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The Oscillatory Basis of Cell Energy Metabolism

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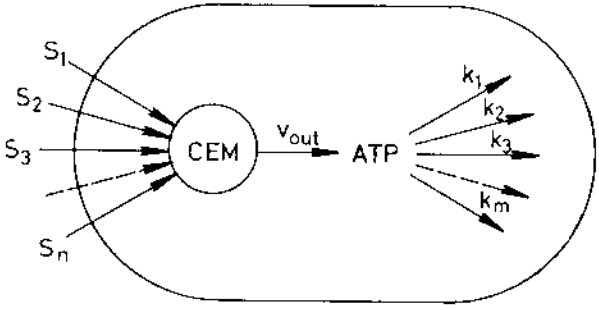
The aim of this paper is to outline a proof that long-period sustained oscillations are necessary for precise stabilization of ATP concentration by cell energy metabolism.

1. The Simplest Model of Cell Energy Metabolism

All living expenses of the cell are covered by a universal intracellular energetic currency -- ATP -- which is generated by a very complex reaction system, the cell energy metabolism (CEM). CEM takes up different initial substrates such as sugars, fatty acids, amino acids etc. from the extracellular medium and then transfers the energy of their chemical bonds to the pyrophosphate bonds of the ATP molecule. Thus the simplest model of these intracellular chemical events may be depicted as follows:



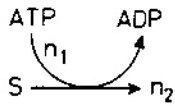
where $S_1, S_2, \ldots S_n$ are initial substrates of CEM; $k_1, k_2, \ldots, k_m$ are rate constants of different ATP-consuming processes; v_{out} is the output velocity of CEM.

2. If I Were God...

If I were God designing an optimal scheme for CEM I should allow for dramatic time-dependent variations of the energy demands of different consumers as well as a very inhomogeneous distribution of the initial substrates in the extracellular medium. Both factors strongly affect the ATP concentration, making it impossible for different consumers to function independently. It is rather evident that the best way to maintain the independence of individual consumers is to stabilize the ATP concentration by means of CEM. This is analogous to the stabilization of voltage in the electrical networks feeding our flats, laboratories and factories.

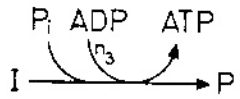
3. CEM as a Stabilizer of ATP Concentration.

Now consider a typical pathway of energy transfer within CEM. Most of the initial substrates are rather stable compounds, which allowed their accumulation during the chemical evolution of the Earth. Therefore the first step in nonphotosynthetic biochemical energy transduction from an initial substrate S is its activation at the expense of ATP to yield unstable intermediates I, and ADP (a discharged form of ATP):



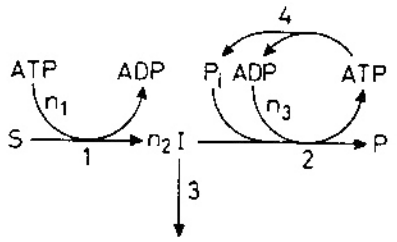
(1)

Generally, the activation of one molecule of S may use $n_1 \geq 1$ molecules of ATP to give $n_2 \geq 1$ molecules of I and n_1 molecules of ADP. The unstable molecules I are now ready to drive a phosphorylation mechanism which can be represented as one overall reaction



(2)

where P_i is inorganic orthophosphate, n_3 is the number of ADP molecules phosphorylated per molecule of I, and P is an end-product. In addition, intermediates I can take part either in a spontaneous decay or in some biosynthetic reactions. Both these cases are leakages of I from the pathway S⟶I⟶P. The simplified scheme of CEM can now be summarized by a kinetic model:



(3)

where 1 is the sparking reaction (1), 2 is the ATP-generating reaction (2), 3 is the leakage, and 4 is a metabolic load representing the total activity of ATP consumers.
Assume that the velocities in (3) can be approximated by simple expressions like

$$v_1 = k_1 \cdot S \cdot A_3, \quad v_2 = k_2 \cdot P_i \cdot I \cdot A_2, \quad v_3 = k_3 \cdot I,$$

$$v_4 = k_4 \cdot A_3, \; A_3 + A_2 = A = \text{const}, \; S = [S], \; P_i = [P_i],$$

$$A_2 = [ADP], \; A_3 = [ATP], \; I = [I], \qquad (4)$$

and that concentrations of S and P_i are constant. A stationary state of (3) is determined by the equation system

$$\frac{d[I]}{dt} = n_2 \cdot v_1 - v_2 - v_3 - 0,$$
$$\frac{d[ATP]}{dt} = -n_1 \cdot v_1 + n_3 \cdot v_2 - v_4 = 0 \qquad (5)$$

which has two solutions

$$a_3 = (1 + l)(L^* - L)/(N - L) \text{ for } 0 \leqslant L < L^* \qquad (6_1)$$

and

$$a_3 = 0 \qquad \text{for } 0 \leqslant L < \infty \qquad (6_2)$$

Here $a_3 = A_3/A$, the relative ATP concentration; $l = k_3/(k_2 \cdot P_i \cdot A_2)$, the relative leakage activity; $L = k_4/(n_1 \cdot k_1 \cdot S)$, the relative load activity; $L^* = (N - l)/(1 + l)$, a critical value of L; $N = n_2 \cdot n_3/n_1 - 1$, the stoichiometric efficiency of CEM.

From (6) it follows that when the leakage is small ($l \leqslant 1$), the concentration of ATP is nearly constant (Fig.1a) over a wide range of L ($0 \leqslant L < L^*$). The overloading of CEM with $L \geqslant L^*$ brings ATP production to an irreversible stop ($a_3 = 0$). With increasing leakage activity the plateau of the load characteristics $a_3(L)$ disappears (Fig. 1b), and beyond some critical value of $l = N$, CEM cannot maintain any stationary ATP concentration.

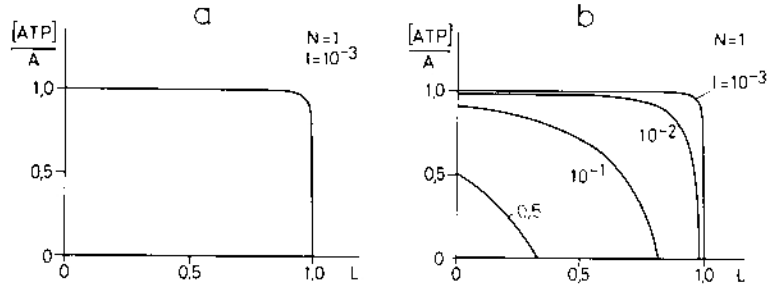


Fig. 1 (a). The stabilization of ATP concentration by CEM (3) within a wide range of relative load activity $0 \leqslant L < L^*$.
(b) Disappearance of ATP-stabilization with increase of relative leakage activity, l.

At a small leakage activity CEM can also buffer ATP against substrate concentration fluctuations. However, such buffering requires S to be maintained at a much higher value than

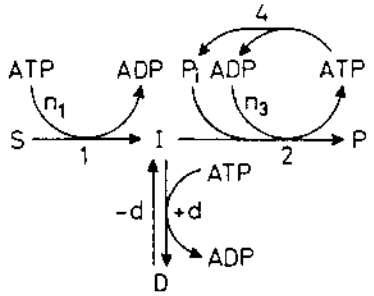
$$S^* = k_4/n_1 \cdot K_1 \cdot L \ . \qquad (7)$$

If S drops below S^*, the production of ATP by CEM stops irreversibly. Analysis of more detailed models [1 - 5] shows that the fine stabilization of ATP concentration is a general property of any type of CEM.

4. Additional Buffering Against Substrate Fluctuations

Although the simplest mechanism of CEM (3) can stabilize [ATP] it is unable to prevent the cessation of energy production caused by even

a relatively brief fall in substrate concentration below S*. This drawback can be reduced simply by inserting a depot for I:

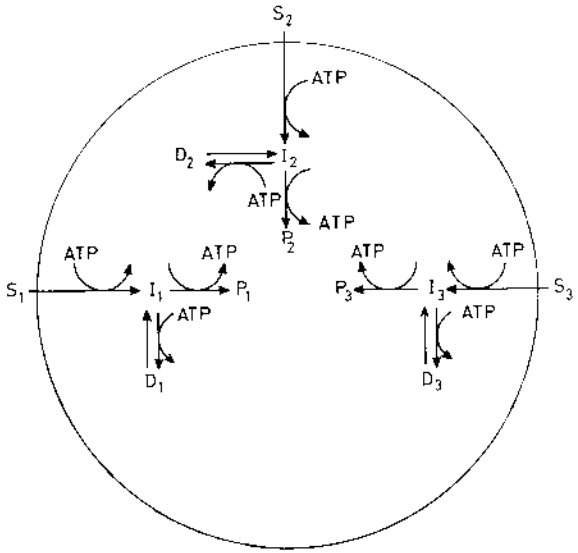


(8)

The deposition I⟶D has to be ATP-dependent for at least two reasons: firstly, to maintain a large excess of D over I, and secondly, it is much more advantageous to have D as a polymer of I-subunits in order to bypass the osmotic effect caused by a large amount of D. As has been shown elsewhere [6,7] a reversible deposition I⟺D with an apparent equilibrium constant K can increase the time constant of I (K + 1) - fold. When K ≫ 1, the deposition mechanism prevents rapid fluctuations in the concentrations of I and ATP if the supply of S is irregular and provides a substitute for S when this is absent for a long time.

5. Backing Several Horses

It may easily happen that a given substrate S is absent so long that the depot is completely exhausted, which will immediately result in irreversible switch-off of CEM. To decrease the probability of such a failure it is necessary to provide the cell with several energetic units similar to (8), but with different initial substrates S_1, S_2, S_3...etc.:

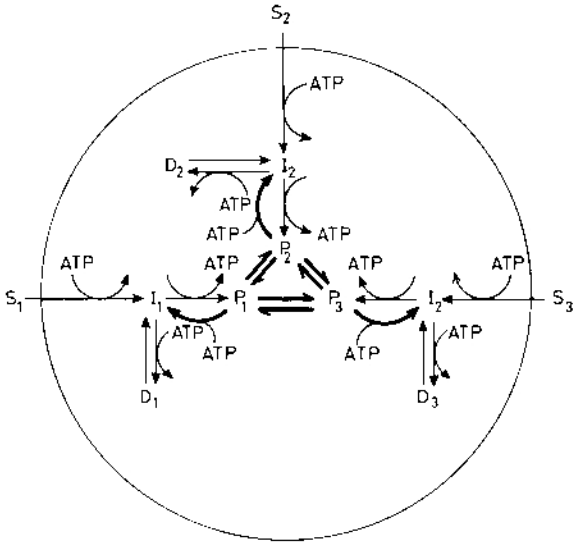


(9)

If the probability p_i of a long absence of substrate S_i is sufficiently low, and if all substrates are chemically different, then the probability p of absence of all substrates will be

$$p = p_1 \cdot p_2 \cdot p_3 \ldots \ll 1$$

Thus the multisubstrate structure (9) of CEM is much more independent of substrate fluctuations than is the single substrate model (8). However, this new structure of CEM (9) has its own drawbacks: firstly, it does not allow any given depot D_i to be restored from substrates other than S_i; and secondly, absence of a given substrate S_i results in the absence of specific intermediates I_i, derivatives of S_i which may be of critical importance to some biosynthetic processes.
To refine structure (9) it is necessary to interconnect end-products and to reverse the ATP-generating steps of each energy unit:

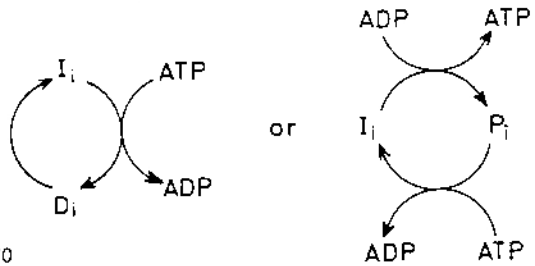


(10)

This refined structure of CEM already looks very similar to the real-life structure of CEM. It has several energetic units interconnected through a central reaction cycle recalling the Krebs cycle. And it looks much more efficient than the previous structure (9). But it is easy to show that it can neither stabilize ATP nor simply export ATP to intracellular consumers. The reason? – A very strong uncontrolled dissipation of energy occurs in many ATP-dependent cycles, futile cycles, within structure (10).

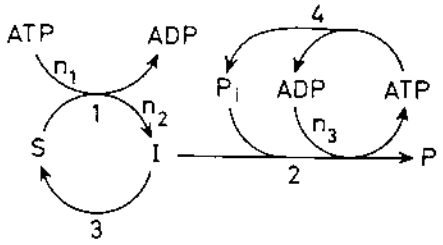
6. Futile Cycles Versus the ATP-Stabilization

Each ATP-dependent cycle of type



(11,12)

is actually a futile cycle if it is not controlled: one turnover of such a cycle is equivalent to a wasteful cleavage of one or more (depending on stoichiometry) molecules of ATP. The effect of this recycling of intermediates upon the main properties of CEM can be seen from the simplest example:



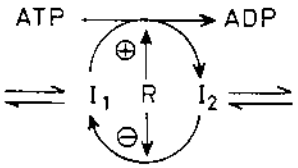
(13)

This model is mathematically equivalent to (3). Its load characteristics $a_3(L)$ can therefore be described by equations (6) in which $l = k_3/k_2 \cdot P_i \cdot A$ and k_3 are relative activity and rate constant of the backward reaction $S \leftarrow I$. From (6) it follows that when forward and backward reactions have comparable velocities ($l \sim 1$), stabilization of ATP concentration is no longer tenable (Fig.1b). Moreover, at $l \geqslant N$, mechanism (13), which contains only one futile cycle, cannot export ATP due to the overconsumption of ATP by this cycle. It is clear that model (10) must be even more sensitive to the futile recycling than the simpler model(13) since it has twice as many futile cycles per energetic unit.

7. A Unique Solution - Temporal Organisation of Futile Cycles

Each cycle of type (11,12) consists of two mutually exclusive processes which have somehow to be separated or organized to prevent futile recycling. In theory, there are only three types of organization of incompatible biochemical processes: spatial organization, organization on the basis of biochemical specificy, and temporal organization.

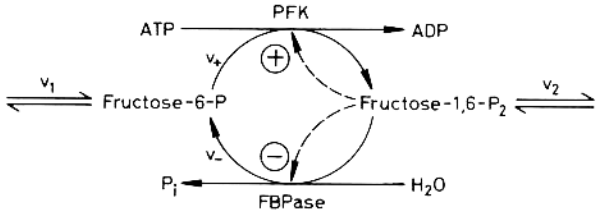
Temporal organization could be autonomous or nonautonomous, and either of these could in turn be periodic or aperiodic. For example, cell divisions can be autonomous if there is no external pacemaking signal or nonautonomous if there is such a signal, and may be either fairly periodic or nearly stochastic. The temporal organization of futiles cycles can easily be realized if the opposing reactions are controlled reciprocally by some regulator R:



(14)

Here the forward reaction $I_1 \longrightarrow I_2$ can go only at a high level of R but the backward $I_1 \leftarrow I_2$ only at a low level. Thus oscillations or fluctuations of R between the two extremes result in the temporal separation of two incompatible processes. Cycle (14) is nonautonomous — it needs some external signals to change the concentration

of R. To transform cycle (14) into an autonomous one it is simply necessary to substitute I_2 for R. Just this takes place in the key futile cycle of the carbohydrate branch of CEM:



(15)

Here the enzyme of the forward reaction, phosphofructokinase (PFK), is activated by its product fructose-1,6-P_2 (FBP), whilst the antagonist enzyme fructose-1,6-bisphosphatase (FBPase) is inhibited by FBP. This type of reciprocal control has a number of far-reaching consequences [8 - 10]. Firstly, it destabilizes and thus makes highly improbable a state with 100%-recycling of the intermediates (Fig. 2). Secondly, it forms two alternative quasistationary steady states of the cycle (15): the glycolytic state O_1 in which net flux is directed to the right ($v = v_+ - v_- > 0$) and the gluconeogenetic state O_3 with the reversed flux ($v<0$). And finally, it forms a hysteretic dependence of the quasistationary net velocity $\tilde{v} = v$ ($v = v_2$) on fructose-6-P (F6P) concentration (Fig.3b), which in turn results in autonomous oscillations around an unstable stationary state O between the two extrema of the characteristics v(F6P) (Fig.4, limit cycle C). Due to the buffering of F6P by the same reserve polysaccharides (glycogen, starch, trehalose etc.) the period of such oscillations can be as long as several hours or even a day, depending on the apparent equilibrium constant of the deposition mechanism (paragraph 4, details in [6,7,11]).

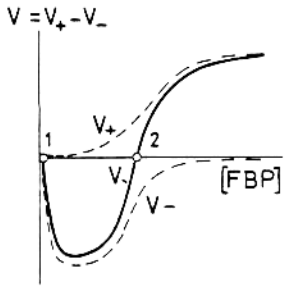


Fig.2 Net velocity $v = v_+ - v_-$ of futile cycle (15) as a function of FBP at a certain nonzero [F6P]. Dashed lines represent velocities of the opposing reactions of the cycle. At points 1 and 2, 100% recling of the intermediates occurs. Point 2 is unstable since a spontaneous increase (decrease) in [FBP] results in further accumulation (depletion) of FBP.

Twice per oscillation period the net velocity v is equal to zero, and 100% recycling mometarily occurs within the cycle (15). However, this recycling occurs only during rapid transitions between the two quasistationary states and therefore the average recycling per oscillation period can be very small.
Returning to the structure (10) we may conclude that it can stabilize [ATP] only when each energy unit has its own autonomous pacemaker, a self-oscillatory futile cycle such as cycle (15). Oscillating intermediate concentrations of such a pacemaker cycle can suppress the recycling in all nonautonomous futile cycles of a given unit via regulatory interactions such as (14).

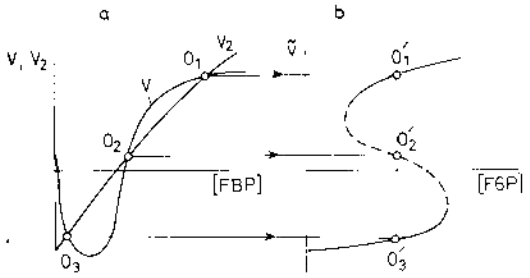


Fig. 3 (a) Three quasistationary states in cycle (15) given by the intersection points O_1, O_2, and O_3 of two graphs representing net velocities of FBP formation, v, and breakdown, v_2.
(b) Hysteretic imput characteristics of the cycle (15). $\tilde{v}$ is the quasistationary value of v at which $v = v_2$.

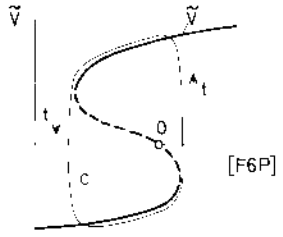


Fig. 4. Limit cycle C around an unstable stationary point O. Arrows indicate direction of the oscillatory motion in the futile cycle (15).

8. Coordination of Oscillatory Units

The coexistence of several self-oscillatory units within a CEM structure such as (10) poses a new question: what kind of coordination between these units is optimal for the whole system? If we limit structure (10) to only two units then the answer can be derived immediately. The two units must oscillate with the same frequency and with 180^0-phase shift. Suppose on the contrary that the two depots D_1 and D_2 oscillate in phase. In such a mode there will be a state during one half of the oscillations period in which both D_1 and D_2 are being restored. But during that state both units produce no ATP at all and neither do they produce products for mutual exchange. Thus during such a state we need extra sources of ATP and products P_1 and P_2, sources which are absent in our minimal model. Thus the only possible mode of interactions of two units is the reciprocal oscillation of two depots. So two-depot CEM looks very like a see-saw: accumulation of D_1 is accompanied by depletion of D_2 and vice versa. For the three-unit case the answer is not yet so clear owing to the multiplicity of oscillatory modes in three-oscillator systems. Among these modes at least one can be realized: two depots oscillate in phase with each other and out of phase with respect to the third. The antiresonant unit must then be powerful enough to support restoration of both other depots at the same time.

9. CEM as the Cell Clock

Thus, the long-period oscillations are of critical importance for the temporal organization of CEM. In addition to this fundamental

function the oscillations can be used as a time-keeping mechanism, the cell clock. Analysis of mathematical models of CEM has shown [12] that the CEM oscillations can have a fairly stable period when a multiplicity of chemostatic negative feedback mechanisms is introduced into the CEM structure.

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