag{5}$$

Moreover, it is clear also that for a high efficiency of glycolytic energy transduction and for stabilization of ATP concentration [1–3] it is necessary to suppress FBPase activity as far as possible, so that

$$v_+ \gg v_- . \tag{6}$$

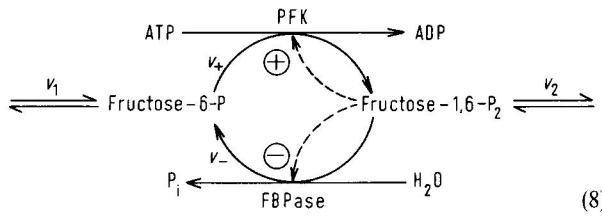
On the other hand, for effective gluconeogenesis too it is necessary to suppress the recycling within (1), but with the reverse relationship between the reaction velocities:

$$v_+ \ll v_- . \tag{7}$$

Thus, for normal functioning of CEM, the mutually exclusive requirements (6) and (7) should be satisfied.

There is only one solution of this problem – the temporal organization of cycle (1), which allows these requirements to be satisfied sequentially in time.

The temporal organization of cycle (1) can be easily realized by means of allosteric reciprocal modulation of the PFK and FBPase activities by FBP:



(8)

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To demonstrate the effect of these regulatory mechanisms on the recycling, consider a mathematical model describing the dynamics of cycle (8) in an open system:

$$\frac{d\sigma_1}{d\tau} = v_1 - v \equiv F_1,$$
$$\varepsilon \frac{d\sigma_2}{d\tau} = v - v_2 \equiv F_2, \tag{9}$$

where $v = v_+ - v_-$ is the net velocity of cycle (1), v_+ and v_- are velocities of the antagonist reactions, v_1 and v_2 are velocities of F6P and FBP exchange, σ_1 and σ_2 are concentrations of F6P and FBP, τ is time, ε is a small time-scale factor. All variables and parameters of the model (9) are dimensionless:

$$v_+ = \frac{v_+}{V_+} = \frac{\sigma_1 - \kappa_+ \sigma_2}{1 + \sigma_1 + \sigma_2} \cdot \frac{Q_+ + a_+ q_+}{1 + Q_+},$$
$$Q_+ = \frac{q_+^{n_+}}{L_+}, \quad q_+ = \frac{1 + \sigma_1 + \sigma_2}{1 + c_{1+}\sigma_1 + c_{2+}\sigma_2},$$
$$L_+ = \frac{L_{0+}}{(1 + c_a \sigma_2)^{m_+}},$$
$$v_- = \frac{v_-}{V_-} = \beta \frac{c_{2-}\sigma_2 - \kappa_- c_{1-}\sigma_1}{1 + c_{1-}\sigma_1 + c_{2-}\sigma_2} \cdot \frac{Q_- + a_- q_-}{1 + Q_-}, \tag{10}$$
$$Q_- = \frac{q_-^{n_-}}{L_-}, \quad q_- = \frac{1 + c_{1-}\sigma_1 + c_{2-}\sigma_2}{1 + c'_{1-}\sigma_1 + c'_{2-}\sigma_2},$$
$$L_- = L_{0-}(1 + d_i \sigma_1)^{m_-}(1 + c_i \sigma_2)^{m_-},$$
$$v_1 = v_{1m} - \kappa_1 \sigma_1, \quad v_2 = \kappa_2 \sigma_2 - v_{2m}.$$

The rate laws for v_+ and v_- are derived in accordance with a generalized Monod-Wyman-Changeux model [5]. They describe not only allosteric reciprocal modulation of the PFK and FBPase activities with FBP but also a similar modulation of this activities with F6P: F6P activates PFK and inhibits allosterically FBPase.

The F6P concentration, σ_1, has to be a slow variable in the model (9) since F6P concentration is well buffered within the cell with the reserved carbohydrates like glycogen on starch, trehalose etc. That means that the FBP concentration, σ_2, varies rapidly (the necessary condition for this is $\varepsilon \ll 1$) and tends to attain quickly a quasi-stationary state in which $F_2 = 0$.

The reciprocal allosteric FBP-dependent regulations of cycle (8) make possible the existence of three such quasi-stationary states, in each of which the condition $F_2 = 0$ is fulfilled. This results in the hysteresis of the quasi-stationary imput characteristic $\tilde{v}(\sigma_1) = v(\sigma_1)_{F_2=0}$ (Fig. 1). Two outer branches of this characteristic are stable and represent two alternative metabolic states of cycle (8) and CEM: the glycolytic state (the upper branch at which $\tilde{v} > 0$), and the gluconeogenetic state (the lower branch at which $\tilde{v} < 0$).

The intermediate branch of the characteristic $\tilde{v}(\sigma_1)$ connecting the two extremes is always unstable at $\varepsilon \ll 1$.

The dangerous stationary state of 100% recycling corresponds to a point of the characteristic at which $\tilde{v}(\sigma_1) = \tilde{v}_+ - \tilde{v}_- = 0$. As it can be seen from Fig. 1, this point belongs to the unstable branch of the characteristic when the extremes

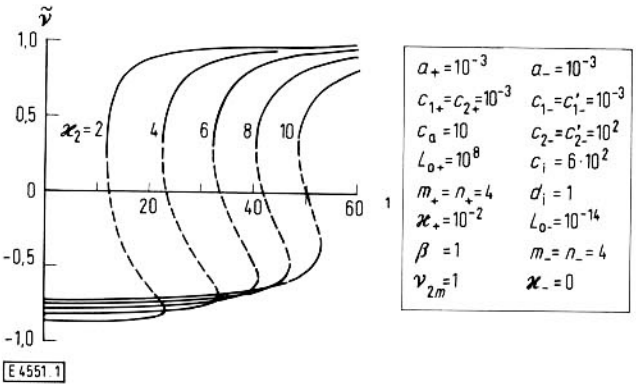

Fig. 1
A family of quasi-stationary input characteristics of the futile cycle (8) plotted for different values of parameter κ_2.
σ_1 is the dimensionless concentration of fructose-6-P, $\tilde{v}$ is the quasi-stationary value of net velocity v at which the condition $F_2 = 0$ for the model (9) holds true. The dashed part of the characteristics corresponds to unstable values of $\tilde{v}$ at $\varepsilon \ll 1$.
Parameter values are $a_+ = 10^{-3}$, $a_- = 10^{-3}$, $c_{1+} = c_{2+} = 10^{-3}$, $c_{1-} = c'_{1-} = 10^{-3}$, $c_{2-} = c'_{2-} = 10^2$, $c_a = 10$, $c_i = 6 \cdot 10^2$, $d_i = 1$, $L_{0+} = 10^8$, $L_{0-} = 10^{-14}$, $m_+ = m_- = 4$, $n_+ = n_- = 4$, $\beta = 1$, $\kappa_+ = 10^{-2}$, $\kappa_- = 0$, $v_{2m} = 1$

are located within two different quadrants of the $(\tilde{v}, \sigma_1)$-plot. Two important conclusions arise from this positioning of the dangerous stationary point. Firstly, this point is unstable, which practically excludes the energetic collapse in CEM caused by the recycling in cycle (8). Secondly, the instability of the stationary state of cycle (8) can form the autonomous periodic or nonperiodic temporal organization of the antagonist reactions of the cycle.

The infinity of the phase plane of the model is obviously unstable since with $\sigma_1 \to \infty$ and $\sigma_2 \to \infty$ both the time derivatives in (9) are negative. From that follows the existence of at least one stable limit cycle within a finite part of the (σ_1, σ_2)-plane when all stationary points of the model are unstable. Fig. 2 represents the domain of existance of a single stable limit cycle C^+ constructed in the (κ_2, v_{2m})-plane by means of numerical solution of the system

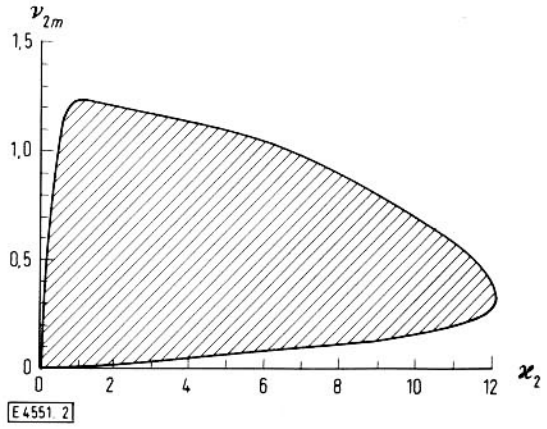

Fig. 2
Domain of existence of stable limit cycle C^+ arround a unique stationary state of the model (9). Parameter values are $\varepsilon = 0.1$, $\kappa_- = 0$, $\kappa_1 = 0$, $v_{1m} = 0$, others are the same as in Fig. 1

$$\frac{\partial F_1}{\partial \sigma_1} = \frac{\partial F_2}{\partial \sigma_2} = 0, \quad F_1 = 0, \quad F_2 = 0. \tag{11}$$

One can see from this figure that in a very large parametric region a unique stationary state (with $\kappa_1 = 0$ the finite and positive stationary state is always unique) is unstable and surrounded by limit cycle C^+. Owing to this limit cycle C^+, concentrations σ_1 and σ_2, net velocity v and the momentary recycling

$$\rho = \begin{cases} v_-/v_+ & \text{if} \quad v_- < v_+, \\ v_+/v_- & \text{if} \quad v_+ \leq v_- \end{cases} \tag{12}$$

oscillate with time τ (Fig. 3). The high amplitude and square-shaped form of the modulator concentration, σ_2, result in a

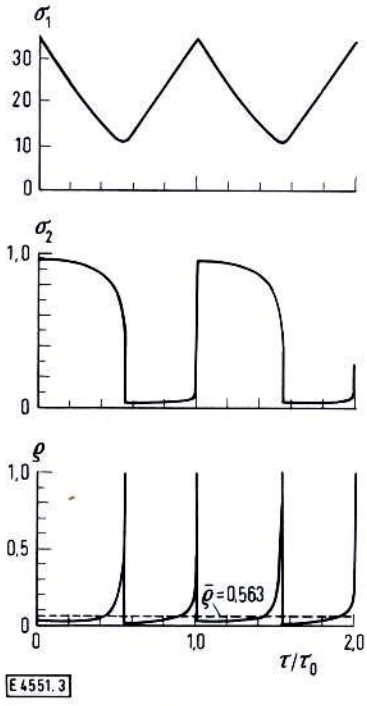

Fig. 3
Self-oscillation in the futile cycle constructed by numerical integration of the model (9).
σ_1 and σ_2 are dimensionless concentrations of fructose-6-P and fructose-1,6-P_2, v is the net velocity of cycle (8), ρ is the momentary intermediate recycling, τ is dimensionless time, $\tau_0 = 56.532$ is oscillation period. Parameter values are the same as in Fig. 1 with exception of $\varepsilon = 0.1$, $\kappa_2 = 2$

very efficient temporal organization of the antagonist reactions of cycle (1), since mean recycling per oscillation period τ_0,

$$\bar{\rho} = \frac{1}{\tau_0} \int_{\tau}^{\tau+\tau_0} \rho(\tau)\, d\tau, \tag{13}$$

is very low ($\bar{\rho} \approx 6\%$).

From Fig. 3 one can see that twice per oscillation period the net velocity v is equal to zero, and 100% recycling momentarily occurs within cycle (8) – just at the moments of switching the cycle from the glycolytic to the gluconeogenetic state and back. Since this high recycling during the reversals of the net flux contributes substantially to the mean recycling $\bar{\rho}$, it is clear that such reversals should occur as seldom as possible, so that very low values of $\bar{\rho}$ are reached. This implies that the oscillation period τ_0 in cycle (8) must be as long as possible.

There is a very simple way of dramatically increasing the oscillation period – the deposition effect [6]. The reversible exchange of F6P via the hexosemonophosphate pool with a huge amount of reserved polysaccharides (Fig. 4) strongly

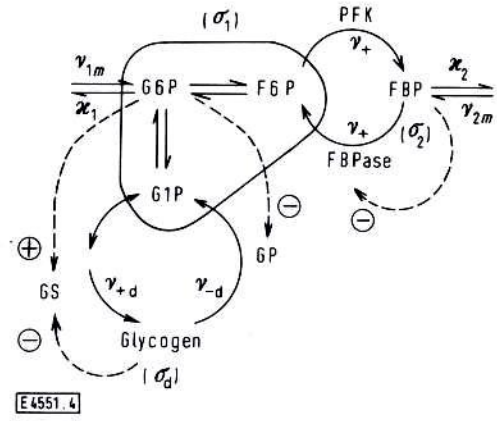

Fig. 4
Kinetic model of glycogen exchange. GS is glycogen synthetase, GP is glycogen phosphorylase, PFK is phosphofructokinase, FBPase is fructose-1,6-biphosphatase. G1P, G6P, F6P, and FBP are glucose-6-P, fructose-6-P, and fructose-1,6-P_2. The dashed arrows show allosteric activation (+) or inhibition (−) of the enzymes

slows down any temporal changes in F6P concentration. Due to this buffering, the oscillation period in futile cycle (8) linked to the polysaccharide depot (Fig. 4) can be of the order of several hours to a day. This can be demonstrated with a very simple model of the glycogen exchange (Fig. 4) as follows:

$$\begin{aligned} \frac{d\sigma_d}{d\tau} &= \mu_d v_d, \\ \frac{d\sigma_1}{d\tau} &= v_1 - v - v_d, \\ \varepsilon \frac{d\sigma_2}{d\tau} &= v - v_2, \end{aligned} \tag{14}$$

where

$$v_d = \left[\frac{\sigma_1^2}{(\kappa + \sigma_1^2)(1 + \sigma_d)} - \frac{\beta \kappa \sigma_d}{(\kappa + \sigma_1^2)(\delta + \sigma_d)} \right] \cdot \beta_d,$$

$$v = v_+ - v_- = \frac{\sigma_2}{\zeta + \sigma_2(1 + \sigma_2^4)},$$

$$v_1 = v_{1m} - \kappa_1 \sigma_1, \quad v_2 = \kappa_2 \sigma_2 - v_{2m}$$

are net deposition velocity (F6P → depot), net velocity of the futile cycle F6P ⇄ FBP, and velocities of hexosemonophosphate and FBP exchange, σ_d is the concentration of reserved polysaccharide (glycogen, starch etc.), σ_1 is the concentration of hexosemonophosphate pool, μ_d is a small time-scale factor. The model (14) takes into account reciprocal allosteric regulation of glycogen synthetase (GS) and glycogen phosphorylase (GP) shown in Fig. 4, and only the substrate inhibition of FBPase by FBP.

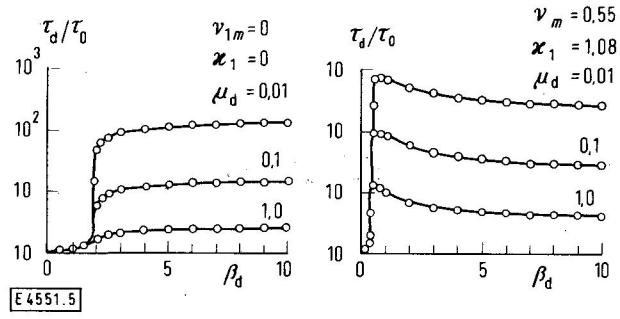

Fig. 5

Fig. 5 shows that as the relative GS activity, β_d, increases from $\beta_d = 0$ to $\beta_d \gg 1$, the oscillation period T_d increases dramatically and than approaches to a certain asymptotic value, $T_{d\infty}$, which can be estimated by linear approximation as follows:

$$\frac{T_{d\infty}}{T_0} = 1 - \frac{\dfrac{\partial \nu_d}{\partial \sigma_1}}{\mu_d \dfrac{\partial \nu_d}{\partial \sigma_d}}. \tag{15}$$

Here T_0 is the oscillation period with $\beta_d = 0$,

$$\mu_d = \frac{K_m(1 + K_1(1 + K_2))}{K_i K_1 K_2} \tag{16}$$

where $K_m \approx 5 \cdot 10^{-5}$ M is the Michaelis constant for PFK, $K_i \cong 1.4 \cdot 10^{-2}$ M is the inhibition constant for the glycogen inhibition of GS, $K_1 = 20.2$ and $K_2 = 0.398$ are the equilibrium constants for the isomerization reactions G1P $\rightleftarrows$ G6P and G6P $\rightleftarrows$ F6P. The partial derivatives have to be taken for $\nu_d = 0$ and for a certain mean value of $\sigma_1 = \sigma_{1\,\mathrm{mean}}$ lying between the abscissae of two extremes of the isocline $\dot{\sigma}_2 = 0$.

The estimate (15) is in a good agreement with numerical integration of the model (9) and predicts the circadian oscillation period $T_{d\infty} \cong 25$ hr with the following conditions: own oscillation period of cycle (8), $T_0 \cong 3$ min, the relative GP activity, $\beta = V_{GP}/V_{GS} = 0.25$, the relative Michaelis constant for GP, $\delta = K_{GP}/K_i \ll 1$, the relative activation constant of GS for G6P, $\kappa = (K_a K_2/K_m)^2 = 0.63$, the activation constant of GS-I, $K_a \cong 10^{-4}$ M.

This oscillation period is generated at the mean glycogen (starch) content of the order of $8.8 \cdot 10^{-5}$ M (~ 1.6 mg/ml) which can be considered as normal for many free living cells.

It appears very likely that the key futile cycle of lipid exchange Acetyl-CoA $\rightleftarrows$ Free Fatty Acid and the key futile cycle of amino acid metabolism Glutamate $\rightleftarrows$ Glutamine can be temporally organized like the cycle (8).

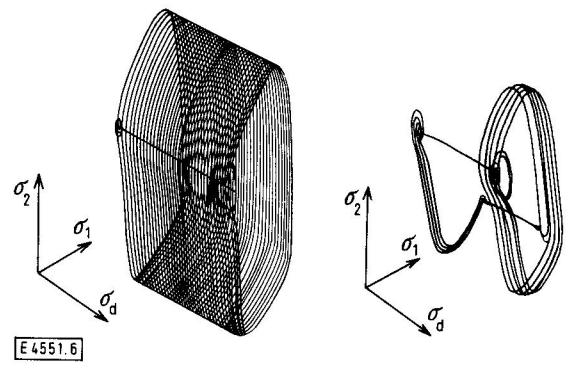

Fig. 6
Left-Highly spiralized stable three-dimensional limit cycle of the model (9) at $\delta = 0.01$, $\varepsilon = 0.025$, $\kappa = 1$, $\kappa_1 = 0.5$, $\zeta = 0.01$, $\kappa_2 = 0.5$, $\mu_d = 0.01$, $\nu_{1m} = 0.3$, $\nu_{2m} = 0.5$, and $\beta_d = 1.1$.
Right-Stochastic trajectory scattering along a certain stable manifold of the model (9) at $\beta_d = 1.11$. Other parameters are the same as in the left-hand figure

Like other models describing the deposition effect [7, 8], model (9) displays very complex behaviour. For instance, in a very narrow region near $\beta_d \sim 1$, one can observe either highly spiralized stable limit cycles (Fig. 6, left) or even stochastic motion (Fig. 6, right). This type of behaviour is due to the nonlinear interaction between two self-oscillatory mechanisms having own oscillation periods of the order of T_0 (the fast oscillator) and of $T_{d\infty}$ (the slow oscillator) [7].

The author is deeply indebted to Prof. Benno Hess and to his coworkers for their hospitality and help. I would like to thank Dr. Svetlana N. Dynnik for computer calculations, Dr. Robin B. Jones for improving my English, and Miss Bettina Plettenberg for their skilful technical assistance. The work has been supported by the Deutsche Forschungsgemeinschaft (Grant No AZ 438 17/7/79).

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