Evidence of an Intracellular Dissipative Structure*

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Data are presented showing the result of the functioning of the mitochondrial respiratory chain in Ehrlich ascites tumour cells incubated with glucose and glutamine.

The very different results obtained depending on the order of substrate addition suggest that the functioning of the respiratory chain in Ehrlich cells far-from-equilibrium maintains an intracellular dissipative structure.

To study the living cell as an open system interacting with the surroundings, non-equilibrium thermodynamics must be used. A key concept in non-equilibrium thermodynamics is that of dissipative structure of Prigogine, defined as the dynamic organization of matter in space and time kept far-from-equilibrium by a continuous dissipation of free energy [1]. Such a way defined, the living cell is a dissipative structure and when the dissipation of free energy is interrupted, it becomes the death.

Recently, Ji [2] has formulated a molecular model of the living cell based on the concepts of dissipative structures and conformons; Ji has named Bhopalator the model. Volkenstein [3] defines conformons as quasi-particles consisting of carriers of electronic charge and treat the conformon as an excitation of longwave phonons. In his molecular model, Ji redefines the conformons as the elementary units of free energy and genetic information that are necessary and sufficient for effectuating molecular mechanisms responsible for the life of the cell. Ji [4] defines intracellular dissipative structures (IDS) as those far-from-equilibrium distributions of chemicals inside the cell that are mantained through a dissipation of free energy.

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Electron transport through mitochondrial respiratory chain and oxidative phosphorilation are processes of a paramount importance in cellular metabolism. This communication presents experimental evidence that the functioning of mitochondrial respiratory chain far-from-equilibrium leads to maintain an IDS. The evidence is based in what could be named "non-commutativity test". A characteristic property of dissipative structures not observed in equilibrium or near-equilibrium systems is the possibility of successive metabolic bifurcations. These bifurcations may drive the system to very different final states depending upon the order of substrate addition.

The experiments were carried out using Ehrlich ascites tumour cells. The tumour was maintained by successive inoculations of 5×10^6 cells in the peritoneal cavity of 2-month old Swiss albino female mice every ten days. The cells to be used in experiments were removed 9–12 days after tumour implantation, washed three times and resuspended in phosphate buffered saline at 200×10^6 cells/ml. The cells were preincubated for 15 min under 95% O_2 and 5% CO_2 atmosphere. Absorption difference spectra of the mitochondrial electronic chain cytochromes in whole cells were recorded at 37 °C using a Beckman DU-8B spectrophotometer equipped with a device to minimize interferences by light scattering. A cuvette containing 2 ml of cell suspension oxidized with 10 mM potassium ferricyanide was used

Table I. Cytochrome redox states for suspensions of Ehrlich ascites tumour cells.

Glucose (5 mM) and glutamine (0.5 mM) were added and cytochrome redox spectra were recorded 5 min after the addition of each substrate. Values are mean ± SEM of at least four separate experiments and represent the percentage of the oxidized cytochrome content in the cells five minutes after the addition of the second substrate, taking the oxidized cytochrome content in control cells respiring endogenous substrates as 100%.

Statistical significances (determined by Mann-Whitney's U test) *versus* cells incubated with first glutamine and then glucose is indicates by *($p < 0.01$).

Substrates added	Cyt *c*	Cyt *c1*	Cyt *b*	Cyt (*a* + *a3*)
None	100	100	100	100
1° Glutamine 2° Glucose	111 ± 3	127 ± 2	85 ± 3	158 ± 4
1° Glucose 2° Glutamine	112 ± 2	113 ± 2*	124 ± 3*	131 ± 6*

as a blank. The difference spectra were recorded 5 min after the addition of the first substrate, 5 mM glucose or 0.5 mM glutamine. Immediately, the second substrate, 0.5 mM glutamine or 5 mM glucose, was added and after 5 min the difference spectra were recorded. The cytochrome redox states were calculated using the method of Vanneste [5].

Data in Table I reveal very different and significative oxidative states of mitochondrial respiratory chain cytochromes depending on the order of addition of glucose and glutamine. These results show a non-commutativity similar to that reported by Ji [6] for the addition of ethanol and 7-hydroxycoumarin to perfused liver. According to Reich and Sel'kov [7], the mitochondrial respiratory chain may operate in equilibrium, near-equilibrium or far-from-equilibrium depending on the time hierarchy of the system. Our results appear to indicate that respiratory chain in Ehrlich tumour cells operates far-from-equilibrium and support Ji's IDS hypothesis.

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