An Effective Hierarchical Model for the Biomolecular Covalent Bond: An Approach Integrating Artificial Chemistry and an Actual Terrestrial Life System

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Abstract Under the AChem paradigm and the programmed self-decomposition (PSD) model, we propose a hierarchical model for the biomolecular covalent bond (HBCB model). This model assumes that terrestrial organisms arrange their biomolecules in a hierarchical structure according to the energy strength of their covalent bonds. It also assumes that they have evolutionarily selected the PSD mechanism of turning biological polymers (BPs) into biological monomers (BMs) as an efficient biomolecular recycling strategy. We have examined the validity and effectiveness of the HBCB model by coordinating two complementary approaches: biological experiments using existent terrestrial life, and simulation experiments using an AChem system. Biological experiments have shown that terrestrial life possesses a PSD mechanism as an endergonic, genetically regulated process and that hydrolysis, which decomposes a BP into BMs, is one of the main processes of such a mechanism. In simulation experiments, we compared different virtual self-decomposition processes. The virtual species in which the self-decomposition process mainly involved covalent bond cleavage from a BP to BMs showed evolutionary superiority over other species in which the self-decomposition process involved cleavage from BP to classes lower than BM. These converging findings strongly support the existence of PSD and the validity and effectiveness of the HBCB model.

Keywords
Collective reutilizability, bond energy, programmed self-decomposition model, unicellular organism, hydrolysis, individual death

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I Introduction

One of the remarkable features of artificial chemistry (AChem) [5], in which analogue chemical reactions are simulated on computers, is that it postulates that all the elementary steps of existing terrestrial life consist of chemical phenomena, with no exceptions [3]. Moreover, AChem takes on the construction of an even closer relationship with existent terrestrial life as its lofty goal [1, 2, 5, 26]. AChem research is thus expected to greatly advance conventional artificial life (ALife) research by providing powerful approaches to studying actual intracellular life activities and their evolution [5, 17, 23, 24, 27]. Among such approaches, network artificial chemistry (NAC), proposed by Hideaki Suzuki [24, 25], can be regarded as a useful paradigm that embraces various possibilities, widely ranging from basic study to application development.

A principal feature of NAC is that it defines interelement relationships by topological networking. Its conceptual framework and approach methodology enable NAC to express a dynamically and continuously changing relationship, which cannot be fully defined by arrays of symbols [4] and lattice structures [29], such as those used in conventional AChem research. NAC also focuses on the strength of interelement linkages introduced as a hierarchy of covalent bonds, hydrogen bonds, and van der Waals force. Then it constructed a model in which the difference in strength of the interelement linkage and the interaction between molecules or intramolecular units introduce molecular structures and molecular reactivity. These two features, based on actual chemical phenomena, have thrown light on the mechanism of cluster generation from molecular folding [25] and suggest that this model attests to the temporal and spatial structure of an actual molecule.

Among the promising features of NAC, we are particularly interested in the hierarchical structure of the strength of interelement linkage (bond energy). By introducing the hierarchical structure of bond energy into chemical systems that constitute the metabolic system of terrestrial life, we can hope to uncover the latent functionality and rationale of the chemical system.

Toward this end, we have used molecular cell biology to build a model of the hierarchical structure of bond energy in the fundamental unit of terrestrial life, that is to say, the cell, a self-organized chemical system consisting of molecules and their interaction and transformation. In concrete terms, we have hierarchically categorized biomolecules in a cell into four classes on the basis of the complexity of the interatomic network. We have also categorized biomolecular covalent bonds, which comprise one class in Suzuki's bond-energy hierarchy, into three classes, based on bond energy. We have found that these two hierarchical structures are fully compatible with one another. According to these classifications, we constructed a hierarchical model for the biomolecular covalent bond—the *hierarchical biomolecular covalent bond* (HBCB) model—and examined its validity and effectiveness as a way to coordinate two independent approaches: study of an existing terrestrial life system, and AChem.

Coordination between building the model, comparing it with an existing terrestrial life system, and examining it by means of AChem throws light on the usefulness of this study, which focuses on AChem. Under the AChem paradigm the model itself can be described as an automaton in the form of a chemical linkage that significantly corresponds to an existing terrestrial life system. If the model succeeds in describing terrestrial life activities, we should be able to examine whether a phenotype of the model actually exists in terrestrial life, by the use of cell biological methods.

However, we cannot examine the evolutionary superiority of the proposed model by comparing it with alternative hypothetical models within the scope of biological studies of actual terrestrial life. One reason is that an organism species as a phenotype of an alternative model may not exist. Moreover, the time scale required for examining evolutionary superiority significantly exceeds the operability of experiments on existing life.

AChem may therefore open the way for ultimate verification. Since AChem presupposes that all the elementary steps of actual terrestrial life consist of chemical phenomena without exception, it is feasible to build an automaton based on chemical linkages in imitation of existing terrestrial life and to observe its behaviors in evolutionary simulations.

In this article, as a first step toward employing such perspectives, we built our HBCB model under an NAC-inspired AChem paradigm in accord with our previously proposed programmed self-decomposition (PSD) model. This model assumes that terrestrial organisms arrange their biomolecules in a hierarchical structure according to the energy strength of their covalent bonds and effectively utilize the hierarchical structure. Next, we examined the existence of a mechanism illustrating this model by means of cell biological and biochemical methods, using existing terrestrial life as experimental material. In parallel with this approach, we developed an experimental AChem system by installing the HBCB model into the SIVA series [19–21], our previously developed artificial ecosystem, and examined whether life activities utilizing the HBCB model had evolutionary superiority.

2 Construction of the Hierarchical Model for the Biomolecular Covalent Bond

2.1 Feasibility of Evolutionary Development of a Hierarchical Structure in the Biomolecular Covalent Bond

The biological polymer (BP) responsible for essential and specific activities that actualize survival and self-reproduction of living organisms has extremely large molecular size. At the same time, BPs have a highly specific structure and function. Therefore, it is extremely difficult to reutilize them, not only in another individual, but even in other tissues or organs in a single individual.

In order for living organisms to reutilize a BP, they must decompose it into smaller elements by cleaving a considerable portion of the chemical bonds that form the BP, and build the required BP with these elements. As the specificity of the structure and function of such elements decreases, their general versatility increases. So, the smaller the elements of the decomposed BP are, the greater the possibility that the decomposed product can be reused. Thus each decomposition product has a different level of versatility as a biological resource. We call such versatility *collective reutilizability*. However, reconnecting the elements into a large-scale biomolecule requires an appropriate amount of bond energy. So the smaller the elements, the larger the amount of energy required for rebuilding. That is to say, a kind of tradeoff takes place.

In biomolecular recycling, this relationship would exert an evolutionary pressure on terrestrial life to keep energy loss as low as possible and make the collective reutilizability of decomposed elements as great as possible in either living individuals or the ecosystem itself. At the same time, there would be more evolutionary pressure to make the amount of released energy as small as possible in the decomposing process and to keep the amount of bond energy possessed by the decomposed elements as large as possible, because the energy to exercise the activities of terrestrial life is fundamentally supplied in the form of chemical bond energy. Such evolutionary pressures might have induced evolutionary selection of a reasonable hierarchical structure of covalent bonds and a biomolecular recycling mechanism effectively utilizing such hierarchical structure. If our hypothesis is borne out, we should be able to identify proofs that reflect the existence of such a mechanism in existent terrestrial life. Therefore, we first attempted to hierarchically classify biomolecules constituting terrestrial life from the viewpoint of system factors of an automaton in the form of chemical linkage.

2.2 Classification of Biomolecules into a Hierarchical Structure

A complex hierarchical structure is observed in the anatomy of terrestrial life. It can be categorized into five classes, from highest to lowest: individual, organ, tissue, cell, organelle. We can hierarchically classify the biomaterials that are the components of the above structures according to the complexity of the interatomic network (Table 1). The five classes are biological polymer (BP), biological monomer (BM), organic biomaterial (BO), inorganic biomaterial (BI), and basic bioelement (BE).

BE, the lowest fundamental class (class V), is composed of only five kinds of elements. The second lowest class (class IV), BI, is composed of substances formed by combining the members of the BE class. BO, the middle class (class III), is composed of substances formed by combining the

Table 1. Hierarchization of biomaterials of terrestrial life based on the complexity of interatomic networks.

Class name	Number of types	Substance examples
I. Biological polymer (BP)	$10^2 - 10^9$	Lipid, polysaccharide, protein, nucleic acid
II. Biological monomer (BM)	Several tens	Glycerol, fatty acid, hexose, amino acid, nucleotide, etc.
III. Organic biomaterial (BO)	More than ten	Pyruvic acid, acetyl-CoA, ribose, etc.
IV. Inorganic biomaterial (BI)	Four	H_2O, CO_2, PO_4^{3-}, NH_4^+
V. Basic bioelement (BE)	Five	C, H, O, N, P

members of the BI class. The members of the BI class and the BO class can exist independently and stably. They have specific chemical properties that clearly differentiate them. The BM class (class II) is composed of substances formed by combining the members of the BO class. The chemical substance of the several tens of types in the BM class is further classified into four groups according to their structure and function. It becomes possible to generate a remarkable diversity through permutation and combination of the members of the BM class. This is how the highest class (class I), BP, is composed. This class plays a fundamental role in self-reproductive activities and contains an extremely large number of members.

The elements of class V do not exist independently as stable biomolecules. In the following section, we focus on the top four classes (classes I–IV) as the hierarchical structure of biomolecules based on complexity (Figure 1a).

We next focus on conversions of the biomolecules to other classes. These conversions are nothing more than the generation and cleavage of covalent bonds, which are essentially attributed to energy absorption caused by bond generation and energy release caused by bond cleavage. Regarding the amount of input and output of bond energy due to covalent bond generation and

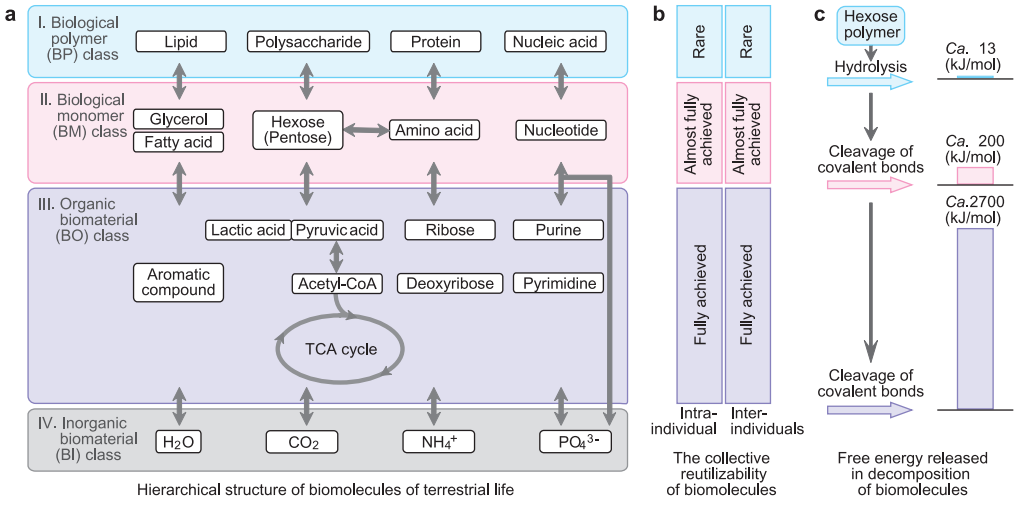

Figure 1. Hierarchization of biomolecules and their collective reutilizability. (a) Hierarchical structure of biomolecules of terrestrial life. (b) The collective reutilizability of biomolecules. Both intra- and interindividual collective reutilizability of BPs is extremely limited. However, almost all BMs, which are classified just below the BPs, are reutilized in most terrestrial organisms. (c) Free energy released in decomposition of biomolecules. Each bar indicates the amount of released energy associated with each step of covalent bond cleavage in the decomposition of a hexose polymer.

cleavage, we note the tendency for the amount to decrease when a conversion occurs between higher classes, and for it to increase when a conversion occurs between lower classes. Thus we can hierarchically categorize covalent bonds between biomolecules into three classes on the basis of bond energy.

Take the example of saccharides (Figure 1c). When a polysaccharide, a hexose polymer, in the BP class is converted to monosaccharides in the BM class, there is a release of energy of approximately 13 kJ/mol accompanied by the cleavage of the glycosidic bond. Similarly, converting a monosaccharide in the BM class to the components of the tricarboxylic acid (TCA) cycle in the BO class generates a release of energy of approximately 200 kJ/mol (when decomposing one glucose molecule into two molecules of lactic acid). Moreover, when BO is converted to BI such as carbon dioxide and water, a release of energy of approximately 2700 kJ/mol (when decomposing two molecules of lactic acid) associated with the cleavage of covalent bonds is generated [11]. Thus, in the molecules that compose terrestrial life, the hierarchy inside the covalent bond can be roughly categorized into three classes based on bond energy. This tendency is observed not just for saccharides, but for all BPs, including proteins, nucleic acids, and lipids. It is noteworthy that the energy release associated with a conversion from a BP to BMs is particularly small. Bonds connecting BMs to build BPs, such as the glycosidic bond and peptide bond, all become broken by hydrolysis, which is a cleavage of bonds by the intervention of water (H_2O) and is characterized by a small energy gap accompanying the reaction.

We find that a four-class hierarchical structure of biomolecules based on complexity and a three-class hierarchical structure of covalent bonds based on bond energy are fully compatible with one another. We previously reported on a prototype version of this hierarchical classification of biomolecules [18, 21].

2.3 Programmed Self-decomposition Model

In exploring the characteristics of the hierarchical structure of biomolecular covalent bonds, we regard biomolecules as resources for composing living organisms. In terms of the collective reutilizability of resources, the circulation of material in a terrestrial ecosystem, namely, its biomolecular recycling mechanism, provides significant insights. That mechanism, examined in this article, is discussed below.

The terrestrial ecosystem forms a nearly closed system in that both its space and substance are limited. Accordingly, to maintain the stability of terrestrial life activities, the space and substance removed from the environment by life activities have to be returned to the environment. That is to say, the ecosystem must be returned to its original state. The mechanism for restoring the terrestrial ecosystem has conventionally been explained by the principle of biological circulation called the food chain [16], which is a biomolecular recycling mechanism for terrestrial life. We have set up a new hypothesis that is complementary to that of the food chain. In our view of the terrestrial ecosystem, besides the restoration of the environment by the food chain, another hidden mechanism is fundamentally built into every life individual, by which it autonomously decomposes itself so as to contribute to the restoration of the environment [18, 21]. We consider the phenomenon of decomposition by the life individual's own effort, called *self-decomposition*, to be a controlled biochemical process of returning substance and space that the individual possesses to the environment for the purpose of restoring the environment to its original state. We call this *programmed self-decomposition* (PSD). We have developed a self-reproductive, self-decomposable (SRSD) automaton using von Neumann's self-reproductive automaton model [30, 31] as a prototype (Figure 2).

Von Neumann's self-reproductive automaton model can be summarized as follows: automaton A produces an automaton according to instruction tape I (information registered on a tape). Automaton B replicates tape I. Automaton C combines with A and B, and controls them. Automaton D is composed of A+B+C. Instruction tape I carries instructions to describe the automaton, and I_D carries the instructions of D. Automaton E, composed of D+ I_D, can reproduce itself. Instruction tape I_{D+F} carries instructions describing both D and F, which can be any given automata.

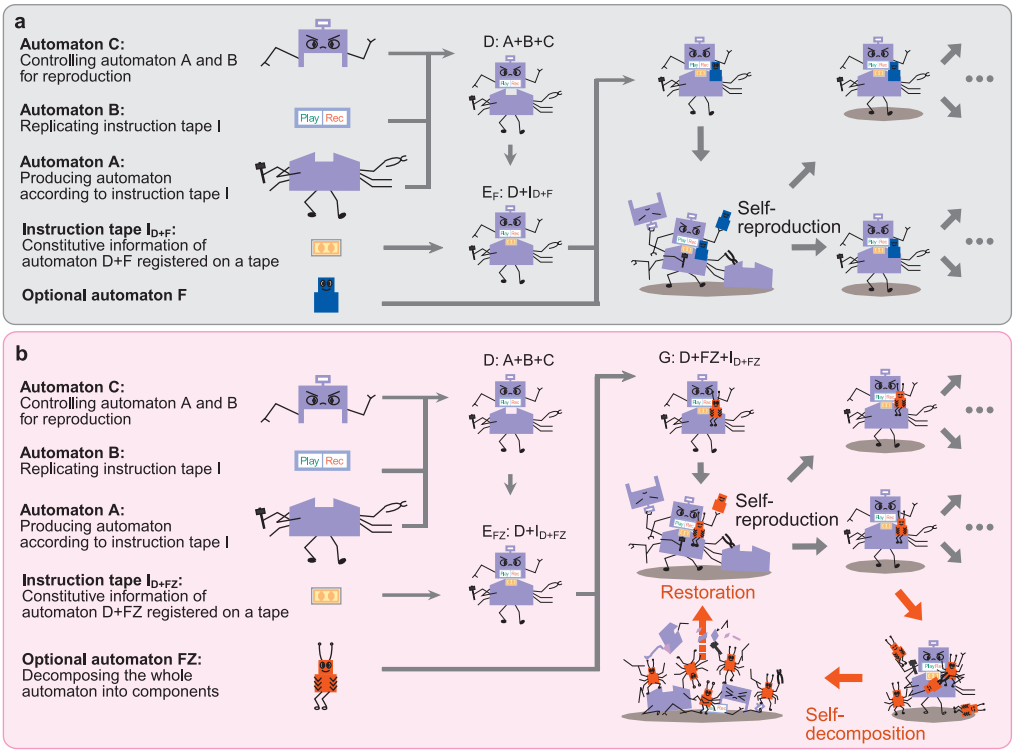

Figure 2. Von Neumann's self-reproductive automaton and Oohashi's self-reproductive, self-decomposable automaton. (a) Von Neumann's self-reproductive automaton model. This model embodies the sequence of self-reproduction of life as a physical machine and its evolution in that process, without the sequence becoming a vicious circle. This is an immortal-type model without an autonomous mechanism for the restoration of the environment to its original state. (b) Oohashi's self-reproductive, self-decomposable (SRSD) automaton model. This model uses von Neumann's self-reproductive automaton model as its prototype. It has a programmed mechanism contributing to the restoration of the environment to its original state through autonomous individual death with self-decomposition, which is an essential feature of terrestrial life.

Automaton E_F, whose instruction tape I_D is replaced by I_{D+F}, can reproduce E_F and produce another automaton F. This model expresses the sequence of self-reproduction of life as a physical machine and its evolution in that process, without falling into a vicious circle. It is also interesting in that automaton E can be compared to a cell, and instruction tape I to its gene. However, this is an immortal-type model without an autonomous mechanism for the restoration of the environment to its original state.

Oohashi's SRSD automaton [18, 21] was developed using von Neumann's self-reproductive automaton E_F as its prototype. Automaton FZ is a modular subsystem comparable to von Neumann's automaton F. It functions to decompose into components the whole automaton in which it is incorporated. Instruction tape I_{D+FZ} carries an instruction describing automaton D and an instruction describing the newly defined automaton FZ for decomposition. Automaton E_{FZ} is comparable to von Neumann's automaton E_F, whose tape I_{D+F} is replaced by tape I_{D+FZ}. Automaton G is composed of E_{FZ} and FZ, that is D+FZ+ I_{D+FZ}. This automaton G can reproduce G itself, and produce FZ as a module within G. FZ is usually latent, but it decomposes G when activated by a certain trigger. Two activation modes are defined for the self-decomposition automaton FZ. The first one is activated by a signal input from outside, indicating unconformity between the life and its habitation environment. The second mode constitutes the end of the life span. If, after a certain length of biological time has passed or a certain set of events has occurred, there is still no signal input from outside to trigger an action, this situation itself becomes an internal trigger that activates

FZ. Note that the concepts of reproduction and decomposition partially include chemical reactions known as anabolism and catabolism.

When we investigated actual life in relation to this SRSD automaton, one significant issue was the cost-benefit comparison between the acquisition of collective reutilizability and the loss of bond energy induced by covalent bond cleavage associated with self-decomposition by automaton FZ. It is possible that the requirement for improvement of efficiency acts as an evolutionary pressure and that the genetic programs of existent terrestrial life involved in a self-decomposition mechanism have evolved toward more efficient biomolecular reutilization.

2.4 Hierarchical Model for the Biomolecular Covalent Bond

When we look on biomolecules as the resource for the material that composes terrestrial life, a highly significant hierarchy is observed in terms of the collective reutilizability of biomolecules (see Figure 1b). Generally, BPs in the highest class have molecular structures that differ across individuals. They differ even across different sites in the same individual. That means that collective reutilizability of BPs is not guaranteed, not only between different individuals, but even between different tissues or organs in a single individual. In contrast, it is noteworthy that the collective reutilizability of biomolecules markedly increases as they are converted to the BM class. They then become reutilizable in every organ and tissue in the same individual, as well as in terrestrial life in general. Of course, this collective reutilizability is increased by a conversion to a class lower than BM (i.e., the BO or BI class) in some cases, but the increase is less than that caused by a conversion from BP to BM.

When we look at the cost-benefit comparison between the acquisition of collective reutilizability and the energy loss induced by cleavage of biomolecular covalent bonds, these two phenomena are seen to be in the above-mentioned tradeoff relationship. Keeping collective reutilizability as high as possible and energy release as low as possible at the point of bond cleavage would strengthen survival capacity. It is possible that this requirement acts as an evolutionary pressure and leaves behind certain universal mechanisms for existent terrestrial life as the fruits of evolutionary selection. It may also be concluded that there exists a rational hierarchy of biomolecules, from the viewpoint of bond energy, with an optimum point at which there is an advantageous decomposition from one particular class to another, as compared to decomposition to a higher or lower class.

We suppose that this significant point corresponds to the process by which BPs are cleaved to BMs. Members in the BM class can be universally reutilized among almost all species, and at the same time, a conversion between this class and the higher BP class is associated with only a very small amount of energy input and output. It can be surmised that an evolutionary pressure toward this significant point has worked effectively during the evolutionary process.

We have made a working hypothesis that existing terrestrial life evolutionarily selects a biomolecular recycling mechanism that optimizes cost-benefit performance by routing through the BM class. In other words, we observe a trade-off between diversity, collective reutilizability, and energy efficiency associated with an interclass conversion in the biomolecular hierarchical structure of terrestrial life. The BM class is a significant point at which collective reutilizability is almost assured for all terrestrial life and, at the same time, the amount of released energy is minimized when converting from the higher class. Therefore, we assume that a biomolecular recycling mechanism that optimizes cost-benefit performance by making the BM class a relay point should be the outcome of evolutionary selection.

We call this hypothesis the *hierarchical model for the biomolecular covalent bond* (HBCB model). If the biomolecular recycling mechanism assumed in the model actually exists and manifests itself, the evolutionary superiority, validity, and effectiveness of the HBCB model as well as the existence of a PSD mechanism in the actual terrestrial life system will be clearly shown. In this study we examine the validity and effectiveness of our HBCB model in simulations of AChem in conjunction with obtaining corroborative evidence for the existence of a PSD mechanism in experiments using terrestrial life system.

3 Investigation of the PSD Model and the HBCB Model Utilizing a Living Terrestrial Life System

3.1 Materials and Methods

3.1.1 Investigation Design

In an experiment utilizing living cells of actual terrestrial life, we looked for the biomolecular recycling mechanism proposed by our HBCB model. We asked if a self-decomposition mechanism existed in a state consistent with the PSD model and if the mainstream of chemical reactions came about as a result of the generation of BMs by hydrolytic cleavage of a BP based on the HBCB model. It is advantageous for us to focus on the self-decomposition process in the experiment. Since various processes, including synthesis and decomposition, progress simultaneously in terrestrial life in general, it is not easy to examine the effectiveness of the HBCB model. On the other hand, the process that leads to death and decomposition consists mostly of reactions leading to self-decomposition and, limitedly, of other, contaminating simultaneous processes. Therefore, we considered the self-decomposition process the most appropriate choice for investigating the biomolecular covalent bond cleavage process in living organisms in as coherent a state as possible.

Accordingly, we first set the following four steps for verification:

1. Our most basic problem was to find an appropriate species from among existing terrestrial life. Using such a species, we then looked for the autonomous degradation of cells that could be interpreted as self-decomposition consistent with the PSD model.
2. We looked for evidence of the expression of self-decomposition corresponding to the genetically programmed process.
3. We looked for evidence that self-decomposition in actual life was not a natural random degradation of biological resources with energy release (exergonic reaction), but rather a controlled active metabolic process with energy consumption (endergonic reaction).
4. We looked for evidence that hydrolytic reactions from a BP to BMs are the main pathway of the autonomous degradation of cells, which can be interpreted as a self-decomposition process.

We set up this four-step verification process as follows.

3.1.2 Test Organism

We selected the protozoan *Tetrahymena pyriformis* strain W [6, 15] for the experimental material for the following reasons:

1. *Tetrahymena* is a unicellular organism, so cell death equals individual death. Experimenting on *Tetrahymena*, therefore, excludes the problem of mixed occurrence of partial death (including apoptosis) and individual death that is inevitable when using a multicellular organism.
2. *Tetrahymena* is a eukaryotic cell and contains various kinds of independent organelles (intracellular functional particles), so *Tetrahymena* can easily be compared with an automaton model that defines the organelle as a functional modular subsystem.
3. *Tetrahymena* has unlimited proliferability without limitation of cell division caused by telomere shortening. Although some species have both a macronucleus and a micronucleus and perform sexual reproduction by conjugation, other species, such as the *pyriformis* used in this study, have only a macronucleus and perform unlimited proliferation by asexual reproduction. In this regard *Tetrahymena pyriformis* is analogous to the automaton model.

4. *Tetrahymena* has well-developed lysosomes, organelles in which various kinds of hydrolytic enzymes densely accumulate. These enzymes degrade a BP into BMs by hydrolytic reaction. This feature of the lysosome corresponds closely to the automaton FZ defined as the functional modular subsystem for decomposition in our SRSD automaton model [18, 21].
5. The authentic type culture and the method of cultivating a pure culture are well established, and there is no symbiosis of other gene systems in the cell. Consequently, this pure culture contributes to a simplification of the experimental condition.

These five characteristics of *Tetrahymena pyriformis* make it an appropriate choice for the experimental material we required for our investigation of the HBCB model and the PSD model.

3.1.3 Induction of Self-decomposition

To achieve clear-cut results in cell biological experiments, it is necessary to construct experimental conditions in which the physiological activity of each cell is controlled in a coherent state so that self-decomposition is released simultaneously in a large population of cultivated protozoa (approximately 10^5 individual cells in 1 ml of culture medium).

Two different types of triggers of self-decomposition are defined in the PSD model [18], namely, an external factor of high unconformity with the environment (the first mode) and an internal factor of natural life span (the second mode).

Self-decomposition as natural death at the end of the life span is a phenomenon readily observed in nature. Since natural death is an autonomous internal phenomenon, it is difficult to avoid having a large majority of normal living cells (individuals) and a small number of self-decomposing cells randomly coexisting in the culture medium. Therefore, it is difficult to conduct a clear-cut experiment that requires precisely controlled conditions without a time lag with respect to the biological activity of each individual cell. Consequently, we could not adopt such a process in this experiment.

On the other hand, induction of self-decomposition by an encounter with an environment highly incompatible with life activity can be manipulated by external factors including factitious operations. Well-controlled experimental conditions can induce expression and progression of self-decomposition in a way that satisfies the experimental objectives. If we wish to establish an experimental method that induces self-decomposition in all cultured cells simultaneously, we must discover an external message that triggers the self-decomposition program inside the gene. In other words, we must locate environmental information signaling inadaptability, and develop a technique for simultaneously distributing such a signal to every cell in a culture. However, since such a signal might have an extremely oppressive effect on all life activities, it is likely that this signal will cause serious damage to the controlled physiological processes that are responsible for self-decomposition. Thus the signal might interfere with the expression or progression of self-decomposition. We determined that the following experimental protocol might overcome such an antinomy. First, an external signal triggering the emergence of self-decomposition is given in a short period of time to activate the genetic program. Next, the culture condition is immediately returned to one appropriate for life, so that the physiological processes responsible for self-decomposition can progress with little damage. Based on this principle, we developed the following method, which we call the impulse shock method.

First, to obtain the homogeneous physiological condition of every cultured cell, we subjected the protozoan *Tetrahymena* [14] cells to a procedure that synchronized the cell cycles according to established methods [32]. Next, we applied two treatments to this culture environment:

1. *Impulse heat shock treatment:* The temperature of the culture medium was rapidly increased to a value unfit for survival, sustained for a short period of time, then returned rapidly to the original optimum condition. In the present experiment, we increased the temperature of the entire culture medium to 39°C, kept it there for 21 min, then returned it to the original temperature of 26°C [28].
2. *Impulse pH shock treatment:* Similarly, the hydrogen ion concentration (pH) of the *Tetrahymena* culture medium was rapidly changed to a value unfit for survival, sustained for a short period

of time, then rapidly returned to the original optimum condition. In the present experiment, we made the culture medium pH 4 by adding hydrochloric acid (acidification). After keeping it there for 450 s, we quickly neutralized the culture medium to its original pH 7 by adding a tris base, which becomes a weak basic buffer. Note that the above-mentioned examples of concrete parameters might slightly change according to minor differences in experimental conditions. The onset of the impulse shock treatment was designated as the 0 hour for various observation and measurement purposes.

3.1.4 Observation of the Phenomenon of Self-decomposition

We observed the timeline of the morphological changes in the cells to confirm that the phenomenon induced in each cell by the impulse shock method was a self-decomposition process. We used the impulse heat shock treatment and supravital staining with acridine orange to stain the intracellular lysosomes [13], which correspond to the automaton FZ in the PSD model. Acridine orange specifically stains lysosomes and their contents in an acid condition. We then observed their behavior under a fluorescence microscope.

3.1.5 Inhibition of the Self-decomposition Process

In order to investigate what kind of biochemical process self-decomposition is, the following procedures were administered immediately after returning the cultured cells to the appropriate culture condition following the impulse shock treatment, and the influence of subsequent decomposition of the cells was investigated in terms of population, individual condition (morphology and mobility), and hydrolytic enzyme activity.

1. To investigate whether self-decomposition is a process programmed inside the gene, we added actinomycin D, an inhibitor of the transcription from deoxyribonucleic acid (DNA) to messenger ribonucleic acid (mRNA), to the culture medium.
2. To confirm if self-decomposition is an endergonic reaction, that is, an active process requiring energy, the supply of oxygen to the medium was restricted for inhibition of energy-requiring metabolic processes.
3. To investigate if the main reaction of the self-decomposition process is hydrolysis of a BP into BMs, we added the reagent chloroquine, a specific inhibitor of the whole hydrolytic enzyme group generated in the lysosome [8]. The lysosome is a strong candidate for a decomposition module FZ in our SRSD automaton model.

3.2 Results

3.2.1 Morphological Changes in *Tetrahymena* Cells and Lysosomes in the Self-decomposition Process Induced by Impulse Heat Shock Treatment

Impulse heat shock treatment of *Tetrahymena* cells brought about morphological changes of the cells and changes in the behavior of the lysosomes that corresponded to the automaton FZ with its self-decomposition function. We observed these changes by means of acridine orange supravital staining, which causes areas in a neutral condition, such as normal cytoplasm and each of the organelles, to appear green, and areas in an acid condition, namely the lysosome and its contents, to appear orange. The following series of self-decomposition processes was observed synchronously in almost all of the cells (Figure 3).

In normal living cells at the 0 hour, the cytoplasm and organelles were stained a similar green, indicating a neutral condition, which is suitable for normal metabolic activities. There were a few

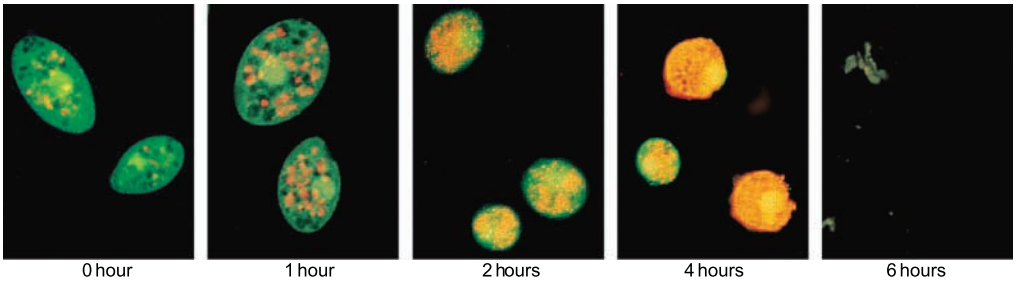

Figure 3. Morphological changes in *Tetrahymena* cells and lysosomes in the self-decomposition process induced by impulse heat shock treatment. To observe the changes in appearance of cells and lysosomes induced by impulse heat shock treatment, the distribution of intracellular pH was visualized by acridine orange supravital staining and photographed under a fluorescence microscope. The green in the photographs indicates the areas or modules in a neutral condition, such as normal cytoplasm. Orange indicates the areas or modules, including lysosomal granules, in an acid condition and the areas throughout which the lysosomal contents were diffused. *0 hour:* Normal living cells. There were a few lysosomal granules. The entire cytoplasm is stained green, indicating a neutral condition. *1 hour:* The number of lysosomal granules increased, indicating an increase in lysosome biosynthesis. The cells lost mobility and swelled slightly. *2 hours:* The number of lysosomal granules further increased. The cells became spherical and decreased in volume, resulting in a further increase in the relative density of lysosomes in a cell. *4 hours:* Lysosomal membranes ruptured, and their contents diffused throughout the whole cell, making the intracellular environment acidic. This condition activated the lysosomal acid hydrolytic enzymes. This finding suggests that hydrolysis turning BPs into BMs was concentrated in this phase. Most of the cell membranes remained, and each cell still included both intracellular components to be decomposed and hydrolytic enzymes under acidic conditions. Such a situation would be reasonable for intensive decomposition of the intracellular contents by lysosomal enzymes. *6 hours:* Cell membranes lysed, and cells decomposed into a homogenate.

particles stained orange, which were considered to be lysosomes. The cells had a pyriform shape and were mobile.

One hour after the impulse heat shock treatment, almost all of the cells had lost mobility and had slightly swelled. Although the cytoplasm and nucleus were green, indicating that they had maintained ordinary life activity under neutral conditions, the number of acidic orange particles (i.e., lysosomes) increased. These observations indicate the biosynthesis of lysosome and its contents, namely hydrolytic enzymes. Lysosomal hydrolytic enzymes were all isolated within lysosomal granules. They are activated specifically in a low-pH (acid) condition but not in a neutral or alkaline condition. We concluded there had been very little hydrolysis of BPs in the cytoplasm during this phase. This finding suggests that this phase is a preparatory stage for execution of self-decomposition.

Two hours after treatment, the number of lysosomal granules had further increased, although the cytoplasm of the cells remained in a neutral condition. In addition, the cells had become spherical and decreased in volume. The synergic effect of these factors resulted in a significant increase in the relative density of the lysosomes in the cell. The increase of acidic lysosomal granules during this period indicated an increase in lysosome biosynthesis, suggesting that an active biochemical process in preparation for hydrolysis of self-decomposition was in progress.

Four hours after the impulse heat shock treatment, the lysosomal membranes had ruptured. The lysosomal contents diffused at high density throughout the whole of each cell and mixed with the BPs. Most of the cell membranes still remained. The intracellular environment in this phase became acidic and the lysosomal enzymes became active, suggesting that hydrolysis was rapidly proceeding. These processes seem reasonable candidates for intensive decomposition of the intracellular contents by lysosomal enzymes. Since the systems to decompose BMs, such as glycolysis and the TCA cycle, are optimized under neutral conditions, it is unlikely for such pathways to have contributed to decomposition in this phase. It could be assumed that the cleavage of covalent bonds associated with hydrolysis using lysosomal enzymes to turn a BP into BMs had played a major role in the observed self-decomposition process. Measurement of the activity of the lysosomal hydrolytic enzymes described below supports this assumption.

Six hours after treatment, the cell membranes had lysed and the cells had decomposed into a homogenate. This finding suggests the completion of the self-decomposition process; the BP of the

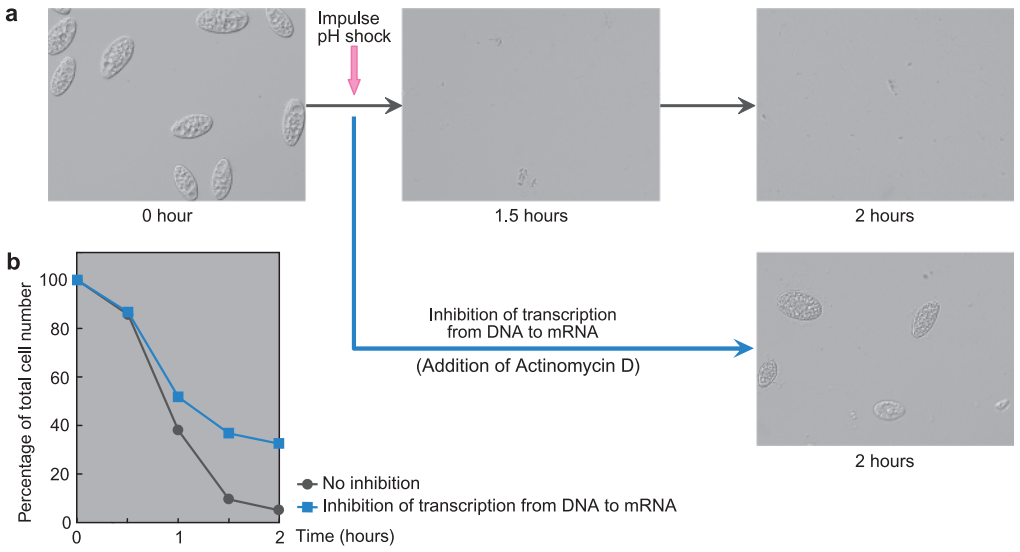

Figure 4. Self-decomposition is an expression of a genetic program. (a) Self-decomposition of cells and its inhibition. Top: Self-decomposition of cells induced by impulse pH shock treatment. Cells rapidly decomposed and became homogenate in approximately 1.5 to 2 h. Bottom: When the expression of genetic programs was inhibited by adding the transcription-inhibiting reagent actinomycin D immediately after induction of self-decomposition by impulse pH shock treatment, some cells remained without self-decomposition, and some of these cells started moving again and began reproducing themselves. (b) Timeline for the decrease in cell number. The number of cells at each time point is shown as the percentage of the number at 0 hour. Cells rapidly decreased in number after the impulse pH shock treatment. Inhibited expression of genetic programs suppressed the decrease in cell number. These findings suggest that the self-decomposition mechanism is a controlled biochemical process that is programmed in the genes and induced by its expression.

cells had decomposed into BMs, which, having a high degree of collective reutilizability, were returned to the environment.

3.2.2 Examination of Whether Self-decomposition Is an Expression of a Genetic Program

To inhibit the expression of a genetic program, actinomycin D, an inhibitor of transcription from DNA to mRNA, was added into the culture medium after the impulse shock treatment; therefore, the large cell population often suppressed the self-decomposition process even though there was insufficient reproduction. Furthermore, a considerable part of the population of the cell was observed to have recovered from this brief arrest of cell movement and reproduction (Figure 4). These experimental results indicate that the decomposition of cells induced by impulse shock treatment requires gene transcription, so that it must be a genetically controlled process according to the central dogma. This finding suggests that gene programs predicated on the PSD model do exist and function as described above.

3.2.3 Examination of Whether Self-decomposition Is an Energy-Requiring, Active Process

When general biological metabolic processes that require an energy supply were inhibited by restricting the supply of oxygen to the culture medium, decomposition of cells was clearly inhibited (Figure 5). This is an important finding because, if decomposition of the cells in this system were merely uncontrolled random degradation with release of energy, decomposition of the cells would progress without an energy supply. On the contrary, the decomposition process did not progress without such an energy supply. This indicates that the observed decomposition is an endergonic, actively controlled process with reduction of entropy. These results, indicating that self-degeneration is a genetically controlled process that requires an energy supply, support our PSD model.

3.2.4 Examination of Whether the Main Reaction of the Self-decomposition Process Lies in Hydrolysis Decomposing a Biological Polymer into a Biological Monomer

The lysosomal hydrolytic enzyme group specifically acts to cleave the covalent bonds of a BP into BMs. The addition of chloroquine, which is a comprehensive and exhaustive inhibitor of these lysosomal hydrolytic enzyme activities, into the culture medium clearly inhibited the self-decomposition of the cells (Figure 5). This means that the hydrolytic activities of the lysosomal enzymes were an essential factor in the observed decomposition. This finding further supports our HBCB model.

3.2.5 Confirmation that Self-decomposition Is an Energy-Requiring, Genetically Regulated Process Mediated by Lysosomal Hydrolytic Reactions

To further confirm that the self-decomposition is an energy-requiring, genetically regulated process and is mediated by lysosomal hydrolytic reactions, we directly measured the activity of the lysosomal enzymes during the self-decomposition process and examined whether the activity change was affected by the inhibition of the expression of the genetic program or an energy-requiring, active metabolic process. Two of the most common marker enzymes, representative of all lysosomal acid

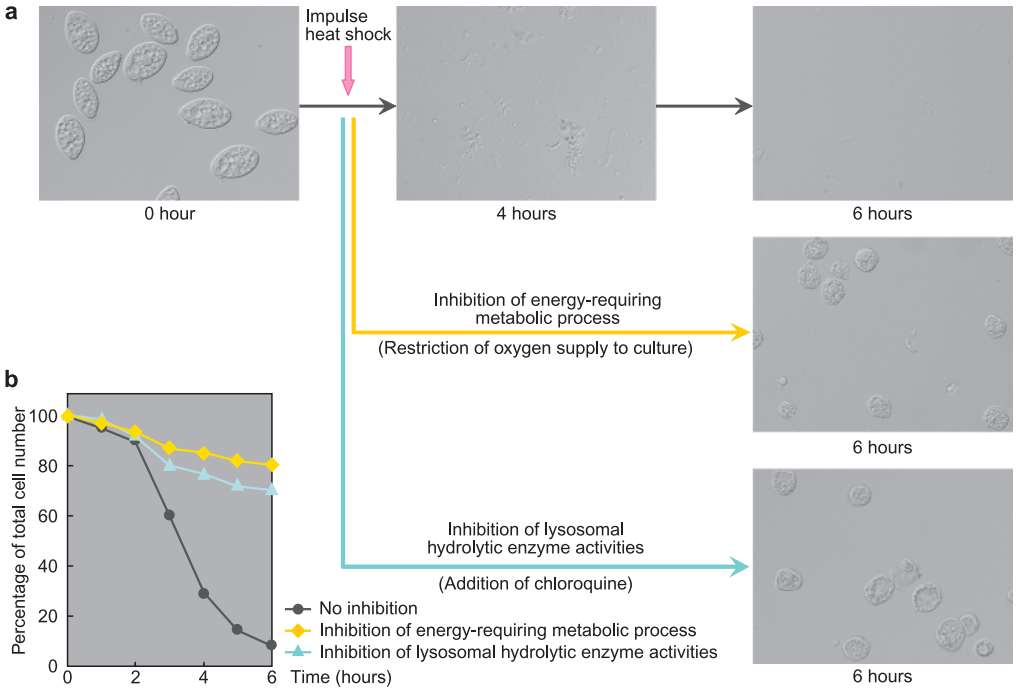

Figure 5. Self-decomposition is an energy-requiring metabolic process involving hydrolysis through lysosomal acid hydrolytic enzymes as one of the main processes. (a) Self-decomposition of cells and its inhibition. Top: Self-decomposition of cells induced by the impulse heat shock treatment. Cells rapidly decomposed and became homogenate in approximately 4 to 6 h. Middle: Inhibition of energy-requiring metabolic processes by restriction of the oxygen supply immediately after impulse heat shock treatment. Self-decomposition of cells was significantly suppressed. Bottom: Inhibition of all lysosomal hydrolytic enzyme activities by the addition of the acidotropic reagent chloroquine immediately after impulse heat shock treatment. Self-decomposition of cells was significantly suppressed. (b) Timeline for the decrease in cell number. The number of cells at each time point is shown as the percentage of the number at 0 hour. Cells rapidly decreased in number after the impulse heat shock treatment. Inhibition of the energy-requiring metabolic processes and inhibition of the lysosomal hydrolytic enzyme activities significantly suppressed the decrease in cell number. These findings suggest that self-decomposition is an endergonic (i.e., energy-requiring) active metabolic process and that hydrolysis, which uses lysosomal enzymes to turn BPs into BMs, plays a major role in the self-decomposition process.

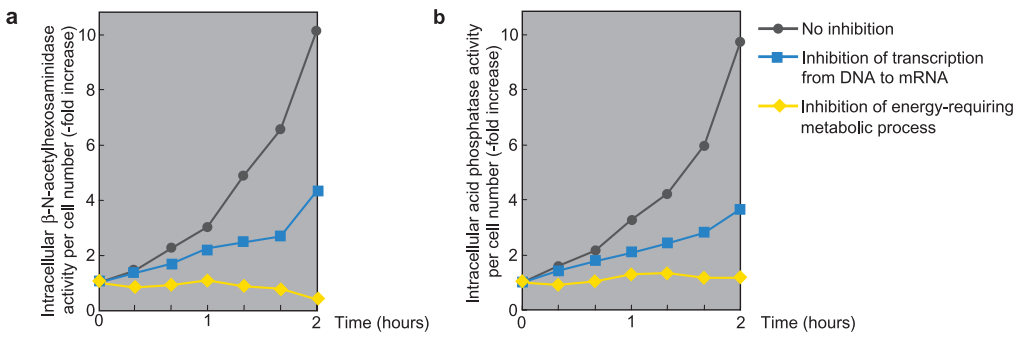

Figure 6. Behavior of the lysosomal acid hydrolytic enzymes in the self-decomposition process shows that self-decomposition is an energy-requiring, genetically regulated process. During the self-decomposition process induced by impulse pH shock treatment, we measured the intracellular activities of the most common marker enzymes of the lysosomal acid hydrolytic enzymes: (a) β-*N*-acetylhexosaminidase and (b) acid phosphatase. The enzymes' activity increased up to approximately 10 times that of the controls 2 h after the induction of self-decomposition. Inhibition of the transcription of genetic programs by actinomycin D (blue line) and inhibition of energy-requiring metabolic processes by restriction of the oxygen supply significantly (yellow line) suppressed the increase in activity of the hydrolytic enzymes. These findings further confirm that self-decomposition is an endergonic (i.e., energy-requiring) genetically regulated process mediated by lysosomal hydrolytic enzymes that decompose BPs into BMs.

hydrolytic enzyme activity, were investigated. The activity of both marker enzymes increased up to approximately ten times that of the control as self-decomposition progressed (Figure 6). This finding, together with changes in intracellular pH distribution (Figure 3), confirmed that self-decomposition proceeded as an accumulation of various hydrolytic reactions from BP to BM. In addition, such increases in enzymatic activity were remarkably suppressed by the inhibition of the expression of the genetic program or an energy-requiring, active metabolic process. This indicates that a causal relationship exists between lysosomal activities and expression of the genetic program or energy consumption. Such results confirm that self-decomposition is an energy-requiring, genetically regulated process mediated by lysosomal hydrolytic reactions.

Such experimental results, which were not contradictory but complementary among different indexes, support the notion that existent terrestrial life utilizes a hierarchical structure as shown by our HBCB model and that existent terrestrial life realizes effective reuse of materials by self-decomposition. As a survival strategy in evolution and selection, terrestrial life might acquire and preserve the mechanism to return BPs to the environment as BMs with a high degree of collective reutilizability through hydrolysis in the self-decomposition process.

As mentioned above, it is impossible in an experiment that uses actual terrestrial life to examine this hypothesis by comparing it with alternative hypotheses in terms of evolutionary superiority. It is necessary to examine the validity of the HBCB model in the context of evolution through computer simulation, making full use of AChem activity.

4 Artificial Chemistry Simulation of Evolutionary Superiority of a Hierarchical Structure of Biomolecular Covalent Bonds

The existence of a PSD mechanism that utilizes the hierarchical structure of biomolecular covalent bonds has been confirmed by our biological experiments using an existent terrestrial life system. This suggests the possibility that decomposition from the BP class to the BM class has been evolutionarily selected. We used our SIVA-T05 (Simulator for Individuals of Virtual Automata—Terra 2005), an experimental AChem system with which we had performed simulation experiments,[1] to examine how evolutionarily advantageous this decomposition process is.

1 Anyone who is interested in obtaining SIVA-T05 can contact the corresponding author by e-mail.

4.1 Architecture of SIVA-T05

4.1.1 The Design Concept of SIVA-T05

We developed a virtual ecosystem series SIVA [19–21] configured with Oohashi's SRSD automaton installed in a finite, heterogeneous environment consisting of virtual biomolecules with chemical reactivity. Since constructing SIVA-III, a pioneering prototype for an AChem system, in 1996 [19], we have continued to develop SIVA as a virtual ecosystem based on AChem. To promote the major purpose of AChem, namely, the achievement of a closer relationship with existent terrestrial life, SIVA-T05, a new version of SIVA, has been developed based on the following design concepts.

1. In accordance with the actual terrestrial environment, the virtual environment that virtual life individuals (VLIs) inhabit in SIVA-T05 has limited amounts of space, materials, and energy. The materials, energy, and temperature are heterogeneously distributed throughout the whole environment.
2. The virtual biomolecules making up a VLI are hierarchically organized according to the HBCB model. In terrestrial organisms, proteins are constructed by polymerization of amino acids, and nucleic acids are constructed by polymerization of nucleotides. Likewise, an element in a certain class in the hierarchical structure of virtual biomolecules consists of elements belonging to the next lower class. In addition, interclass conversion, such as synthesis and decomposition, is associated with release and absorption of energy corresponding to each class.
3. As in terrestrial life, virtual biological polymers and monomers are categorized into two groups: the constitutive information group, which serves as the gene-preserving information about structure and function of the VLI, and the functional module group, which serves as proteins or enzymes expressing life activities in the VLI. The constitutive information group and the functional module group correspond to the genotype and the phenotype in terrestrial life. Virtual biomolecules belonging to the functional module group are synthesized according to the information described by virtual biomolecules belonging to the constitutive information group.
4. A functional automaton for self-reproduction and self-decomposition acts as the phenotype of a VLI, and a virtual genome acts as the genotype. The functional automaton consists of virtual biomolecules belonging to the functional module group, whereas the virtual genome consists of ones belonging to the constitutive information group.
5. A VLI reproduces itself by using materials and energy existing in the virtual environment. Activities of a VLI depend on the amount of materials and energy as well as the temperature in its habitation point.
6. For each VLI, the optimum environmental conditions are defined a priori. A VLI cannot express its life activities when actual environmental conditions at its habitation point markedly deviate from its optimum ones.
7. A VLI can be set to decompose itself when the environmental conditions at its habitation point deviate from the optimum for a VLI, or when it has lived out its life span. Materials and energy released in association with the decomposition of a VLI are restored to the environment.
8. Mutation can occur in the virtual genome and may change the optimum environmental conditions of a VLI. This may enable a VLI to live in an environment in which it originally could not. That is to say, evolutionary adaptation can occur.

In the following sections, we describe how these concepts are implemented in SIVA-T05.

4.1.2 Environmental Design of SIVA-T05

To simulate the characteristics of a terrestrial environment with limited amounts of materials and energy distributed in a finite space, the virtual space of SIVA-T05 is designed to be a two-dimensional lattice consisting of 16 × 16 (= 256) spatial blocks. A single spatial block is defined as 8 × 8 (= 64) pixels for habitation points. One habitation point is occupied by one VLI and vice versa (Figure 7a). Environmental conditions can be independently defined for each spatial block, and those of the 64 habitation points in the same spatial block are configured to always be homogeneous. Since all VLIs in one spatial block share the same environmental conditions, the population of VLIs in that block significantly affects the local condition. Consequently the divergence of local environmental conditions across the whole ecosystem is gradually emphasized along with the proliferation of VLIs, as would happen in a terrestrial ecosystem.

The temperature gradient and the initial distribution of virtual energy and four kinds of virtual inorganic biomaterials (see Section 4.1.3) consisting of VLIs are heterogeneous across the whole ecosystem (Figure 7b). No substances other than virtual inorganic biomaterials exist in the initial environment. To simulate the effects of solar energy and its diffusion and radiation in the terrestrial ecosystem, a predefined amount of energy per time unit is refilled, and the total amount of energy in each spatial block must not exceed a predetermined threshold. The amount of refilled energy and the upper limit of total energy are set at appropriate levels so that a simulation does not become meaningless, that is, not so small that no VLI can live stably, and not so large that all VLIs can always live without any failures. In order to compare the reproduction processes among the four different species of virtual life under identical conditions, as below, all the conditions of the four spatial blocks at the center of the virtual ecosystem are set to be identical at the outset.

4.1.3 Composition of Virtual Biomolecules

In SIVA-T05, we have designed a new type of virtual life based on the HBCB model. Table 2 shows the design of the hierarchical structure of virtual biomolecules based on the complexity of the interatomic network of actual biomolecules making up terrestrial life.

Virtual biological polymers (VPs) and virtual biological monomers (VMs) are categorized into two groups: the functional module group and the constitutive information group, which correspond to the phenotype and the genotype, respectively, in terrestrial life.

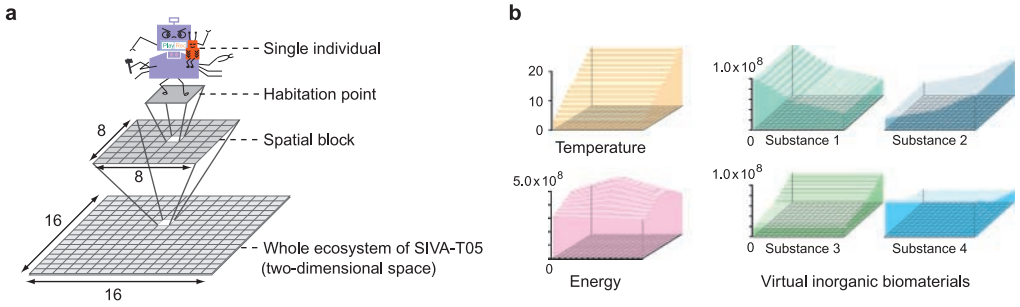

Figure 7. Environmental conditions of the virtual ecosystem SIVA-T05 are designed to be finite and heterogeneous. (a) Spatial design. The virtual space of SIVA-T05 is a two-dimensional lattice consisting of 16 × 16 (=256) spatial blocks, each of which consisted of 8 × 8 (=64) habitation points. One habitation point is occupied by one VLI and vice versa. Environmental conditions of each spatial block can be independently defined, and those of the 64 habitation points in the same spatial block are configured to be always homogeneous. (b) Spatial distribution of environmental conditions. Upper left: Distribution of environmental temperature. Lower left: Initial distribution of energy stocked in each spatial block. A predefined amount of energy is refilled from outside the virtual ecosystem to simulate the effect of solar energy. Right: Initial distribution of four kinds of virtual inorganic biomaterials (VI). Each substance flows between neighboring spatial blocks to restore the environment to the initial condition when the amount of a substance goes above or below that of the predetermined level. All the environmental conditions of the four spatial blocks at the center of the virtual ecosystem are set to be the same at the beginning in order to compare the reproduction processes of the four different species of virtual life that are initially seeded under identical conditions.

Table 2. Hierarchization of virtual biomolecules composing virtual life based on the complexity of the interatomic network.

Class name	Functional module group		Constitutive information group
Virtual biological polymer (VP)	Polymerized functional units		
Functional unit	Functional word (command)	Temporary information word (variable, relational operator, etc.)	Virtual codon
Virtual biological monomer (VM)	O P Q R (4 kinds)	I J K L 0 1 2 3 4 5 6 7 8 9 (14 kinds)	W X Y Z (4 kinds)
Virtual organic biomaterial (VO)	A B C D (4 kinds, uppercase letters)		
Virtual inorganic biomaterial (VI)	a b c d (4 kinds, lowercase letters)		

Basically each substance in a certain class consists of several elements belonging to the next lower class. For example, a virtual organic biomaterial (VO) consists of several virtual inorganic biomaterials (VIs), and a VM consists of several VOs. Several VMs constitute a functional unit, which is a subclass of the VP class, and several functional units constitute a larger VP. In the present simulation experiments, we designed five VMs as a single functional unit. A functional unit serves as one word in the SIVA language in the functional module group, while it serves as a virtual codon (V_{codon}) in the constitutive information group (see Section 4.1.4).

The amount of released energy associated with decomposition of a virtual biomolecule into elements in a lower class, and the absorbed energy associated with synthesis of a biomolecule in a higher class from elements, are defined as shown in Table 3. Note that the hierarchical structure of virtual biomolecules based on complexity corresponds closely to that based on bond energy. In principle, released energy and absorbed energy do not have the same value in actual terrestrial life. To simplify the experimental conditions, however, the same value was used for both in this experiment. The relationship between the amount of bond energy associated with different interclass conversions was also simplified. The bond energy associated with conversion between the VP class

Table 3. Amount of energy released or absorbed by the conversion between virtual biomolecular classes.

Conversion between classes	Amount of energy (units per molecule in the lower class)
Virtual biomolecular polymer (VP) and virtual biomolecular monomer (VM)	0.1
Virtual biomolecular monomer (VM) and virtual organic biomaterial (VO)	1
Virtual organic biomaterial (VO) and virtual inorganic biomaterial (VI)	10

and its subclasses (the functional unit classes) is the same as the one associated with conversion between VP and VM.

4.1.4 Function of Virtual Life and Features of Virtual Life Activities

Oohashi's SRSD automaton is installed as an artificial life form in SIVA-T05 (Figure 8). The VLI consists of a virtual genome and functional automata. The virtual genome is a VP of the constitutive information group and corresponds to instruction tape I in Figure 8, whereas the functional automata are VPs belonging to the functional module group and correspond to automata A, B, C, and FZ in Figure 8. The virtual genome covers the functions of preservation, replication, and transcription of structural and functional information about a VLI, while the functional automaton covers various life activities of the VLI, such as synthesis, decomposition, and reproduction.

The virtual genome consists of the sequence of four kinds of VM (W, X, Y, Z in Table 2) corresponding to the nucleotide in terrestrial life (Figures 8 and 9a). In the virtual genome, five VMs constitute a functional unit, which serves as a V_{codon}. Namely, each V_{codon} is defined to correspond to one of 18 kinds of VM (I, J, K, L; O, P, Q, R; 0–9 in Table 2) of the functional module group (i.e., virtual amino acid: VAA). The sequence of V_{codon}s defines the sequence of the VAAs in a functional automaton. The sequence information regarding all the automata is described in the virtual genome. For the reproduction of a VLI, automaton B replicates the whole virtual genome and automaton A synthesizes a functional automaton. Mimicking the transcription from DNA to mRNA in actual terrestrial life, automaton A first copies the corresponding part of the V_{codon} sequence and then converts the transcript into a VAA sequence. Mutation can occur in either of these copying processes (see Section 4.1.6).

SIVA-T05 executes the functions of the automata described by the SIVA language as an interpreter, and thus the life activities of VLIs are expressed. First, a functional unit consisting of a sequence of five VAAs serves as a ⟨word⟩ in the SIVA language. A ⟨word⟩ can be categorized as a functional word, which serves as an executable ⟨command⟩, or as a temporary information word (Table 2). A ⟨command⟩ as a functional word covers a substantial part of the life activities of a VLI,

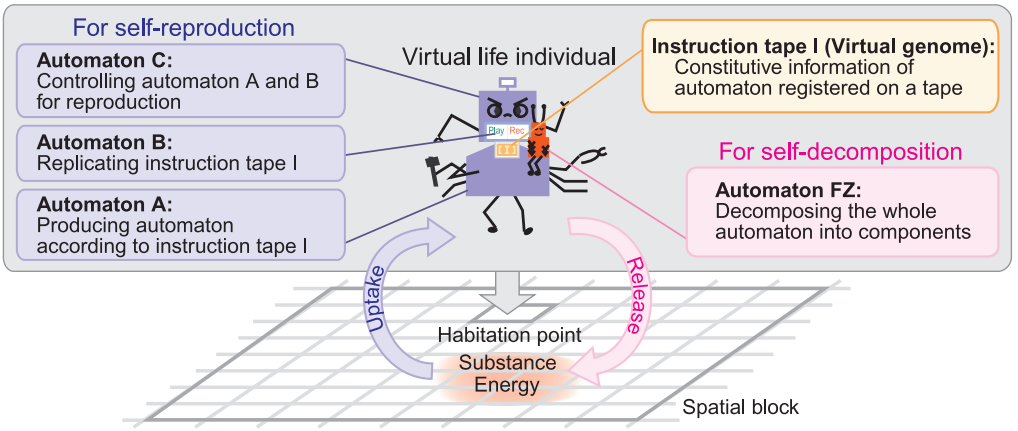

Figure 8. Relationship between life activities of virtual life individuals (VLIs) and the environment in SIVA-T05. Oohashi's self-reproductive, self-decomposable automaton is implemented in the VLI in SIVA-T05. Each VLI consists of functional automata for self-reproduction [D (=A+B+C)], those for self-decomposition [FZ], and an instruction tape [I_{D+FZ}] (i.e., a virtual genome) that is a blueprint of all the automata. Automaton A produces all the functional automata described in the virtual genome. Automaton B replicates the virtual genome. Automaton C constitutes a daughter VLI, combining the automata newly synthesized by automaton A and the virtual genome replicated by automaton B, and divides it from the parental VLI. Automaton FZ decomposes a VLI when it encounters environmental conditions unsuitable for survival or when it lives out its life span. A VLI can reproduce itself by uptake of substances and energy that exist in the spatial block to which its habitation point belongs. During self-decomposition, the substances and the energy generated by the decomposition of virtual biomolecules constituting the VLI are restored to the spatial block. The occupied space (i.e., the habitation point) is also released for utilization by another VLI.

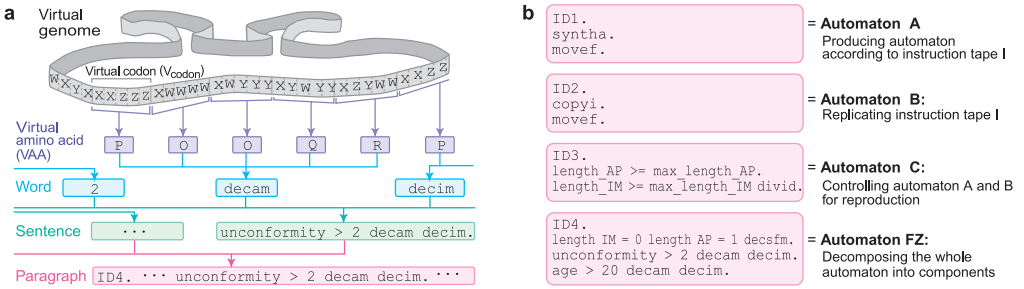

Figure 9. Examples of SIVA language statements that describe Oohashi's self-reproductive, self-decomposable (SRSD) automaton. (a) Synthesis of a functional automaton based on the virtual genome. The functional automaton and the virtual genome in Oohashi's SRSD automaton model correspond to the phenotype and the genotype of terrestrial life. Functional automata are synthesized from the information contained in the virtual genome. In the virtual genome, a sequence of the four different kinds of the five VMs in the constitutive information group forms a virtual codon (V_{codon}). Each V_{codon} corresponds to one of the VMs in the functional module group [viz., a virtual amino acid (VAA)], and the sequence of VAAs in the functional automaton is determined by the sequence of the V_{codon}s. A sequence of five VAAs corresponds to one word in the SIVA language. One or more words constitute a sentence, and one or more sentences constitute a paragraph. One paragraph corresponds to one functional automaton. (b) An example of a functional automaton described in the SIVA language used in the present simulation. There is an ⟨ID⟩ at the beginning of each paragraph. `ID1`, `ID2`, `ID3`, and `ID4` correspond to automata A, B, C, and FZ in Figure 8, respectively. The meanings of the ⟨command⟩s and ⟨variable⟩s used in this description are as follows: ⟨command⟩ `syntha`: reading information in the virtual genome and synthesizing a functional automaton; `movef`: moving an automaton to the next tag on the virtual genome; `copyi`: duplicating the virtual genome; `divid`: dividing a VLI; `decsfm`: decomposing itself (i.e., automaton FZ) into VMs; `decam`: decomposing a functional automaton into VMs; `decim`: decomposing the virtual genome into VMs. ⟨variable⟩ `length_AP`: the total number of functional automata existing in a VLI; `max_length_AP`: the maximum number of automata that a VLI can possess (usually corresponding to twice the total number of automata described in the virtual genome); `length_IM`: the total length of the virtual genome existing in a VLI; `max_length_IM`: the maximum length of the virtual genome that a VLI can possess (usually corresponding to twice the total length of the original virtual genome); `unconformity`: index representing the degree of unfitness; `age`: index representing the age of a VLI in TC, the time unit of the simulation.

for example, `syntha` for synthesizing a functional automaton based on the information described in the virtual genome, `copyi` for replicating the virtual genome, `divid` for dividing a VLI, and `decam` for decomposing functional automata. A temporary information word can be a ⟨variable⟩ (e.g., `length_AP` representing the total number of functional automata in a VLI), ⟨relational operator⟩ (e.g., `>=` meaning the left side is greater than or equal to the right side), ⟨number⟩ (ranging from `00000` to `99999`), ⟨period⟩ (`.` , representing the end of a sentence), or ⟨ID⟩ (from `ID0` to `ID255`, representing the beginning of a paragraph).

One or more words constitute a ⟨sentence⟩, which has to include zero or more ⟨command⟩s and one ⟨period⟩ at the end. Before a ⟨command⟩, a ⟨sentence⟩ can include one or more conditional phrases, each of which consists of a combination of a ⟨variable⟩ or ⟨number⟩ and a ⟨relational operator⟩. When there is no conditional phrase in the ⟨sentence⟩, ⟨command⟩s are directly executed in the order described in the ⟨sentence⟩. If a ⟨sentence⟩ includes any conditional phrases, a ⟨command⟩ is executed only when all the conditional phrases are true but not when any of the conditional phrases is false.

A collection of ⟨sentence⟩s constitutes a ⟨paragraph⟩. Namely, one ⟨paragraph⟩ consists of zero or more ⟨sentence⟩s with one ⟨ID⟩ at the beginning. The ⟨sentence⟩s in a ⟨paragraph⟩ are executed in the order described in the ⟨paragraph⟩. Since the results of the conditional phrase judgments are reset when the ⟨sentence⟩ ends, they do not affect the execution of the next ⟨sentence⟩. The structure of ⟨sentence⟩ and ⟨paragraph⟩ can be described as follows in Backus-Naur form:

⟨sentence⟩ ::=
([⟨variable⟩ ⟨number⟩] ⟨relational operator⟩ [⟨variable⟩
⟨number⟩])* ⟨command⟩* ⟨period⟩

⟨paragraph⟩ ::=
⟨ID⟩ ⟨period⟩
⟨sentence⟩*

One ⟨paragraph⟩ described in SIVA language corresponds to a functional automaton in the life activities of a VLI. Figure 9b gives an example of the descriptions of a functional automaton used in the present simulation experiment. Each functional automaton in Figure 8 corresponds to a ⟨paragraph⟩ as follows: Automaton A corresponds to `ID1` and synthesizes functional automata based on the information in the virtual genome by executing `syntha`. Automaton B corresponds to `ID2` and replicates the virtual genome by `copyi`. Automaton C, corresponding to `ID3`, constitutes a daughter by combining the newly synthesized automata and the replicated virtual genome, and divides it from a parent by executing `divid` when all the functional automata have been synthesized and all the virtual genomes are replicated, that is, when both the conditional phrases `length_AP >= max_length_AP` and `length_IM >= max_length_IM` become true (see the legend of Figure 9 for details of each variable). Automaton FZ, corresponding to `ID4`, decomposes the virtual genome and all the functional automata except for itself by executing `decim` and `decam` when the environmental conditions become unsuitable for the VLI's survival (namely, when the conditional phrase `unconformity > 2` becomes true) or when the VLI lives out its life span (namely, when the conditional phrase `age > 20` becomes true). Furthermore, automaton FZ decomposes itself by executing `decsfm` when the whole virtual genome and all the functional automata other than itself have been decomposed (namely, when both the conditional phrases `length_IM = 0` and `length_AP = 1` become true). Apart from these ⟨paragraph⟩s describing functional automata, there is another ⟨paragraph⟩ (viz., `ID0`), which defines various parameters specific to each VLI, including the optimum temperature.

Each VLI expresses its life activities by executing all ⟨paragraph⟩s during one *time count* (TC), the unit of virtual time in SIVA-T05. The order in which a VLI in the virtual ecosystem expresses its life activities within one TC is randomly determined at every TC.

4.1.5 Relationship between Environmental Conditions and Activities of Functional Automaton

All activities of the functional automata are designed to be affected by the environmental temperature and the amounts of substances and energy according to the properties of chemical reactions in actual terrestrial life (Figure 10). First, activities of the functional automata are set to be

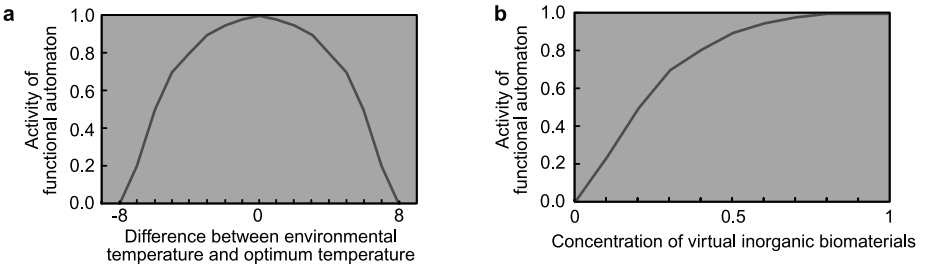

Figure 10. Design of the relationship between environmental conditions and the activity of a functional automaton. The activity of each functional automaton constituting a VLI is designed to be maximal when a VLI exists in the optimum environment specific to it and to decrease when its environmental conditions deviate from the optimum state. (a) Relationship between the activity of a functional automaton and environmental temperature. The activity is designed to decrease according to a pseudo-Gaussian curve as the environmental temperature deviates from the optimum temperature for the VLI. (b) Relationship between the activity of a functional automaton and the concentration of virtual inorganic biomaterials (VI). As the concentration of VI necessary for self-reproduction increases, the self-reproduction activity of the functional automaton asymptotically increases according to a pseudo-Michaelis-Menten equation.

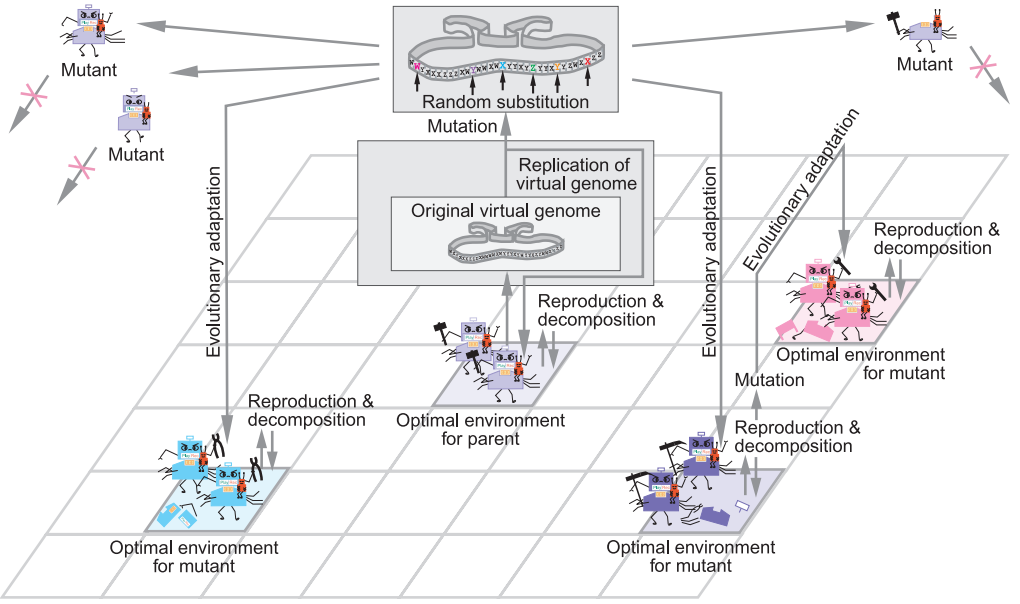

Figure 11. Concept of mutation and evolutionary adaptation employed in SIVA-T05. When VLIs replicate the virtual genome for self-reproduction in SIVA-T05, at a certain probability a VM in the replicated virtual genome is randomly replaced by another type of VM belonging to the constitutive information group (i.e., W, X, Y, or Z). Most new phenotypes produced as a result of mutation provide negative change or no positive change in life activities, including environmental adaptability. To a certain extent, however, a positive change in life activities can be expected to occur and a mutant emerge whose optimal environmental conditions differ from those for its parent. Such a mutant can live in a spatial block with environmental conditions under which its parental individual cannot. In this article, we refer to this phenomenon as evolutionary adaptation.

maximal when the temperature of the habitation point is optimum for the VLI and to decrease according to a pseudo-Gaussian curve when the temperature deviates from the optimum [12].

Next, activities of the functional automata related to self-reproduction are designed to asymptotically increase as the concentration of VI increases in accordance with the Michaelis-Menten equation [12], which describes the relationship between substrate concentration and chemical reaction rate. An actual chemical reaction in terrestrial life often shows some maximal points rather than monotonic increase, because, as typically seen for hydrogen-ion concentration (pH), an increase in concentration of a certain substance can affect a state of the other substances, such as the ionization state. In this experiment, however, we adopted the simpler relationship for the first step in our investigation.

Table 3 lists the different amounts of energy required for the synthesis of the functional automaton or the replication of the virtual genome according to the class to which the virtual biomolecular materials belong. As the amount of energy necessary for reproduction increases relative to the amount of available energy in the local environment, the activities of the functional automaton decrease. In actual terrestrial life, energy necessary for life activities can be stored in life individuals as the bond energy of biomolecules such as adenosine triphosphate (ATP). In this simulation experiment, however, VLI can utilize only the energy existing in the environment, for simplification.

To implement changes in the functional automaton activities in SIVA-T05, the probability of successful execution of a ⟨command⟩ is designed to decrease as the difference between the environmental temperature and the optimum temperature for the VLI becomes greater, or as the amounts of substances and energy necessary for reproduction increase relative to the amounts in the environment. Under these conditions, if the expression of life activities (i.e., the execution of ⟨command⟩s) fails, then `unconformity`, which is a variable representing unfitness with respect to the environment, increases by 1. Similarly, when there is VLI division, if the adjacent pixels of a parent VLI's habitation point are already occupied by other VLIs, it fails to divide itself and

increases `unconformity` by 1. When `unconformity` becomes greater than the predetermined threshold (namely, when the conditional phrase `unconformity > 2` of `ID4` in Figure 9b becomes true), the self-decomposition process starts. Self-decomposition also starts when the variable `age` representing the age of a VLI reaches the predetermined life span (namely, when the conditional phrase `age > 20` in `ID4` becomes true). The substances and energy released by the self-decomposition are returned to the spatial block where the VLI exists.

4.1.6 Mutation and Evolutionary Adaptation

SIVA-T05 can simulate various types of mutation occurring in the genes of actual terrestrial life. Figure 11 illustrates the concept of mutation and evolutionary adaptation in SIVA-T05. In the present simulation we used only the VM substitution that occurs when copying the virtual genome. There are two situations in the life activities of VLIs in which substitution can occur: when automaton B replicates the virtual genome for reproduction (i.e., replication of DNA in terrestrial life), and when automaton A copies the part of the virtual genome necessary for the synthesis of a functional automaton (i.e., transcription from DNA to mRNA in terrestrial life). When automaton B replicates the virtual genome, the VM in the replicated virtual genome is replaced by another type of VM belonging to the constitutive information group at a predefined probability `mis_copyi_rate`. When automaton A makes a transcript of one part of the virtual genome, the VM is similarly replaced at a predefined probability `mis_syntha_rate`. Thus the former case induces changes in phenotype based on changes in genotype, while the latter one induces changes in phenotype without changes in genotype. In both cases, the composition of the VMs, VOs, or VIs making up the functional automata of the VLIs may consequently change. The optimal temperature defined for each VLI may also change. A new phenotype produced by the results of mutation may often have no effect or even a negative effect on the life activities of the VLIs. In rare cases, however, a new (i.e., mutant) VLI emerges that can live in an environment in which no antecedent (i.e., parent) VLI had ever before been able to exist. Only when such a characteristic is caused by changes in the genotype can it be passed on to offspring. In the framework of the simulation on SIVA-T05, therefore, we define evolutionary adaptation as the phenomenon whereby offspring can live in a place with environmental conditions under which its parental individual cannot. If mutation produces a sequence of VMs that cannot be interpreted in SIVA language, SIVA-T05 neglects it.

4.2 Methods of Experimental Simulation Using SIVA-T05

We conducted two experimental simulations using the AChem system SIVA-T05 to examine the efficiency of the HBCB model. We evaluated reutilization efficiency of resources produced in the PSD process in relation to the destination class of the decomposed VP.

For this experiment, we set three mortal species M-I, M-O, and M-M as initial virtual lives, each of which had a different destination of decomposed VP by its automaton FZ. Here M-I is a mortal species whose automaton FZ decomposes VP into VI. Similarly, M-O and M-M are mortal species decomposing VP into VO and VM, respectively. Across these species, automata A and B were also set to be able to utilize virtual biomolecules belonging to different classes for synthesis or replication. That is, only VI was available for M-I; VI and VO for M-O; and all of VI, VO, and VM for M-M. When multiple kinds of virtual biomolecules belonging to different classes are available, the species utilized them in ascending order of energy amount required for the synthesis, namely, in the order VM, VO, VI.

We seeded each VLI of the three species separately at the center habitation point in three ecosystems with identical environmental conditions and conducted simulations of reproduction and evolution to test the vitality of such species. As a control, we also performed another simulation with identical conditions using immortal species I-N whose automaton FZ for self-decomposition was inactivated. I-N is an immortal species with no decomposition. Automata A and B of the species I-N could utilize only VI as material. Life activities of these primitive VLI were designed to fit the initial environmental conditions of the center habitation points where they were seeded.

It is possible that, in the evolutionary process, these four species of VLI would coexist in an identical ecosystem at the same time, sharing both space and substance. Therefore, we seeded each VLI of the four species in four spatial blocks with identical environmental conditions located at the center of one ecosystem and simultaneously started their simulations. Accordingly, these four VLIs did not have contact with one another at the starting point. We also performed another simulation in which the four species were seeded in the same spatial block. In this simulation, the VLIs of four different species had contacted one another when the number of VLIs of each species was only one or two. Since the success or failure of proliferation at this very early stage decided the whole simulation process, the results of this simulation—governed, as it was, by chance—became unstable. Therefore, four VLIs of different species were seeded in different spatial blocks so that they had contact with one another only after some stable proliferation.

To evaluate the results of the simulations, we compared the life activities of the species from the viewpoint of changes in the size of the habitation area, the number of individuals, the frequency of reproduction, the accumulated number of reproductions, and the frequency of mutation. See our previous report [21] for details of other conditions for the simulations.

4.3 Results of Experimental Simulations

4.3.1 Validity of the PSD Mechanism

Figure 12 shows successive changes of the VLI distribution when each species proliferated in an independent but identically conditioned ecosystem.

At the beginning of the simulation, species I-N with no self-decomposition mechanism proliferated prosperously. However, as the habitation area expanded, the gap between the environmental conditions

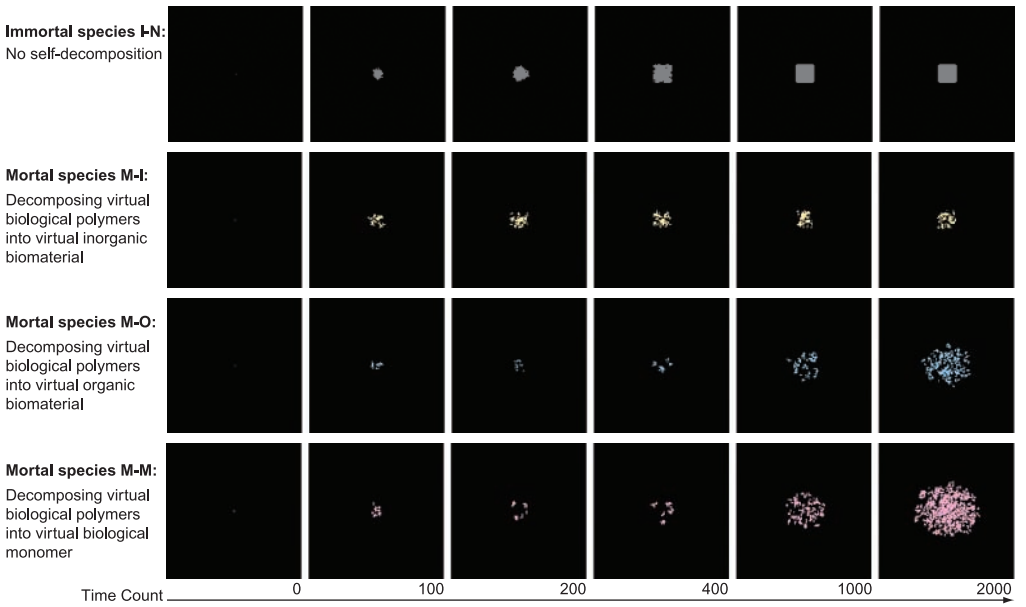

Figure 12. Mortal species in which virtual biological polymers were decomposed into virtual biological monomers in the self-decomposition process expanded their habitation area most prominently (results of simulations in which four species proliferated in separate ecosystems with identical environmental conditions). Immortal species I-N (first row in gray) with no self-decomposition mechanism stopped self-reproduction and expansion of habitation area after time count (TC) 400. VLIs of mortal species M-I (second row in yellow), M-O (third row in blue), and M-M (bottom row in red), with a self-decomposition mechanism, repeatedly reproduced and decomposed themselves until the end of the simulation (TC 2000). Species M-M, with a self-decomposition process oriented to the virtual monomer (VM) class, conspicuously expanded its habitation area. Increase in population and expansion of the habitation area were more prominent for species M-M than for species M-I and M-O, in which decomposition was oriented to classes lower than VM.

of the habitation area and their optimal conditions increased, and thus the requirements for adaptation increased. Indicative of this tendency, the immortal species I-N with no self-decomposition mechanism reached the limit of its adaptive area at TC 400 and stopped reproduction and expansion of its habitation area.

At the same time, the VLIs of mortal species M-I, M-O, and M-M, having a self-decomposition mechanism, repeatedly reproduced and decomposed themselves until the end of the simulation (TC 2000). These cells formed dynamically stable populations, receiving the full benefit of their restoring substance and space to the ecosystem by the self-decomposing mechanism. A large number of mutations occurred, reflecting the repeated replication of the virtual genome associated with reproduction. Thus they continuously produced offspring with a new virtual genome and a corresponding phenotype that enabled them to advance into areas where the initial VLIs could not survive. As a result, they succeeded in increasing population and expanding their habitation area.

The results of this experimental simulation were completely consistent with our previous findings [20, 21] that show the validity of the self-decomposing mechanism in a finite, heterogeneous habitation environment, which is a major characteristic of a terrestrial ecosystem.

4.3.2 Effect of Hierarchization of Biomolecular Covalent Bonds

Species M-I, M-O, and M-M showed significant difference in reproduction and evolution (Figures 12 and 13). Note that species M-I, M-O, and M-M each had a different type of self-decomposition mechanism with a different destination for the decomposed VP. At the end of the simulation

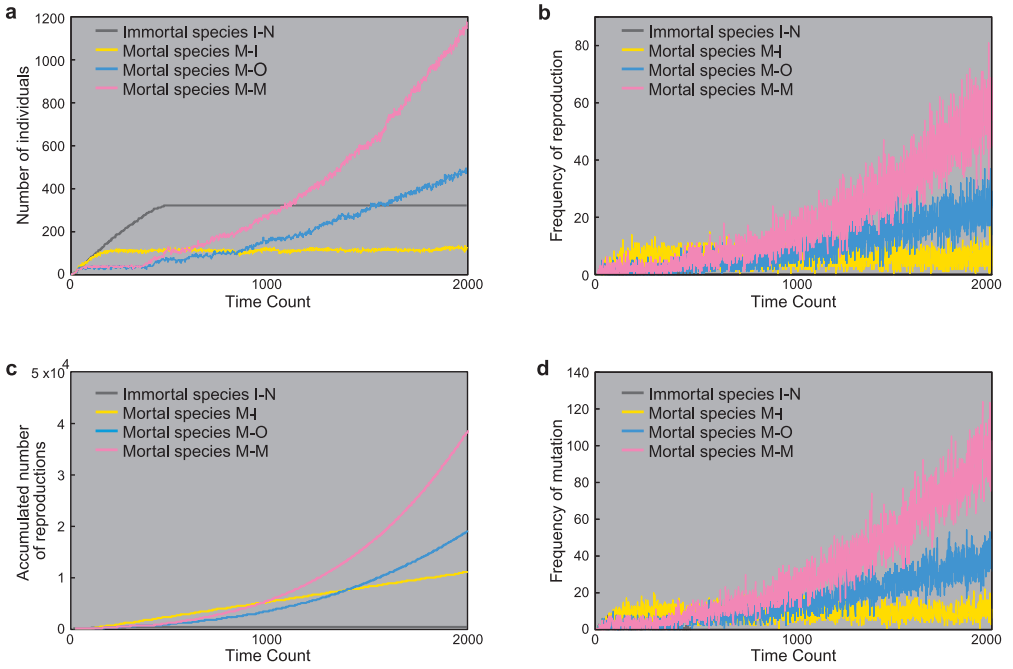

Figure 13. Mortal species in which biological polymers were decomposed into virtual biological monomers in the self-decomposition process showed a prominent advantage in reproduction and evolution (results of simulations in which four species proliferated in separate ecosystems with identical environmental conditions). (a) Number of individuals. (b) Frequency of reproduction. (c) Accumulated number of reproductions. (d) Frequency of mutation. Immortal species I-N (gray), with no self-decomposition mechanism, stopped reproduction, and mutation did not occur after TC 480. Mortal species M-I (yellow), M-O (blue), and M-M (red), all with a self-decomposition mechanism, repeatedly reproduced and decomposed themselves until the end of the simulation (TC 2000). Mutations also repeatedly occurred. Species M-M, with a self-decomposition process oriented to the VM class, showed greater advantage in all the indexes than did species M-I and M-O, both with decomposition oriented to classes lower than VM.

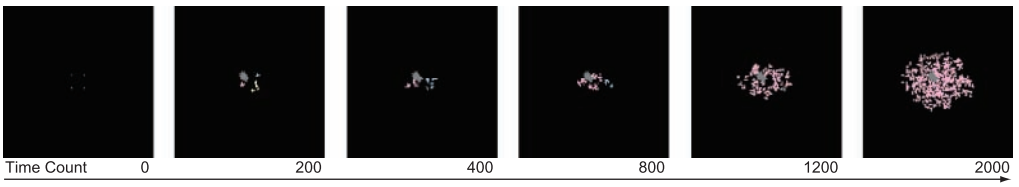

Figure 14. Mortal species in which biological polymers were decomposed into virtual biological monomers in the self-decomposition process survived and expanded their habitation area most prominently (the result of a simulation in which four species in an identical ecosystem coexisted simultaneously and interacted). When all the species of VLIs coexisted in an identical ecosystem, mortal species M-M (red), in which biological polymers were decomposed into VMs, showed prominent advantages over the other three species. Immortal species I-N (gray), with no self-decomposition mechanism, stopped reproduction after TC 200. Its habitation area was smaller than when it was seeded in a separate ecosystem. Mortal species M-I (yellow) and M-O (blue), in which the self-decomposition process was oriented to classes lower than the VM class, were exterminated by the middle of the simulation. Mortal species M-M survived and expanded its habitation area up to nearly the same size it had when seeded in a separate ecosystem, and thus showed the greatest advantage in reproduction among the four species.

(TC 2000), three species showed clear difference in both population and habitation area. By that measure, they showed greater advantage of reproduction in the following order: [species M-M oriented to VM class] > [species M-O oriented to VO class] > [species M-I oriented to VI class].

Species M-O and M-M showed themselves to be inferior to species M-I until about TC 400. Two factors can be mentioned in this regard. First, species M-O and M-M used VIs as a resource for reproduction but did not return them to the environment. This one-way consumption made the environmental conditions recede from the optimum, making reproduction disadvantageous at the beginning of the simulation. Second, because the VLIs of each species inhabited the optimum habitation area at the beginning of proliferation, differences in reutilizability of resources did not greatly affect the results.

To understand differences among the four species, we compared changes in the number of individuals, the frequency of reproduction, the accumulated number of reproductions, and the frequency of mutation (Figure 13). The number of VLIs of species I-N with no self-decomposition function reached a ceiling at 320 individuals by TC 480. On the other hand, the VLIs of species M-I, M-O, and M-M continued reproduction and decomposition until the end of the simulation (TC 2000), when they showed marked differences in proliferation. Reproduction of species M-M showed overwhelming superiority in all of the indexes.

Generation of VMs by self-decomposition induced a large-scale reduction of the reproduction cost of not only their own direct offspring but also of all VLIs inhabiting the same environment, compared with generation of VOs or VIs by self-decomposition. This is because VOs and VIs have a high degree of reutilizability compared to what VMs have, but involve a larger amount of energy release and absorption accompanied by synthesis and decomposition of VPs. Such generation enables all the VLIs in the whole ecosystem to efficiently reproduce themselves. Frequent reproduction should induce the occurrence of more mutation and accelerate evolutionary adaptation. These effects would enhance the activity of species M-M more than that of other species and allow species M-M to advance into new areas where its initial VLIs could not survive.

As shown above, the effect of the self-decomposition mechanism on reproduction and evolution was most prominent when the destination of the self-decomposition was oriented to VM. On the contrary, when the destination was oriented to VO or VI, some effects of self-decomposition on reproduction and evolutionary adaptation were still preserved, but they were much less prominent.

We obtained even clearer results when the four species proliferated simultaneously in one ecosystem (Figures 14 and 15). The reproduction of immortal species I-N was extremely constrained from the beginning of the simulation and reached a ceiling at TC 250. Species M-I, M-O, and M-M showed almost the same proliferation at the beginning, although species M-I showed a slight advantage over the others. After approximately TC 300, however, species M-I decreased its habitation area due to pressure from the other species. It was exterminated at TC 367.

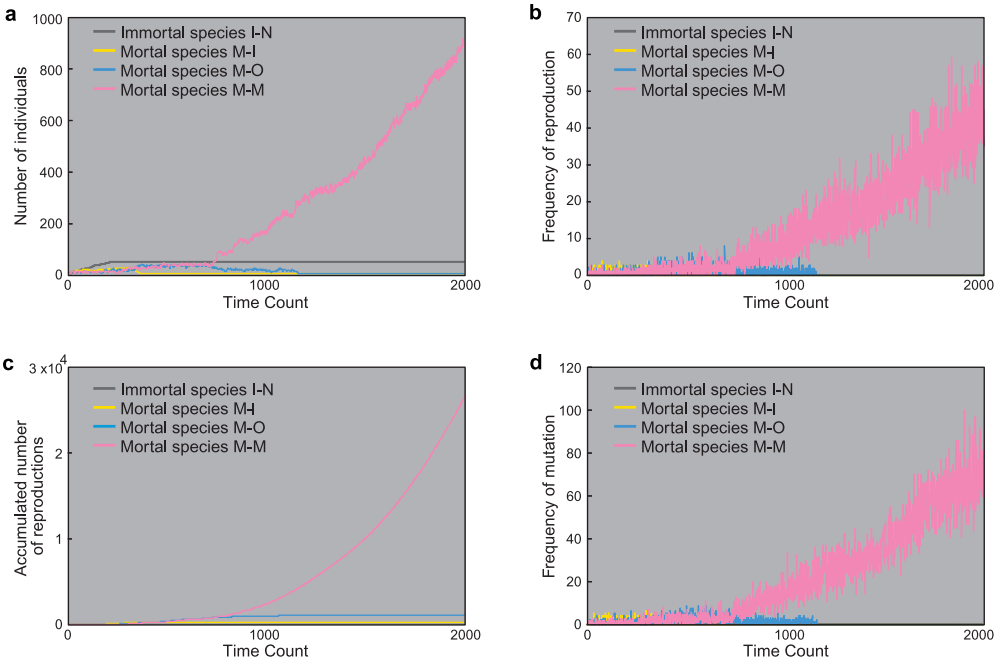

Figure 15. Mortal species in which biological polymers were decomposed into virtual biological monomers in the self-decomposition process showed a prominent advantage in reproduction and evolution (result of a simulation in which four species in an identical ecosystem coexisted simultaneously and interacted). (a) Number of individuals. (b) Frequency of reproduction. (c) Accumulated number of reproductions. (d) Frequency of mutation. Immortal species I-N (gray), with no self-decomposition mechanism, stopped reproduction and mutation after TC 250. The two mortal species M-I (yellow) and M-O (blue), in which biological polymers were decomposed into a class lower than VM, were exterminated in the middle of the simulation (species M-I: TC 367; species M-O: TC 1167). Mortal species M-M (red), in which self-decomposition was oriented to the VM class, showed the greatest advantage in all the indexes among the four virtual species.

Species M-O strove for survival evenly with species M-M until approximately TC 600, but was exterminated at TC 1167 due to pressure from species M-M. Species M-M increased its speed of reproduction from approximately TC 800. It showed less prominent proliferation at the beginning, however, but became prominent gradually. It became the most successful population by TC 800 and continued to expand its habitation area until the end of the simulation (TC 2000). Such results were consistent with our previous study in which a virtual life form with a self-decomposition mechanism surpassed one without it. Moreover, the results suggested that among the three self-decomposable species, the lower the energy loss for the acquisition of collective reutilizability, the more capable the species becomes for reproduction and evolution. The results of this simulation correspond well to the results of the biological experiment in which the PSD mechanism observed in actual terrestrial life involved hydrolysis as the main process that decomposes a BP into BMs.

The simulations using the AChem system SIVA-T05 suggest that VLIs with a PSD mechanism attained superiority in reproduction and evolutionary adaptation over VLIs without the PSD mechanism because they had an advantage in the recycling of habitation space and biomaterials and thus accelerated evolutionary adaptation. This result supports our previous findings as well as the possibility that the self-decomposition mechanism observed in biological experiments utilizing terrestrial life (protozoa) may exist as a consequence of evolution.

In this experimental simulation based on the HBCB model, we also showed that the effects of self-decomposition on the acceleration of evolutionary adaptation significantly differed according to destination classes of the decomposition of VPs. The results showed that decomposition from the VP class to the VM class had the greatest effect and provided the most prosperity. This suggests that

the species attaining a high collective reutilizability of resources with minimum energy loss by decomposing BPs into BMs, as actual terrestrial life does, may be evolutionarily selected from among other species that decompose BPs into various other materials.

The HBCB model, developed as a way to introduce a hierarchical structure for biomolecular bond energy, which in turn is central to Suzuki's NAC, provided valuable findings for understanding actual terrestrial life. The simulation also confirmed the efficiency of this model.

5 Discussion

The concept of the AChem system proposed by Suzuki [24], namely, a hierarchy based on the bond energies between chemical elements, has greatly inspired us. Applying his concept and our knowledge of cell biology, we categorized biomolecules into four classes based on the complexity of the interatomic network. We also categorized covalent bonds between biomolecules into three classes based on bond energy. Covalent bonds are the major bond energy of actual biomolecules and make up one of the classes in Suzuki's hierarchy [24, 25]. We found that these two classifications fully corresponded with one another. Incorporating characteristics of an AChem system that is tightly coupled with actual biomolecules, we constructed the HBCB model to complement our previously proposed PSD model. In the present study, we carried out two complementary experiments to examine the validity and effectiveness of both our PSD and our HBCB models. We examined models in a biological experiment on cells, utilizing actual terrestrial life, and in an AChem simulation with controls over the evolutionary time scale.

An essential property of AChem is the close correspondence with specific characteristics of chemical substances and reactions constituting terrestrial life [5]. Consequently, the AChem system can effectively compare and couple artificial life with an actual terrestrial one [25, 27]. A successfully designed AChem system is expected to provide a nearly equivalent counterpart to certain forms of existent terrestrial life. This characteristic of AChem made this approach possible.

Our biological experiment revealed that existent terrestrial life actually possesses programmed individual death with self-decomposition. The results indicate that self-decomposition is an energy-requiring, genetically regulated process and mainly consists of hydrolytic decomposition of a BP into BMs. Moreover, it suggests that terrestrial life is equipped with a biomolecular recycling mechanism that utilizes the hierarchy of covalent bonds. These results support the existence of a PSD mechanism and the validity of the HBCB model.

In simulation experiments in which we used SIVA-T05, mortal virtual species with a self-decomposition mechanism showed clear advantages in reproduction and evolution over immortal virtual species without a self-decomposition mechanism. This finding validates the effect of a PSD mechanism in a finite, heterogeneous habitation environment, which is a major characteristic of a terrestrial ecosystem. In addition, the simulation experiments showed that the virtual species, in which the self-decomposition process mainly involved covalent bond cleavage from the VP class to the VM class, showed evolutionary superiority over other species in which the self-decomposition process involved covalent bond cleavage from the VP class to a lower class than VM.

Thus the results of cell biological experiments using terrestrial life and the results of the simulation experiment of the HBCB model using SIVA-T05 almost completely supported and complemented one another. The overall results strongly support the hypotheses proposed in the HBCB model. That is, existent terrestrial life has developed a hierarchical structure based on bond energy strength, of which the biomolecular recycling system makes use. Moreover, the balance between energy efficiency and collective reutilizability may have been optimized as a result of the evolution of the SRSD automaton.

The fruitful outcome of this study could not have been acquired by the single use of either an artificial ecosystem employing computers, or biology employing living organisms. A discernible benefit of computer simulation studies using ALife is that, by modeling and simulating various hypotheses that are theoretically possible although not testable in real terrestrial environments, we

can evaluate the effectiveness and validity of such hypotheses without resorting to the real world [7, 10, 22]. This advantage allows for the study of problems otherwise out of reach, such as those related to the ecosystem or evolution. Conventional ALife studies may not fully take into account the fact that actual terrestrial life activities without exception consist of chemical phenomena [7, 10, 22]. Indeed, the majority of ALife studies during a certain period may have regarded that fact as a kind of constraint, and tried to dissociate themselves from actual existing life [9]. As a result, conventional approaches solely employing ALife are not likely to meet expectations of contributing to profound understanding of actual life.

By organically integrating the two different approaches from artificial and actual life under the paradigm of AChem, which takes cognizance of the fact that the basic processes of terrestrial life without exception consist of chemical phenomena, it is expected that further insight can be acquired that will exceed any obtained through a single approach. We believe that such positive integration will contribute to the field of AChem.

6 Conclusion

In focusing on the hierarchical structure of biomolecular covalent bonds based on bond energy in terrestrial life, we have constructed the HBCB model to complement our previously proposed PSD model. We examined the validity and effectiveness of the models by organically coupling biological and AChem experiments.

The biological experiments revealed that terrestrial life is equipped with biomolecular recycling systems that utilize the hierarchy of covalent bonds. The results support the existence of programmed individual death with self-decomposition. In addition it is suggested that self-decomposition is an energy-requiring, genetically regulated process and mainly consists of hydrolytic decomposition of a BP into BMs.

The AChem experiment revealed that, in a finite and heterogeneous environment such as that on Earth, life that has a biomolecular recycling mechanism with PSD predominates over life without such a mechanism. Such superiority is most conspicuous when the cleavage of covalent bonds embodying the self-decomposition process is oriented to the level of the process in which a VP is decomposed into VMs, where the decomposed products acquire almost complete collective reutilizability and energy loss is minimized: This is the most plausible process based on the HBCB model. These results support the validity and effectiveness of the HBCB model.

This approach, based on an integration of AChem and a terrestrial life system, promises to deepen our understanding of terrestrial life from an evolutionary perspective.

Acknowledgments

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