COMPARTMENTALIZATION OF SELF-REPRODUCING MACHINERIES: MULTIPLICATION OF MICROSYSTEMS WITH SELF-INSTRUCTING POLYMERIZATION OF AMINO ACIDS

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Abstract. A theoretical model is presented for the self-instructing polymerization of free amino acids which proceeds inside microsystems which are phase-separated from the solution of thermal polyamino acids. It is shown theoretically that a compartmentalized microsystem fixes inside itself only the process with a faster macromolecular multiplication as time passes, even if the catalytic polymerization alone could spontaneously decrease the corresponding reaction rate. The compartmentalized machinery of macromolecular multiplication cannot reach its stationary state. The machinery is inevitably multiplied and alternates with those with either faster rates of macromolecular multiplication or slower rates of macromolecular degradation during their time development. These results are based upon the dynamic process that any material system acts by itself so as to remove any flow disequilibrium, that is, to maintain the continuity of material flow.

1. Introduction

Many experiments on chemical and prebiotic evolution have been intended to narrow the gap between simulated protobiogeneses (Fox and Dose, 1977), which stand repeated tests, and the contemporary biological machineries of protein synthesis with both polypeptides and polynucleotides. In fact, recent experiments on simulated protobiogenesis may have reached such a level as to suggest that Fox's microspheres, phase-separated and compartmentalized in the solution of lysine-rich thermal polyamino acids, perform the self-instructing polymerization of free amino acids when ATP is supplied (Fox and Nakashima, 1980). The compartmentalized microsystems make their own polyamino acids. This observation now raises the new theoretical problem of why and how the testable self-instructing polymerization of free amino acids could transform itself into the contemporary form of polymerization coded in terms of polynucleotides, and if this really happens in the course of development.

A well-known theoretical model on the coded polymerization is Eigen's hypercycle (Eigen, 1971; Eigen and Schuster, 1977, 1978a, b). Hence, the theoretical problem of bridging the gap between the self-instructing and the coded polymerizations of free amino acids is reduced to hypothesizing the possible appearance of hypercyclic systems where proteins and nucleic acids are present under the condition that the self-instructing polymerization initially occurs in the medium of free amino acids, polyamino acids and energy-rich nucleoside phosphates.

Simulated protobiogeneses suggest that the cell-like structures or, protocells, are first phase-separated as compartmentalized microsystems and reproduced before the multi-

plication process of macromolecules emerges inside the protocells (Fox and Dose, 1977). Simulated protocells lack the function of reproducing macromolecules at their initial stages, but the hypercyclic process, on the other hand, is capable of reproducing macromolecules. Nevertheless, a theoretical hypercycle cannot determine its own boundary conditions or external constraints, that is, the compartmentalized structure within which the multiplication of macromolecules proceeds (Epstein and Eigen, 1979; Küppers, 1979). In order to narrow the gap between the self-instructing and the coded polymerizations of free amino acids, a means of incorporating the multiplication process of macromolecules autonomously into a locally compartmentalized structure must be found.

The present problem can be stated as: Why and how compartmentalized hypercycles came into existence? In this paper, we shall attempt to describe the operational process of materializing an autonomous compartmentalization of macromolecular multiplications. The basis of the theoretical investigation is upon the experiments indicating (Fox and Nakashima, 1980). that the self-instructing polymerization of free amino acids may proceed inside microspheres with lysine-rich thermal polyamino acids when energy-rich ATP is fed.

2. Formation of Microsystems

Compartmentalizations of locally condensed peptide bond linkages may occur in both types of solutions of thermal polyamino acids (Fox, 1976) and amino acids with metal ions (Yanagawa and Egami, 1978). A possible theoretical interpretation for the formation of such compartmentalized microsystems is that if the polymerization due to peptide bond synthesis is not in a complete balance with the hydrolysis, the solution of polyamino acids will become unstable against a spontaneous disturbance forming microscopic compartments of locally condensed peptide bond linkages (Matsuno, 1980). Both the accumulation of polyamino acids and the number of peptide bond linkages inside the compartmentalized microsystems increase with time so long as the solution remains in a nonequilibrium or disequilibrium state, lacking the balance between the polymerization and the hydrolysis (Matsuno, 1980).

Since both the polymerization and the hydrolysis of polyamino acids are responsible for compartmentalization of phase-separated microsystems, a most simplified process includes three different kinds of polyamino acids A, B and AB following the reaction

$$A + B \underset{k_2}{\overset{k_1}{\rightleftarrows}} AB, \tag{1}$$

where water participating in polymerization and hydrolysis is deleted for simplicity, and k_1 and k_2 are the corresponding reaction coefficients in the mass action approximation. The reaction coefficients are by no means exogenous parameters. They determine their own values endogenously as the reaction proceeds. The underlying autonomous process is that the smaller the hydrolytic decay rate k_2 becomes, the more stable the reaction

system will be (Matsuno, 1979). In fact, a spontaneous formation of locally condensed peptide bond linkages decreases the average hydrolytic decay rate k_2 of the reaction system.

An impetus for the compartmentalizations is the imbalance or the disequilibrium between polymerization and hydrolysis. So long as the disequilibrium between the two elementary processes continues to survive, phase-separated microsystems can be formed and multiplied without being accompanied by the multiplication and the evolution of the constituent polyamino acids. We need other reactions for realizing the macromolecular multiplication and evolution.

3. Self-Instructing Polymerization

Based upon the observation that the self-instructing polymerization of free amino acids energized by ATP proceeds inside microspheres made of lysine-rich thermal polyamino acids (Fox and Nakashima, 1980; Nakashima and Fox, 1980), we postulate the catalytic reaction

$$a_1 + a_2 + \cdots + a_p + AB \xrightarrow{f_A} A + AB \tag{2}$$
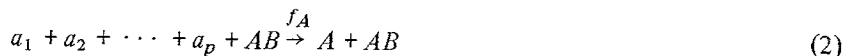
in addition to a simple reaction of polymerization and hydrolysis (1), where a_i ($i = 1, 2, \cdots, p$) represents each of energy-rich free amino acids and f_A is the corresponding reaction coefficient within the mass action approximation. The energy source for the energy-rich building materials $\{a_i\}$ is due to ATP. The catalytic reaction is modelled after the polymerization catalyzed by lysine-rich polyamino acids.

Now, imagine that an arbitrary microsystem S, compartmentalized in the solution of polyamino acids A, B and AB, makes its own polyamino acids A by taking energy-rich free amino acids into itself from the outside environment. The mass action approximation, which we follow, prescribes the rates of change in the numbers n_A, n_B and n_{AB} of polyamino acids A, B and AB inside microsystem S. The number rate of polyamino acid A polymerized per unit time from the building elements coming into S from the outside environment is

$$\dot{n}_A^{(in)} = f_A n_{AB}. \tag{3}$$

The degradation rate of polyamino acid A going out of S per unit time is expressed as

$$\dot{n}_A^{(deg)} = g_A n_A, \tag{4}$$

where the value of the degradation coefficient g_A depends upon the structure which S maintains by itself. In fact, the structure of S has to be determined in a self-consistent manner along with the interaction parameters.

A specific problem we shall consider is the autonomous mechanism which determines both the interaction parameters f_A and g_A endogenously.

First of all, the incoming flow $\dot{n}_A^{(in)}$ must keep the balance with the outgoing flow

$$\dot{n}_A^{(out)} \equiv \dot{n}_A^{(deg)} + \dot{n}_{A,Ac}, \tag{5}$$

in which $\dot{n}_{A,Ac}$ represents the flow of accumulation of polyamino acid A inside S, otherwise the law of material conservation would be violated. The law of material conservation, however, also implies that even if the flow disequilibrium

$$\Delta \dot{n}_A \equiv \dot{n}_A^{(in)} - \dot{n}_A^{(out)} \tag{6}$$

$$\neq 0 \tag{7}$$

happens to occur by any chance, microsystem S must immediately act so as to remove the imbalance through the autonomous adjustments of the interaction parameters f_A and g_A. The adjustable parameters within the mass action approximation are only those which describe the strengths of interaction among measurable quantities.

To see the underlying dynamic process of removing flow disequilibrium, let us suppose that an initial state at time $t = t_1 - 0$ is slightly in disequilibrium

$$\Delta \dot{n}_A = \Delta \dot{n}_A^{(1)} \; (\neq 0) \tag{8}$$

and that each adjustment of removing flow disequilibrium takes place at every time interval of a sufficiently small Δt. The adjustment at $t = t_i + 0$ ($t_i = t_0 + i\Delta t, i = \cdots, -2, -1, 0, 1, 2, \cdots$) is taken to remove the disequilibrium at $t = t_i - 0$.

Define the quantities

$$\Delta f_A^{(i)} \equiv f_A(t_i + 0) - f_A(t_i - 0), \tag{9}$$

$$\Delta g_A^{(i)} \equiv g_A(t_i + 0) - g_A(t_i - 0), \tag{10}$$

$$\Delta \dot{n}_{A,Ac}^{(i)} \equiv \dot{n}_{A,Ac}(t_i + 0) - \dot{n}_{A,Ac}(t_i - 0), \tag{11}$$

where f_A, g_A and $\dot{n}_{A,Ac}$ are functions of time. Then, the disequilibrium at time $t = t_i - 0$ with

$$\Delta \dot{n}_A^{(i)} = f_A(t_i - 0)n_{AB}(t_i) - g_A(t_i - 0)n_A(t_i) - \dot{n}_{A,Ac}(t_i - 0) \tag{12}$$

is removed by the adjustment either

$$\left. \begin{array}{l} \Delta f_A^{(i)} = -\Delta \dot{n}_A^{(i)}/n_{AB}(t_i) \\[2mm] \Delta g_A^{(i)} = 0 \end{array} \right\} \tag{13}$$

or

$$\left. \begin{array}{l} \Delta f_A^{(i)} = 0 \\[2mm] \Delta g_A^{(i)} = \Delta \dot{n}_A^{(i)}/n_A(t_i) \end{array} \right\} \tag{14}$$

at time $t = t_i + 0$. At this point, we note that the time interval Δt is chosen to be suffi-

ciently small so that the adjustments of both the parameters f_A and g_A cannot occur simultaneously. In addition, non-autonomous disturbances are not assumed for the time being.

Expressions (13) and (14) lead to

$$\Delta f_A^{(i)} = -\alpha_f^{(i)} \, \Delta \dot{n}_A^{(i)} / n_{A\,B}(t_i), \qquad (\alpha_f^{(i)} = 0 \text{ or } 1) \tag{15}$$

$$\Delta g_A^{(i)} = \alpha_g^{(i)} \, \Delta \dot{n}_A^{(i)} / n_A(t_i), \qquad (\alpha_g^{(i)} = 0 \text{ or } 1) \tag{16}$$

with

$$\alpha_f^{(i)} + \alpha_g^{(i)} = 1 , \tag{17}$$

where $\alpha_f^{(i)}$ and $\alpha_g^{(i)}$ are random variables taking only the values 0 or 1 as being subject to (17), unless otherwise specified.

The adjustment at time $t = t_i + 0$ in turn causes a change in the accumulation rate of polyamino acid A inside S as

$$\Delta \dot{n}_{A,Ac}^{(i)} = \Delta f_A^{(i)} n_{A\,B}(t_i) - \Delta g_A^{(i)} n_A(t_i) \tag{18}$$

and, consequently, a new disequilibrium at time $t = t_{i+1} - 0$ results with the flow imbalance

$$\Delta \dot{n}_A^{(i+1)} = -g_A(t_i + 0)\dot{n}_{A,Ac}(t_i + 0)\Delta t . \tag{19}$$

In obtaining (19), we have assumed that the number $n_{A\,B}$ of polyamino acid AB inside S determines its own value immediately as equilibrating both the reactions of polymerization and hydrolysis in (1). This assumption is plausible since the presence of microsystems is a result of equilibration between the two reaction processes. On the other hand, the flow disequilibrium (19) is due to the imbalance between the catalytic polymerization and the associated degradation. Equation (19) tells that the previous flow disequilibrium (12) induces a new one at a later time in a recurrent manner.

Supposing that the time derivatives $\dot{f}_A$ and $\dot{g}_A$ exist at the limit $\Delta t \to 0$, one obtains

$$\ddot{n}_{A,Ac} = \dot{f}_A n_{A\,B} - \dot{g}_A n_A \tag{20}$$

with the aid of (18). The result of the sequential adjustments removing flow disequilibrium now leads to

$$\dot{f}_A = \frac{\alpha_f(t)g_A}{n_{A\,B}} \left(\int_{t_1}^{t} (\dot{f}_A n_{A\,B} - \dot{g}_A n_A) \, \mathrm{d}t + \dot{n}_{A,Ac}(t_1 - 0) \right), \tag{21}$$

$$\dot{g}_A = -\frac{\alpha_g(t)g_A}{n_A} \left(\int_{t_1}^{t} (\dot{f}_A n_{A\,B} - \dot{g}_A n_A) \, \mathrm{d}t + \dot{n}_{A,Ac}(t_1 - 0) \right), \tag{22}$$

with

$$\{(\alpha_f(t) - 1)^2 + (\alpha_g(t))^2\}\{(\alpha_f(t))^2 + (\alpha_g(t) - 1)^2\} = 0 \,, \tag{23}$$

where α_f and α_g are real stochastic variables subject to the constraint (23). The accumulation of polyamina acid A inside S follows

$$\dot{n}_A = \int_{t_1}^{\prime t} (\dot{f}_A n_{AB} - \dot{g}_A n_A) \, dt + \dot{n}_{A,Ac}(t_1 - 0) \,. \tag{24}$$

The coupled equations (21)–(24) provide an autonomous mechanism determining all of the interaction parameters f_A and g_A and the measureble quantity n_A in an endogenous manner. The stochastic variables α_f and α_g, on the other hand, are operational parameters representing the indefiniteness of the intensive capacity which microsystem S maintains in itself.

The present autonomous process of removing flow disequilibrium gives the inequalities either

$$\left.\begin{aligned} \dot{f}_A &\leqslant 0 \\ \dot{g}_A &\geqslant 0 \\ \dot{n}_A &\leqslant 0 \end{aligned}\right\} \tag{25}$$

or

$$\left.\begin{aligned} \dot{f}_A &\geqslant 0 \\ \dot{g}_A &\leqslant 0 \\ \dot{n}_A &\geqslant 0 \end{aligned}\right\} \tag{26}$$

depending upon the initial condition either $\dot{n}_{A,Ac}(t_1 - 0) < 0$ or $\dot{n}_{A,Ac}(t_1 - 0) > 0$, If the first set of inequalities (25) is the case, the inequality

$$\dot{g}_A(t') > \dot{g}_A(t) \quad \text{for} \quad t' > t > t_1 \tag{27}$$

will follow provided that $\alpha_g(t) = \alpha_g(t') = 1$.

Since the stochastic variable α_g takes only the values either 1 or 0 and since the time derivative $\dot{g}_A$ is always a non-negative function according to the premise, the inequality (27) turns out to imply that the degradation coefficient g_A will diverge as time increases. This gives a trivial consequence, resulting in an extinction of microsystem S. The premise $\dot{g}_A \geqslant 0$ is thus invalidated for surviving microsystems. So long as microsystems can emerge and continue to survive, the inequalities (26) must be satisfied.

Surviving microsystem S, within which the multiplication of macromolecule A proceeds, exhibits such a unidirectionality that the reaction coefficient f_A for self-instructing polymerization increases as a function of time, and that the degradation coefficient g_A decreases as a function of time. The autonomous process of removing flow disequilibrium, an expression of the law of material conservation, forces microsystem S

to fix inside itself only the reaction process with faster macromolecular multiplication over time. The self-instructing polymerization compartmentalized inside microsystem S allows and materializes only the reaction process that alternates with those of faster reaction rates during development, in spite of the fact that the self-instructing polymerization alone could decrease its reaction rate spontaneously. If a spontaneous transformation of macromolecular structure is accompanied by an increase in the rate of the self-instructing polymerization, the new structure will be fixed, and the newly fixed species will be just an exemplification of molecular evolution.

The autonomous process of removing flow disequilibrium also forces the surviving microsystem S to fix its own structure of compartmentalization so that the degradation velocity of outgoing macromolecules may decrease with time. The structure of compartmentalization allows and materializes only such a transformation with smaller degradation velocity during its time development.

A principal finding in this section is that the compartmentalized machinary within which the self-instructing polymerization proceeds can exhibit macromolecular evolution through its multiplication process, although the compartmentalized structure also depends upon the latter process.

4. Multiplication of Microsystems

The dynamic process of removing flow disequilibrium, developed in the preceding section, is autonomous in the sense that the microsystem acts so as to remove the flow disequilibrium which it caused. The autonomous process of removing flow disequilibrium creates a new disequilibrium to be removed in the same manner. However, the possibility also exists that the flow disequilibrium will appear spontaneously without being affected by the previous operations. An example of disturbances giving the flow disequilibrium with non-autonomous origns is seen in a spontaneous change in the environmental condition outside the microsystem. This results in affecting either the catalytic polymerization of polyamino acids or the degradation of the multiplied macromolecules, or both. One should note that once a non-autonomous disturbance has spontaneously occurred, its effect is absorbed in the later autonomous process of removing flow disequilibrium.

Consequently, the autonomous process of removing flow disequilibrium in the presence of non-autonomous disturbances determines the interaction parameters f_A and g_A as following

$$\dot{f}_A = \frac{\alpha_f(t) g_A}{n_{AB}} \left(\int_{t_1}^{t} (\dot{f}_A n_{AB} - \dot{g}_A n_A) \, dt + \dot{n}_{A,Ac}(t_1 - 0) \right) + \dot{f}_A^{(ex)} \qquad (28)$$

$$\dot{g}_A = -\frac{\alpha_g(t) g_A}{n_A} \left(\int_{t_1}^{t} (\dot{f}_A n_{AB} - \dot{g}_A n_A) \, dt + \dot{n}_{A,Ac}(t_1 - 0) \right) + \dot{g}_A^{(ex)} , \qquad (29)$$

$$\dot{n}_A = \int_{t_1}^{t} (\dot{f}_A n_{AB} - \dot{g}_A n_A) \, dt + \dot{n}_{A,Ac}(t_1 - 0) , \qquad (24)$$

where $\dot{f}_A^{(ex)}$ and $\dot{g}_A^{(ex)}$ represent the exogenous contribution arising from spontaneous changes in the environmental conditions.

In order to estimate the contribution of non-autonomous disturbances to the autonomous process of removing flow disequilibrium, consider a particular case such that

$$\dot{f}_A^{(ex)} = 0 , \tag{30}$$

$$\dot{g}_A^{(ex)} = \begin{cases} x & t_2 < t < t_3 \\ 0 & \text{otherwise} \end{cases} \tag{31}$$

with $t_2 > t_1$. Supposing that the time difference $(t_3 - t_2)$ is sufficiently small so as to satisfy

$$\dot{g}_A = \dot{g}_A (t_2 - 0) + x \quad \text{for} \quad t_2 < t < t_3 , \tag{32}$$

one obtains

$$\dot{g}_A (t_3 + 0) = \alpha_g (t_3 + 0) \left\{ \dot{g}_A (t_2 - 0) - g_A (t_3) \left(\frac{n_{AB}}{n_A} \dot{f}_A (t_2 - 0) - \right.\right.$$
$$\left.\left. - (\dot{g}_A (t_2 - 0) + x) \right) (t_3 - t_2) \right\} , \tag{33}$$

where $\dot{g}_A (t_2 - 0)$ represents a non-zero value of $\dot{g}_A (t)$ at the limit of approaching $t = t_2$ from below.

If the inequality

$$\dot{g}_A (t_3 + 0) > 0 \tag{34}$$

is the case, the autonomous process of removing flow disequilibrium without being subject to further non-autonomous disturbances at times later than $t = t_3$ will eventually lead to an extinction of microsystem S through the divergence of the degradation coefficient g_A, as discussed previously. The condition for extinction (34) is rewritten as

$$x > -\dot{g}_A (t_3 - 0) + \frac{n_{AB}}{n_A} \dot{f}_A (t_2 - 0) - \frac{\dot{g}_A (t_2 - 0)}{(t_3 - t_2) g_A (t_3)} . \tag{35}$$

The present result concludes that any stationary microsystem in the sense of

$$\dot{f}_A = \dot{g}_A = 0 \tag{36}$$

is vulnerable against any non-autonomous disturbances increasing the degradation velocity of macromolecules leaving the microsystem, which produce the extinction of the microsystem itself. The same vulnerability holds for the non-autonomous disturbances decreasing the rate of self-instructing polymerization.

Thus the stable stationary states are unattainable for microsystems when non-autonomous disturbances originating in the external environment are not prohibited. As any microsystem loses its structural identity during development, multiplication of microsystems is a form of losing the structural identity associated with each microsystem,

and experimental evidence suggests that the multiplication proceeds through buddings (Fox and Dose, 1977; Yanagawa and Egami, 1978) or generation of endoparticles (Brooke and Fox, 1977).

The compartmentalized machinery of multiplying macromolecules through the self-instructing polymerization cannot avoid the multiplication of itself and its alternation by others. The machinery, within which the macromolecular multiplication and evolution proceed, always alternates with those either with an increase in the rate of macromolecular multiplication or with a decrease in the rate of macromolecular degradation during development.

5. Discussion

We have introduced a theoretical model aimed at explaining in a consistent manner both the protocellular and the macromolecular multiplication and evolution. The underlying dynamics responsible for the multiplications and the evolutions is simply the law of material conservation in the context that any material system acts by itself so as to remove any flow disequilibrium, which would violate the continuity of material flow.

The autonomous process of removing flow disequilibrium makes it inevitable that the compartmentalized machineries, or protocells, within which the macromolecular multiplication and evolution proceed, are also multiplied and alternated during development. One cannot apply a fixed boundary condition, such as a constant over all organizations (Eigen, 1971) or constant fluxes (Epstein and Eigen, 1979), to a self-reproducing machinery which will eventually lose its structural identity. The compartmentalization of self-reproducing machineries is of an emergent character and is determined consistently inside the macromolecular processes.

The modelled self-instructing polymerization of free amino acids inside a compartmentalized self-reproducing machinery is autocatalytic. The process of macromolecular multiplication can exist even if the coupling between polypeptides and polynucleotides is absent. Hence, if a spontaneous appearance of polynucleotides in the self-reproducing machinery is accompanied by either an increase in the rate of the catalytic polymerization or a decrease in the degradation velocity of outgoing macromolecules, then the polynucleotides will be fixed in the machinery.

Acknowledgments

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