Sustained Rhythm and Directed Self-Locomotion under Thermodynamically Open System*

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1 Nature of Life

We maintain our life under energy uptake by food and excretion. This simple fact implies that living organisms are always exchanging energy and mass with outer environment, and stay in these flows, in other words life is a representation of non-equilibrium and non-linear system. Macroscopic and dynamic phenomena are obvious in living organisms in thermodynamically open conditions. Examples are beautiful patterns appearing on surfaces of animals, fish or insects, development processes where one cell selectively differentiates to variety of cells destined for their own roles, and then dramatically give rise to a body with complex structure and functions in irreversible manner, moreover a cardiac rhythm, electrical encephalogram and circadian rhythm succeeding from birth to death, and active movement of amoeboid cells seeking better environment etc. These spatial and temporal structures are spontaneously formed in flows of energy and mass, and are characterized by the presence of time arrow such as self-organized pattern formation or self-repairing. Spatio-temporal structures in thermodynamically open system exhibit marked difference from thermodynamically stable equilibrium-state determined by the minimum of free energy as a crystal structure.

Here we show an example of the relationship of energy dissipation with spatio-temporal structure in a living system. Figure 1 describes patterns shown in migrating acellular slime mold and it's intracellular chemical mediator [1]. Acellular slime mold exists as amoeboid cells that migrate seeking better environmental conditions and feeding. This amoeboid cell, called plasmodium, is a single cell, which grows to a few centimeters, occasionally several tens of centimeters. Figure 1a shows a plasmodium migrating to the left on the page, and polar distributions of energy metabolite: ADP (adenosine-5'-diphosphate) and ATP (adenosine-5'-triphosphate) according to the migrating direction.
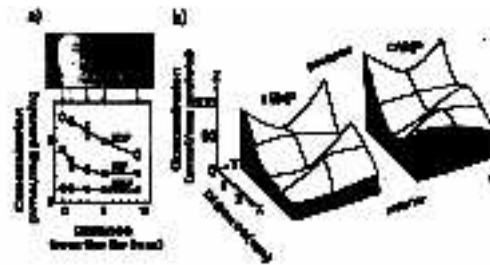

Figure 1. Spatial pattern of chemical species in moving *Physarum polycephalum.* a) One-dimensional distribution of adenine nucleotide. b) Two-dimensional distribution of cGMP and cAMP. (modified from Ueda, T. *et al.* [1])

Figure 1b shows 2-dimensional (2-D) patterns of intracellular chemical mediators: cAMP (cyclic adenosine-3',5'-mono-phosphate) and cGMP (cyclic guanosine-3',5'-monophosphate) where the plasmodium migrates from upper-left to lower right. Patters of cAMP and cGMP correspond well to the one of cytoplasm flowing rhythmically. cAMP concentration monitored at a fixed position in frontal portion of plasmodium oscillates in 1 to 2 min period as same as cytoplasmic flow, however showing 1/3 phase difference. Previous study revealed that the behavior *i.e.* chemotaxis and cell motility, and differentiations such as spore formation take place coherently over a whole cell of acellular slime mold plasmodium in spite of the cell size over 10 cm, and

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those phenomena were controlled by oscillation patterns and rhythms of intracellular chemical mediators such as ATP and cAMP, and cytoplasmic flows [1]–[3]. Consequently from such experimental evidence, we can recognize that the spatio-temporal order generated in living organisms directly relates to intracellular biochemical reactions.

2   Chemical Reaction Network and Spatio-temporal Pattern

Living systems consist of plenty of molecules and more than thousand of chemical reactions between them. In general, a collision between molecules is an elementary process of a chemical reaction, indicating that a reaction rate is usually proportional to a probability of collision. The probability of collision is proportional to products of concentrations of molecules, which leads to "the mass-action-law". For a reaction between A and B chemical species, the reaction rate is described as (1), a nonlinear differential equation of first order in terms of time where ξ_a and ξ_b are concentration of A and B respectively, and h is the reaction rate.

$$\frac{d\xi_a(t)}{dt} = \frac{d\xi_b(t)}{dt} = -h\xi_a(t)\xi_b(t) \tag{1}$$

Generally, in living organisms more than a few thousands of chemical compounds exist and they form complex chemical reaction networks. Each elementary reaction is nonlinear in terms of reaction rate that scales in products of concentrations. Thus the reaction networks may involve highly nonlinear properties. Because detailed balance must be maintained in each elementary process even in complex chemical reaction networks in a condition of thermal equilibrium, directional (stable) chemical reaction flux should not be generated in the networks. This indicates that stable rhythms or self-organized spatial patterns should not be generated as in living organisms. However the situation essentially changes in non-equilibrium conditions. Variety of spatial and temporal structures will be generated in complex network systems. When fast reactions and slow reactions coexist in elementary processes

of reaction networks, fast reactions, in general, can be eliminated adiabatically. Therefore, the characteristics of networks in slow time scale can be described by renormalizing fast reactions in slow reactions. If the characteristics of networks can be contracted to two variables by adiabatic elimination of variables, a closed stable limit cycle can be described in phase space. In case of contraction to three variables, spiral evolutional process or spatio-temporal chaos can be described. In this manner, Prigogine attempted to characterize dynamical properties of living systems by a small number of variables [4].

We assume that the characteristics of chemical reaction networks in a living organism can be contracted to two slow variables, u and v, that represent concentrations of activator and inhibitor respectively. We can describe the following reaction-diffusion equation, taking D as a diffusion coefficient.

$$\begin{aligned} \frac{\partial u}{\partial t} &= f(u, v) + D_u \nabla^2 u \\ \frac{\partial v}{\partial t} &= g(u, v) + D_v \nabla^2 v \end{aligned} \qquad (2)$$

Notice that the function f or g generally involves non-linear terms of over third order, because many (second order) elementary reaction rate processes based on volume effects are contracted as mentioned above. Equation (2) enables us to describe time evolutional concentric circles or spirals mathematically and spatio-temporal patterns observed in Belousov-Zhabotinskii reaction at $D_u \approx D_v$. A self-generating and self-repairing Turing pattern is expected at $D_u \ll D_v$, and in fact De Kepper et al. demonstrated these patterns in real aqueous chemical reaction in 1990s. At $D_u \gg D_v$, spatio-temporal chaos is generated, thus the system expresses more complicated behavior; nerve excitation transduction equation belongs to this category.

3 Nonlinear Characteristics of Biomacromolecules

It has been known that specific nature of various spatio-temporal patterns of living organisms, such as traveling waves shown in heart,

slime mold or fertilized eggs, intercellular synchronization, dynamics of groups of organisms or ecosystem and further spatio-temporal patterns in neural networks can be described in a frame of the reaction-diffusion equation shown above. On the other hand, many studies have been conducted for Belousov-Zhabotinskii reaction recognizing it as a real space model of metabolic reaction in living organisms. Spatio-temporal patterns as concentric circles and spirals have been reported in various experimental systems by modifying combination of chemical compounds. From these experimental studies, it has gradually become evident that active molecules i.e. radicals must exist as intermediates in reaction networks in order to introduce over third order non-linearity to contracted terms of reaction. Such a highly active molecular species are ordinary dangerous compounds for living cells, therefore the scenario that living cells utilize them for generating rhythms or spatio-temporal structures seems to be unreasonable.

Authors consider that non-linear properties utilized by living organisms relate not only to a rate process based on mass action law but also to mesoscopic structure such as biomacromolecules or membrane [5]. DNA and some types of proteins are known to show on/off type structural transition according to concentration change of ions or ATP essential in energy metabolism. A system that transits discontinuously between two states is categorized as the first order phase transition by Landau's phase transition theory. If on/off type transition is described as a change of an order parameter η between 0 to 1, the free energy F can be expressed as a fourth order function of η.

$$F \sim \frac{1}{4}\eta^4 - \frac{1}{2}\eta^3 + \frac{1}{4}\eta^2 + \alpha\eta \tag{3}$$

where α is a variable depending on environment as mentioned below. To simplify the discussion, other coefficients are selected approximately. An order parameter η can be regarded as a density reformed in zero-th dimension. When R_c and R_g are the first dimensional size of a chain molecule in random coil-like expanding form and folding form respectively, an order parameter for chain molecule in size R is described as $\eta = (R^{-3} - R_c^{-3})/(R_g^{-3} - R_c^{-3})$.

In the first order phase transition, the central limit theorem is held when N, the number of elements in a system is infinite: Avogadro number. Thus the states of $\eta = 0$ and 1 do not coexist, except for the point of phase transition: fluctuation is not explicit in macroscopic static physical properties. However, because N is finite in each DNA or protein molecule, η becomes a fluctuating physical value. Therefore we must note that the phase transition observed in an ensemble of molecules can not be characterized as a point but a finite width.

Each biomacromolecule is a mesoscopic system that consists of 10^4 to 10^{10} atoms. This situation neglects discontinuity derived from quantum theory and enables us to describe the state of a biomacromolecule as a continuous function in terms of η like as equation (3). Assuming that a slope of free energy in terms of an order parameter η determines change rate of an order parameter η, temporal change of the system can be shown as equation (4) where k is constant.

$$\begin{aligned} \frac{d\eta}{dt} &\cong -k\frac{\partial F}{\partial \eta} = \\ &= -k\left\{ \eta \left(\eta - \tfrac{1}{2} \right) (\eta - 1) + \alpha \right\} \end{aligned} \qquad (4)$$

The authors' recent studies found that the above aspect of phase transition is useful at least for describing a collapse transition of long chain DNA ($\eta = 0$ and $\eta = 1$ correspond to unfolded coil and collapsed coil respectively) [6]–[12].

4 Dialogue between Environment and Biomacromolecules

Figure 2 shows the experimental data of a collapse transition of a DNA molecule (here 166×10^3 base pairs T4DNA) according to the concentration change of trivalent biological polyamine (spermidine). Each molecule undertakes on/off type transition. Most of cells existing on the Earth contain DNA molecules of over 10^6 base pairs (Mega base pair, Mbp). Because DNAs exist as unfolded coils in good solvent, water and are highly ionized, and counter ions such as K^+ and Na^+ freely drift in water, electroneutrality is maintained for the whole solution.

On the contrary negative electrical charges on a DNA must form ion pairs with counter ions in order to be collapsed compactly. In other words obvious amount of ions such as K^+ or Na^+ must be captured according to a collapse transition of DNA. Consequently, it becomes clear that the collapse transition of DNA is an on/off type and large amount of ions move before and after the transition. Taking this fact in consideration, we discuss a simple cell model below.
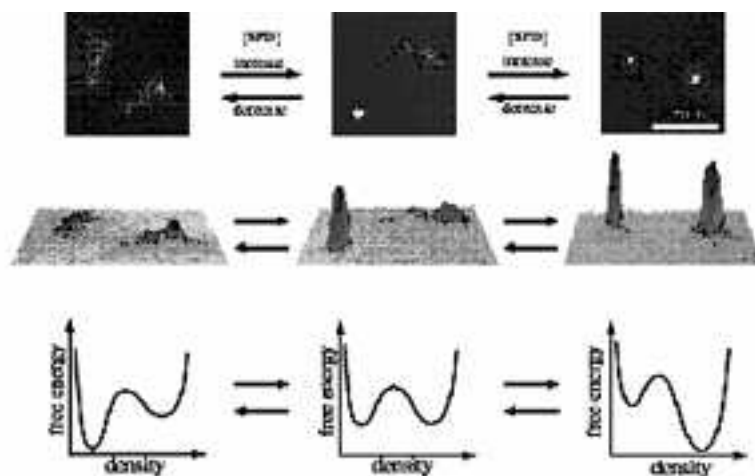

Figure 2. Discrete coil-globule transition of DNA molecules induced by spermidine. Top: Fluorescence image of T4DNA molecules. Middle: Quasi-three-dimensional profile of light intensity, corresponding to top images. Bottom: The change in the free energy profile.

As shown in figure 2, multivalent cation causes a collapse transition of DNA. In fact it has become clear that multivalent cations dissolved in solution bind to DNA according to a collapse transition, by the results of detailed experiments. Because the size of cell is finite (μm scale), a switching of amount of ions bound to DNA implies drastic change of ion concentration of "environment" (in this case, cytoplasmic solution). Reminding that polyamines used in the experiment shown in figure 2 are ubiquitous through prokaryotic to eukaryotic cells, here we regard

concentration c as an environmental parameter.

Living cells require energy metabolism to maintain their own life. A role of currency of energy exchange is played by ATP, which possesses negative charge. Therefore let's take intracellular concentration of ATP, x as a parameter which exhibit the state of energy. Polyvalent cations decrease the effective concentration activity in the solution by binding with ATP. By introducing constant K and s relating to binding equilibrium, the concentration of polyamines in environment forms a function of x like as (5).

$$c(x) = \frac{s}{Kx + 1} \qquad (5)$$

Reminding that logarithm of c corresponds to chemical potential, α is described as a linear function of $\ln(c)$ because an environmental parameter α given in equation (3) corresponds to the chemical potential of polyvalent cations in this model. It is already known that the translation entropy change derived from exchange between polyvalent cations and univalent cations most significantly contributes in the free energy change where translation entropy scales in $\ln(c)$.

$$\begin{aligned} \frac{d\eta}{dt} &= -k\eta \left(\eta - \frac{1}{2}\right)(\eta - 1) \\ &\quad + k\{a\ln(Kx + 1) - b\} \end{aligned} \qquad (6)$$

Concluding the above discussion, temporal change of the order parameter of DNA leads to equation (6), provided that a and b are constant.

Considering that cells are thermo-dynamically open system, we define an energy parameter x most simply as follows, provided that A and B are constant.

$$\frac{dx}{dt} = A - B(1 - \eta) \qquad (7)$$

Equation (7) represents the change where chemical energy consumption is sustained in the collapsed state and promoted in the unfolded state. In fact the gene expression is sustained and the energy

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consumption rate comes to the minimum when DNAs existing in a cell are compactly collapsed.
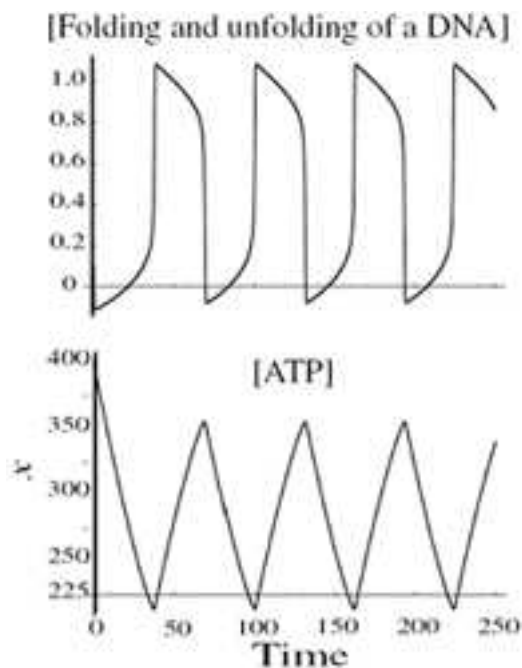

Figure 3. Periodic oscillation of higher order structure of DNA. Collapsed globular state and elongated coil state is created in order parameter $\eta = 1$ and $\eta = 0$, respectively. In this simulation, following parameters are used for initial condition; $a = -0.3$, $b = -0.4$, $K = 0.01$, $A = 5$, $B = 10$, $k = 10$, $(\eta, x) = (0.1, 400)$.

Two variables in equation (6) and (7) give rise to a limit cycle (bifurcation) when the parameter A concerning energy influx exceeds a threshold. Simulations of spontaneous rhythms derived from this model are shown in figure 3. The above model substantially indicates that living system breaks translation symmetry of time and generates rhythms dissipating energy by use of non-linear functions incorporated in the characteristics of giant DNA molecules. We recently observed

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self-oscillation of single giant DNAs (data not shown) in actual experiment with laser trapping, as shown in figure 4 [13]. Rhythmic conformational change in a DNA is introduced by the local temperature gradient generated by the absorption of the photo energy of the infrared laser.
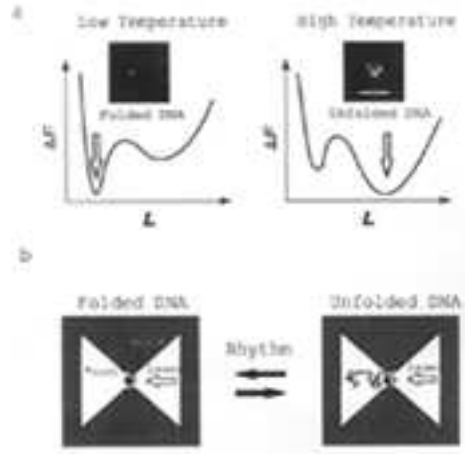

Figure 4. Schematic representation of rhythmic conformational change in a giant DNA chain. a) Folding/unfolding transition of a giant DNA chain in poly (ethylene glycol) (PEG) solution with a change in temperature, where ΔF is the free energy difference and L is the long-axis length of a DNA chain. A giant DNA chain exhibits a discrete transition between the folded collapsed state and the unfolded coil state with a change in either temperature or the concentration of condensation agents (folding/unfolding transition). In PEG solution, the folded DNA chain tends to unfold with increasing temperature. Fluorescence images of folded and unfolded DNA chains are also shown. Scale bar, 5 μ m. b) Rhythmic conformational change in a giant DNA chain. Under thermodynamically open conditions with local heating with a focused CW laser, it is considered that a DNA chain in PEG solution undergoes a rhythmic folding/unfolding transition between two states because the transition is sensitive to the temperature gradient around the focus.

Once non-linear rhythms happen in individual cells, as mentioned

above, various spatio-temporal structures will be spontaneously generated in the level of a multicellular system, a body of organisms. No rhythm takes place without consuming chemical energy as ATP in living system. Understanding the living system as a non-equilibrium and non-linear system, we approach the issue – how does autonomous system of combined various molecules, such as molecular motor, is alive. This trial has just started.

5 Spontaneous Rhythms Developed in A Dissipation System

We have discussed a hypothesis that bistable mesoscopic system generates a limit cycle oscillation under non-equilibrium and non-linear open condition, for the systems including DNAs. Here we introduce two experimental results where mechanical oscillatory motions are promoted.

Figure 5 shows spontaneous rhythmic movement of oil-water system driven by oscillation of interfacial tension accompanied with the repetitive change of the contact angle [14], [15]. Without a spatial guidance, the interface moves randomly (Figure 5). On the other hand introducing achiral shape of rotor, we can regulate the direction of the motion in deterministic manner (data not shown). The rhythmic change of the interfacial tension is caused through high non-linearity of transportation process of surfactant molecules from aqueous phase into organic phase in a far-from-equilibrium condition on concentration of the surfactant. Based on experiments, the mechanism of the motion is summarized as follows:

1) Surfactant molecules in aqueous phase tend to diffuse to interface and form monolayer at the interface, interfacial tension decreased gradually. In the process, the contact angle among oil-water, water-substrate, and oil-substrate is held over 90 degree to keep Young's equation.

2) When the interfacial tension reaches lower critical value, the monolayer collapses and cationic surfactants migrate into organic phase with anionic chemical, I^{3-}, in cooperative manner, forming reversed micelles or W/O emulsions.
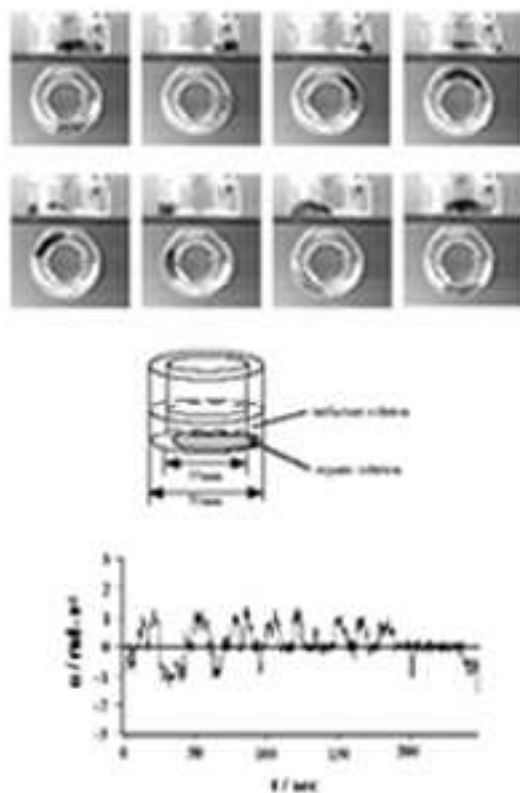
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Figure 5. Spontaneous motion of an oil droplet (2 ml) under an aqueous phase (10 ml) in an annular container with a time interval of 0.6 sec. The upper and lower parts of each picture show the side view and bottom view, respectively. The aqueous phase is 1 mM trimethyloctadecylammonium chloride solution, and the organic phase is a 2 mM iodine solution of nitrobenzene saturated with potassium iodide. b) Time trace of the angular velocity of the center of mass for the oil droplet. The movement was digitized with a time interval of 1/10 sec. The angular velocity was obtained as the first derivative by a 9 point least-square fitting. This indicates that the oil droplet tends to maintain one-directional motion for a while, but then switches to another direction. (partly from Yoshikawa, K. *et al.* [14])

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In the moment, the interfacial tension increases rapidly, and the contact angle decreases below 90 degree. As the result, the interface becomes "shrink" state, the oil droplet is pushed from the side of the interface with inverted contact angle.

3) Accompanied by the gradual decrease of the interfacial tension, the Young's equation recovers due to the increase of contact angle above 90 degree.

4) Then, the next cycle begins.

In living organisms, vectorial process such as force generation in muscle and active transport through membrane is done in direct conversion of chemical energy. This system demonstrates the coupling of dissipation of chemical energy and vectorial process is combined in a non-linear dynamical system with the boundary condition of broken chiral symmetry. This insists that non-equilibrium fluctuation can generate directional locomotion in asymmetric potential whereas the most of discussion are based on thermal equilibrium fluctuation for a thermal ratchet model [16]–[18]. Notice that "asymmetrical boundary condition" and "non-linear oscillation," like limit cycle, are important to realize this artificial chemical motor.

The locomotive machine of the living organisms employs highly effective transformation from chemical energy to mechanical energy under isothermal conditions. Molecular pumps incorporated in cells are also such highly effective molecular machines. These proteins might be working in the same principle as mentioned above for the molecular motor.

6   Conclusions

In this article, we have taken an architectural approach to living phenomena with information of characteristics of the element (biomolecule). Functions of biomolecules have been previously discussed focusing mostly on the key-and-keyhole like network logic based on strong and specific interactions of ligands. However we here insisted that talking between biomolecules and environmental "field" based on weak and global interactions arisen from many small ions and ATP, is

essential to understand the dynamical properties of living system. This also leads to the idea that the size of the space in which biomacro-molecules generate dynamic changes must be significant because the concentration of substances in the field must change drastically in a small system like a cell. Where we should go next is to find principles underlying cell-sized small systems where non-linear dynamic phenomena take place based on the interaction between biomacromolecules and surrounding field. New directions will grow in a fusion of mathematical science and material science.

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