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Alzheimer's Disease as Subcellular 'Cancer'

—— *The Scale-Invariant Principles Underlying the Mechanisms of Aging* ——

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Alzheimer's disease (*AD*) is characterized by the slow onset of neurodegeneration leading to dementia in many elderly people.[1] The pathological hallmarks of AD are: the extracellular *β-amyloid* deposition in the *senile plaques*;[2] the β-amyloid deposition in cerebral blood vessel walls especially in *hereditary cerebral hemorrhage with amyloidosis of the Dutch type* (*HCHWA-D*);[3] the intracellular *neurofibrillary tangle* formation composed of *paired helical filaments* (*PHF*), the principal component of which is a hyperphosphorylated form of the microtubule-binding protein, *tau*;[4] and neurological dysfuction and neuronal cell death in limited regions and pathways of the central nervous system.[5] Note that β-amyloid is a truncated form of a cell surface integral membrane glycoprotein: amyloid precursor protein (APP).[6] Despite these hallmarks, the pathogenesis of AD has been poorly understood.

In the present paper, a theory of aging is proposed to give a coherent account of the origins and causes of neurodegeneration common to the diverse neurodegenerative disorders such as AD and *prion* (*proteinaceous infectious particles*) *diseases*[7),8] in comparison with the pathogenesis of cancers.[9] Surprisingly, the self-aggregation of denatured proteins —such as β-amyloid, PHF and prions— responsible for neuronal cell death resembles, in many respects, the development (or the clonal evolution) of malignant cells at the expense of the entire organism harboring them. Although neurodegenerative disorders and cancers apparently differ in pathology, they nevertheless seem to follow the same principles regardless of the level and scale of the biological organization. It is the general principles of *heritable variations* and *natural selection*[10] as well as the general principles of *self-organization*[11),12] that operate, not only on different molecules, but also at different hierarchical levels and scales of the biological organization, independent of the details of diseases.

Traditionally, natural selection, along with self-organization, has been thought to underlie 'creative' aspects of biological phenomena such as the origin of life,[13] adaptive evolution of viruses,[14] immune recognition[15),16] and brain function.[17] It therefore must be surprising to find that the same principles will also underlie 'non-creative' aspects, for example, the development of cancer[18),19] and the aging of complex organisms. Although self-organization has extensively been studied in nonliving things such as chemical reactions[11] and laser physics,[12] it is undoubtedly true that the similar sources of the order are available to living things at different levels and scales.[20]

Several paradigm shifts are, however, required to realize how the general principles of natural selection can be extensible to non-DNA molecules which do not possess the intrinsic nature of self-reproduction. One of them is, from the traditional, *genetic inheritance view* that DNA (or RNA) molecules are the *ultimate* unit of heritable variations and natural selection at any organization level, to the *epigenetic* (*nongenetic*) *inheritance view*[21),22] that any non-DNA molecule can be the target of heritable variations and molecular selection to accumulate in certain biochemical environment. Because they are all enriched with a β-sheet content, ready to mostly interact with one another, different denatured proteins like β-amyloid, PHF and prions can individually undergo self-templating or self-aggregating processes out of gene control.[23),24] Other paradigm shifts requisite for a breakthrough in the etiology of neurodegenerative disorders will be discussed.

As it is based on the scale-invariant principles, the present theory also predicts plausible mechanisms underlying quite different classes of disorders such as *amyotrophic lateral sclerosis* (*ALS*), *atherosclerosis, senile cataract* and many other symptoms of aging. The present theory, thus, provides the consistent and comprehensive account of the origin of aging by means of natural selection and self-organization.

Towards the Frontiers of Theoretical Physics

What is life? This long-standing problem has attracted many physicists especially since Erwin Schrödinger published the famous book in 1944. Nevertheless, this problem has not been solved yet. This is not because we lack complete knowledge of elements such as DNA, proteins and other molecules, but because we lack a theoretical framework provided by a new view of life. Indeed, we have been too much familiar with the traditional reductionists' view that requires identifying the elements at different levels of biological organization and understanding the relations between the different levels. On the basis of this traditional reductionism, however, we cannot understand how living things are different from nonliving things, because both are equally made of material molecules. To understand life itself, we must identify not only the 'elements' but also the 'elementary processes' within the organism. Only then, normal states and disease states and even senescent states can be clearly interpreted in terms of dynamic changes in the elementary processes as well as plastic changes in the elements. Regardless of whether the external environment remains constant or not, the organism is continually subject to the intrinsic variability at any level and scale. It therefore must be emphasized that transients and variations are essential and advantageous to life, because changes in the environment are important for successful adaptation. In this sense, we should not always expect that any biological system will show the same responses to the same stimuli. This situation apparently violates the reproducibility of the same events under the same conditions, though it has been central to physics. Now we need paradigm shifts in the theoretical physics as well as modern biology. Along this line, the present paper proposes a theory to attack the problem of aging. Unfortunately, the problem of aging has been largely neglected by most biologists, probably because the aging process is too complicated to seek a simple explanation. It therefore must be surprising that there is a simple explanation of aging, and that the general principles govern not only the aging of higher organisms but also the origin of life and even brain function. Now is the best time to attack the long-standing problem: what is life?

§ 1. Introduction

The pathogenesis of AD has been a long-standing mystery since first described by A. Alzheimer in 1907. This is not because we lack complete knowledge of components —such as molecules, organelles, cells or individuals— at different levels of organization, but because we lack a global view integrating the fragments of knowledge at all levels and scales. Since life has become *hierarchically* organized during both evolution[25] and development,[26] we need the global view to understand the pathogenesis of AD and many other complex biological phenomena. Lessons learned from past breakthroughs in biology and medicine can give insights into better understanding of how we attack the long-standing mystery of AD.

Without complete knowledge of molecules or cells, we have indeed made successive breakthroughs in immunology and oncology in the context of natural selection since Charles Darwin's book[10] of "*The Origin of Species by Means of Natural*

Selection" in 1859. It has progressively been understood that Darwin's principles of heritable variations and natural selection —which govern the long-term evolution of all living organisms on the earth— can also govern the short-term evolution of all dividing cells in the multicellular organism. The paradigm underlying this genetic inheritance view is that any self-reproducing entities —such as living organisms and dividing cells— adaptively evolve through successive rounds of mutations and natural selection acting *ultimately* on genes. *Clonal selection theory*[15] in immunology and *clonal evolution theory*[18),19)] in oncology have been proposed in this context. Of course, this is not a result of the simple application of the general principles of natural selection to immunology and oncology, but a result of the independent rediscovery of the same principles in the cellular society.

Here we have to appreciate two important points. First, the general principles indeed govern adaptive evolution of biological organization regardless of its hierarchical level. Although life has become hierarchically organized during evolution and development, it nevertheless must be a surprise that the same principles govern adaptive behavior of biological organization at different hierarchical levels. Second, adaptive evolution of dividing cells, for example, at a hierarchically lower level is not always promised to favor the hierarchically higher organism harboring them, as in the case of cancer. It seems to be paradoxical! However, understanding this paradox will advance investigations of many symptoms of aging as well as normal living states.

1.1. *Paradigm shifts in modern biology*

One purpose of this work is to realize that Darwin's principles of natural selection can and should be extended to the intracellular society involving complex biochemical reactions of molecular metabolism. This, of course, requires several paradigm shifts, though they are more or less dependent on each other and thus it may not be appropriate to consider each of them separately. For convenience, however, eight important paradigm shifts are mentioned in order.

The first, concerning the relevance of variations among components of the biological organization, is from the *instructionists' view* to the *selectionists' view*.[17),27),28)] The instructionists' view is that the construction and ongoing operation of the biological organization require the instruction from its genes and/or its environment. According to this view, variations are simply noise with little essential importance. The selectionists' view, in contrast, is that the biological organization takes advantage of preexisting variations for successful adaptation to changes in its environment through natural selection. Thus variations are functionally important for adaptive behavior of the biological organization. This issue, referred to as "instructionist versus selectionist", will be discussed in § 2 in the context of aging.

The second, concerning the selectionism, is from the *genetic inheritance view* to the *epigenetic inheritance view*.[21),22)] Since the astonishing discovery of the self-templating structure of DNA molecules,[29)] we have been too much familiar with the *central dogma*[30)] that all amino acid sequences are determined by DNA base sequences via RNA templates. It is therefore considered that all the infectious pathogens, like viruses in the simplest microbes, must contain DNA or RNA as genetic material for

the self-replication and transmission of themselves.[31] Although the genetic inheritance view that DNA (or RNA) molecules are the *ultimate* unit of heritable variations and natural selection has been successful in biology as discussed in § 3, the central dogma as well as the traditional view of infectious pathogens[31] appears to be no longer complete enough, because there is increasing evidence for infectious prions[7),8)] lacking nucleic acids. We therefore need the epigenetic inheritance view that non-DNA molecules potentially become direct targets of heritable variations and molecular selection. This will be intensively discussed in § 4.

The third, concerning the information-rich molecules, is from the *genetic information view* to the *epigenetic information view*. Consider cellular constituents at the molecular level. Besides nucleic acids, there are other cellular constituents such as proteins, sugars and lipids: proteins serve as the main machineries of molecular recognition[32] and catalysis[33] and also determine the shape and structure of the cell;[34] sugars are energy stores and structural fibers and also serve as markers for molecular recognition[35] as well as tags for intracellular protein transport;[36] lipids aggregate to form membranes and serve as second messengers for signal transduction.[37] It therefore turns out that cells are so *heterogeneous*. Of course, as established by the genetic information view, DNA stores the genetic information required to make enzymes acting on metabolic pathways of these molecules. It has, however, little direct influence not only on regulation of the metabolic pathways but also on dynamic processes such as intracellular transport and signal transduction. These cellular constituents are thus considered as information-rich molecules. This opens up the epigenetic information view, in which cellular constituents themselves store the epigenetic information out of genetic control. It is therefore important to emphasize that —just like nucleic acids are continually subject to genetic mutations and natural selection during evolution of life— all the other information-rich molecules are potentially exposed to epigenetic mutations and natural selection at any process within living organisms.

Though the rest of this paper will mostly focus on proteins, it should be kept in mind that the same logical structure of the following arguments can be applied to other information-rich molecules such as sugars and lipids, which will provide us with deep insights into the mechanisms underlying many symptoms of aging. This will be discussed in § 5.

The fourth, concerning the sources of the order found in living organisms, from the *single-force view* to the *multiple-force view*.[13] From the single-force viewpoint, either natural selection or self-organization is considered as the dominant sources of the order depending on the time scale. When natural selection acts on individuals of a population, as in the case of the origin of species, the order will emerge gradually, for evolution takes place gradually through the changing composition of populations from generation to generation.[38] When self-organization occurs, the order emerges spontaneously by phase transitions, as in the case of crystallization of a collectively self-reproducing molecular system.[13] In contrast with this single-force view, the multiple-force view is that both forces are considered at the same time as the sources of the order. Consider the long-lived cells: for example, nerve cells and muscle cells. As they are nondividing cells (i.e., permanent cells), both natural selection and

self-organization are potentially capable of operating in the intracellular society, with cumulative effects. The observed cellular states (which reflect the emergent order through natural selection and self-organization) therefore depend on the cell's past history. An important point to be stressed here is that the emerging 'order' will not always favor the preexisting cellular states.

The fifth, concerning the states of nondividing cells such as nerve cells, is from the *static view* to the *dynamic view*. The static view is that adult nervous system is static because of the inability to generate new nerve cells, and that the plasma membrane of nerve cells is dismissed as a passive, permeable barrier. This static view is far from the truth. The dynamic view, on the contrary, is that neurons undergo dramatic changes in the morphology during early childhood as well as late adulthood in response to environmental influences. The plasma membrane not only plays a dynamic role in an intracellular signal pathway,[36] but also undergoes the *endocytic-exocytic cyles*. Indeed, a bit of the plasma membrane is continually internalized in the process of *endocytosis* and it is also added to the cell surface in the converse process known as *exocytosis*.[39),40] Such dynamic cellular processes — responsible for the remarkable adaptability of nerve cells to environmental stimuli — are, of course, not determined by immediate gene instructions. Instead, they are driven by the coordinated activity of a complex network of protein metabolism, since proteins serve as the main machineries of molecular recognition and catalysis, and also determine the shape and structure of the cell. This issue will be discussed in § 5.

The sixth, concerning the approach to living organisms, is from either the *reductionists' view*, as practiced by molecular biologists, or the *holists' view*, as practiced by ecologists, to the *unified view*[41] involving both of them interactively. As life has become hierarchically organized during both evolution and development, it is necessary to have this unified view to understand complex biological phenomena.

The seventh, concerning the reductionism, from the *elementary unit view* to the *elementary process view*. In the elementary unit view, most attempts are made to identify the elementary units (or simply, the elements) at different levels of biological organization. This view, however, cannot provide useful insights into understanding of how living things are different from nonliving things, for both are composed of the material elements. Also, it is very difficult to understand how disease states emerge out of living organisms, if there is no difference at the level of the elements even after the onset of the disease. In the elementary process view, attempts are made not only to identify the elements but also to understand how the elementary processes are organized into the functions of living organisms. On the basis of this view, we can understand how disease and senescent states differ from normal states, and also grasp the general principles underlying the functional mechanisms of different organisms at different levels and scales.

The eighth, concerning the origin of life and its evolution, is from the *simple self-reproducing molecule view*[42] to the *complex self-regulatory network view*.[13),43] The simple self-reproducing molecule view, on the one hand, is that life started as simple self-reproducing RNA or RNA-like molecules and such simple molecules were organized into complex networks of biochemical reactions over the course of evolution. The complex self-regulatory network view, on the other hand, is that life started as

complex self-regulatory (or self-sustaining) networks of biochemical reactions and simple self-reproducing molecules, like DNA and RNA, evolved later out of such complex networks.

The complex self-regulatory network view strongly suggests that any molecule does *not* necessarily have the self-reproducing nature *a priori* as long as reaction networks, as a whole, are self-sustaining and hence ultimately self-reproducing.[13] *Why? Because there is no essential difference between constituent molecules and their biochemical environment concentrated by many kinds of molecules, independent of whether molecules or their environment would be self-reproducing.* It is such 'relativity' that seems to be a Copernican revolution in the modern biology as well as in the origin of life. An immediate consequence of this revolution is that a self-reproducing molecular system lacking genes can adaptively evolve through heritable variations and natural selection.

According to the emerging view reflecting a convergence of these new views mentioned above, I got some idea to frame a theory of aging. The main idea of my theory is this: Life has become not only hierarchically organized but also heterogeneously organized during both evolution and development in such a way that a number of molecules other than genes (DNA or RNA molecules) are embedded in biological organization as its necessary components. Most of them are organized into complex self-regulatory networks of metabolism. Unlike DNA (or RNA) molecules, non-DNA molecules themselves are not intrinsically self-reproducing. Even then, the environmental networks are continually acting on some particular non-DNA molecules through the self-regulatory nature of the networks themselves. Such non-DNA molecules can be the targets of heritable variations and natural selection as if they were the self-reproducing molecules. They can be selfish through natural selection like subcellular 'cancer'.

1.2. *What is aging?*

Another purpose of this work is to open up new vistas into fundamental mechanisms underlying not only normal states but also disease states and even senescent states, for all these states have evolved since the origin of life. Rather than considering each of them separately, as previously appreciated, we should now consider such a diversity of biological states as general phenomena characterized by their degree of cohesiveness among different levels of organization. On the basis of the main idea mentioned above, I will propose a theory of aging to attack the long-standing mystery of AD, prion diseases and other neurodegenerative disorders. This new theory will also account for the pathogenesis of quite different diseases such as amyotrophic lateral sclerosis (ALS), atherosclerosis, senile cataract and many other symptoms of aging.

Although aging or senescence is a widespread phenomenon, it has been largely neglected by most biologists and thus it remains one of the most poorly understood of biological phenomena. (Most gerontologists have traditionally stressed the difference between 'aging' and 'senescence'.[5] Throughout this paper, the term 'aging' is not distinguished from the term 'senescence' like most biologists.[44]) What is aging? Burnet (1976, p. 86)[45] wrote: "*No one has yet produced a satisfactory explana-*

tion of the whole process, and probably no one ever will". Kirkwood[46] also wrote: "*Aging is a complicated process and it may be a mistake to seek too simple an explanation*". However, my answer is very simple: Aging is a concerted *process* of natural selection and self-organization operating on different components — such as molecules, organelles, cells and organs — at different hierarchical levels of biological organization. This is the conclusive account of the origin of aging by means of natural selection and self-organization. In this sense, the aging of higher organisms is not the special phenomena; instead, it is one of the general phenomena typical of life like the origin of life and its evolution and even the brain function.

To date, many theories of aging have been proposed.[5),44)~55)] I think it, therefore, important to point out briefly how my theory is different from others especially the *evolutionary theories of aging*,[44),47)~50)] for they also are based on the general principles of natural selection. Assuming that genetic variation(s) would affect *age-dependent characters* like survivorship and fecundity, the evolutionary theories suggest that evolution of aging occurs as a *result* (not a process) of natural selection modifying the *time* of onset of the variant gene(s). However, these theories must be circular or incomplete as they already assume 'aging' in a general sense in terms of age-dependent characters. It is not chronological 'age' or 'time',[56] but living states emerging out of the complex networks of molecular metabolism that determine the time of action of gene(s).[46] The evolutionary theories of aging are therefore viewed as a simple application of the general principles of natural selection to aging. In contrast, as I stressed before, my theory proposes that aging itself is a concerted process of natural selection and self-organization acting on different components at different hierarchical levels. This is one of the rediscoveries of the general principles of natural selection operating at various levels in different biological systems. In an extreme sense, therefore, *aging itself is considered as an 'evolutionary' process.*

§ 2. Instructionist versus selectionist

The issue "instructionist versus selectionist" has been discussed in biology. However, such an issue has never been discussed in gerontology, probably because so many theories of aging have been proposed along quite different lines of evidence and they seem to be so complicated. In this section, I will discuss how the present theory of aging is different from others in the light of this issue.

All the theories of aging proposed so far are considered to be situated between the two extreme instructionists' views. The one is the *gene instructionists' view* that aging, like development, is directly controlled by genes. The *programmed theories* such as the evolutionary theories of aging are based on this view since they ultimately require deleterious genes, though the evolutionary theories also stress the importance of the effects of natural selection upon genetic variations affecting age-dependent characters.[44),47)~50)] However, as discussed before, assuming such life-history characters falls into a circular argument because it is essentially equal to assuming some time-keeping mechanisms responsible for aging. Furthermore, it turns out that most biological processes — even cell differentiation, for example, during development — are no longer directly controlled by immediate gene instructions (see § 4 in more

detail).

The other is the *environment instructionists' view* that organism's components — such as genes, proteins, protein-synthesis machinery and many other molecules — are continually subject to variations, not due to instruction from genes, but due to instruction from the organism's internal and/or external environment. The *error theories* such as the *somatic mutation theory*[55] and the *error catastrophe theory*[46),53),54] are based on this view. While the former assumes that aging is due to genetic mutations in somatic cells, the latter assumes that aging is the result of a progressive breakdown in accuracy in protein synthesis. Both are, however, refuted by experiments.[44),50]

Although many other theories of aging are also plausible, most of them seem incomplete or redundant or controversial. Indeed, none of them can clearly explain one of the most mysterious aspects in aging: variations. Different organisms, for example, even those belonging to the same species, often undergo different processes of aging under the same conditions. As long as theories are based on the instructionists' view, any variation is simply interpreted as noise with *no* functional importance. The opposite is true, when we adopt the selectionists' view. This view stresses, as in the case of the origin of species, that preexisting variations among individuals within a population are centrally important for successful adaptation of the selected individuals to changes in the environment because such variations are always subject to natural selection. In the next section, though still based on the traditional, genetic inheritance view, I will discuss how the selectionists' view has been successful in immunology and oncology.

§ 3. Genetic inheritance systems

Not only adaptive evolution of the complex organisms,[10] but also competitive survival of free-living bacterial cells in the simplest organisms[57] and even adaptive behavior of infectious pathogens,[58] like viruses[31] in the simplest microbes, all seem to require genetic material (DNA or RNA) for their self-replication and transmission. Therefore, it has long been thought that adaptive evolution requires genes as the necessary unit of transmission and heritable variations at any organizational level ranging from lower level of molecules to higher level of organisms. This genetic inheritance view as well as the selectionists' view has been extremely successful in immunology and oncology.

3.1. *Clonal selection theory in immunology*[15]

A multicellular organism is developed as a clone derived from a single fertilized cell with progressive differentiation of cell structure and function. The body, as development proceeds, is composed of two different classes of cells: dividing, inter-differentiated cells like lymphocytes and liver cells; and nondividing, post-differentiated cells such as nerve cells and muscle cells. Interestingly, there are parallels between the organization of dividing cells within a multicellular organism and that of a group of organisms in an animal society. Because variations and heritability are held in the cell lineages within the organism, the general principles of natural selection — which are known to govern adaptive evolution of organisms in an

animal society — are also capable of governing adaptive behavior of dividing cells as members of a cellular society.

In fact, as long as dividing cells persist, the body is inevitably exposed to different clones of variant cells through successive rounds of genetic mutations and natural selection. Our immune system takes advantage of such a preexisting pool of variant lymphocytes as 'induced' rather than 'constitutive' defense strategy according to the clonal selection theory. (Strictly speaking, our immune system depends on the *gene recombination* as well as *hyper-mutations* to produce a large repertoire of preexisting variations.[59])

3.2. *Clonal evolution theory in oncology*[18),19)]

Surprisingly, according to the clonal evolution theory in oncology, the same principles of natural selection allow any dividing cell to be malignant independent of its state of differentiation. Experimental studies have, indeed, revealed that many of cancers arise from a single abnormal cell and become more dangerous through this microevolutionary process.

It is true that cancers differ depending on the cell type from which they derive. However, several common features are shared by different cancers:[60]

(i) Many cancer cells are characterized by the abnormally high rates of mutation, possibly because of intracellular defects in *DNA repair systems*.[9]

(ii) Because of such abnormally high mutability, most malignant tumor cell populations are highly 'heterogeneous' in many respects such as drug-resistance[61] and metastatic capacity,[62] and thus capable of evolving at an astonishing rate when subject to new selection pressures (e.g., medical or surgical treatment).[9]

(iii) There are many ways in which cancerious behavior arises for each cell type.[9]

(iv) There is almost always a long delay between the initial event (e.g., exposure to radiation or mutagens) and the onset of disease because genetic mutations must be accumulated in the clonal descendants of a progenitor cell.[9]

(v) Although the accumulation of multiple mutations is required for the development of cancer, there are two mutational routes towards misbehavior: a stimulation of *oncogenes* and an inhibition of *tumor suppressor genes*.[63]

3.3. *Dual effects: development versus cancer*

A concept of oncogenes is paradoxical. What are normal functions of these genes, whose aberrations are so dangerous? It turns out that most *proto-oncogenes* (intact genes before converted into oncogenes by mutations) code for components of the mechanisms that regulate the social behavior of cells in the body, and that functions of these genes are essential to the development of the body.[63] Proto-oncogenes, in a sense, have dual effects: indeed, proto-oncogenes are necessary for the ontogeny of the body, whereas the same genes, once converted into oncogenes, cause malfunctions. Of course, the ontogeny of the body occurs due to an epigenetic change (see § 4), that is, a change in the gene expression pattern without a change in the DNA sequence. Cancer is thus considered as one of the 'hidden' properties emerging through successive cycles of mutations and natural selection at the level of dividing

cells.

Now, there arises one important problem: what mechanism keeps time? Indeed, it is not necessary to assume the chronological 'time' or 'age' to account for the development of cancer. The answer is this: it is random mutations created in DNA molecules that must be one of the time-keeping mechanisms. In general, DNA molecules undergo many mutations due to thermal fluctuations, reactive metabolites (including reactive forms of oxygen), ultraviolet light from the sun, and so on. Although most of them are eliminated by DNA repair systems, only a few stable mutations accumulate in the DNA. The accumulated mutations keep time and finally trigger the onset of disease.

In this context, let us recall to mind the evolutionary theories of aging. These theories have presupposed *pleiotropic* genes[48] that are favorable *early* in life but have cumulative bad effects *later on*.[46] As I repeatedly mentioned, the assumption of the chronological 'time' or 'age' falls into a circular argument, for it implies 'aging'. Without the assumption of the chronological 'time' or 'age', in contrast, we can now understand the dual effects of genes such as proto-oncogenes.

3.4. *Dual effects: immune recognition versus cancer*

In addition to the dual effects of genes over the lifetime of individuals, the genetic inheritance systems themselves have dual effects as mentioned above: on the one hand, the immune system takes advantage of a preexisting pool of variant lymphocytes; on the other hand, any dividing cell can be selfish like cancer at the expense of the whole individual.

When we take into account the epigenetic inheritance systems, along with the genetic inheritance systems, complex phenomena may be expected. However, it turns out that the general principles will also underlie such complex phenomena and similar dual effects will appear. This issue will be discussed below.

§ 4. Epigenetic inheritance systems

The following evidence suggests how the epigenetic inheritance systems operate at various levels and scales in almost all the living organisms, together with the traditional, genetic inheritance systems.

4.1. *Evidence for the epigenetic inheritance systems*

First, not all of proteins and amino acids are made by genetic coding. Some of antibiotics in bacteria[25] and amino acid neurotransmitters[64] in higher organisms are synthesized entirely by enzymes. Although the enzymes themselves are made by genetic coding, the products of these enzymes are not directly encoded by genes. In contrast with this enzymatic protein synthesis, which appears to be exceptional, enzymatic protein degradation is always the case: indeed, most proteins are constantly being degraded by digestive cellular enzymes called proteases[32),65] to prevent the buildup of abnormal proteins and to facilitate the recycling of amino acids. Since this protein degradation — though out of the central dogma — is a highly 'selective' process, it is as important to the adaptability of a cell to a changing environment as

is the protein synthesis. (Here, the term 'selective' means that misfolded and abnormally assembled proteins are degraded by the protein-degradation systems, whereas correctly folded and normally assembled ones remain intact.)

Second, the infectious agents — causing certain degenerative disorders of the central nervous system, pathology of which resembles that of AD — can indeed lack genetic material; and their only known constituent is a prion protein (PrP^{SC}).[7),8)] But even so, prions show virus-like characteristics such as self-replication and susceptibility to species barriers. How are prion diseases transmissible with no genetic material? It turned out that the prion protein (PrP^{SC}) is a variant of a normal cellular protein (PrP^{C}) which is encoded by the chromosomal gene (PrP gene) of a host. Though PrP^{C} and PrP^{SC} are encoded by the same PrP gene, the two isoforms significantly differ in conformation: one is α-helix-based normal isoform (like the coiled structure) and the other is β-sheet-based abnormal isoform (like the plane structure). Infection is considered to occur when exogenous prions containing PrP^{SC} would act as templates or specific 'enzymes' to promote the conversion from normal isoforms to abnormal ones without the aid of genetic instructions.[23)] Probably because precise matching between protein-protein interactions are required for this conversion, it would be responsible for susceptibility to species barriers.

Third, the cells cannot construct their own intracellular organelles such as the Golgi apparatus, endoplasmic reticulum and peroxisomes *de novo*: they require the prior presence of specific proteins embedded in membranes that make the individual organelles unique.[66),67)] How are the organelles constructed in the cells? Rather than the genetic information that specifies the organelle's proteins, the interactions of the organelle's proteins with other proteins that underlie molecular recognition can mediate the transport of appropriate molecules to the organelle necessary for its own construction. Although amino acid sequences of organelle-specific proteins can be traced to DNA sequences, it seems likely that dynamic processes mediated by the protein-protein interactions do not reflect direct or immediate regulation by genes.

Fourth, the surface patterns of ciliated protozoa, once damaged accidentally or changed by surgical interference, may be transmitted through many binary fissions.[68),69)] Of course, this transmission occurs independent of any change in DNA sequence; rather it occurs due to one of the structural inheritance systems.[22)] It is a three-dimensional structure that acts as a template for a daughter structure.

Fifth, cell differentiation in multicellular organisms usually occurs without detectable changes in DNA sequence: our nerve cells and our muscle cells and our liver cells are all genetically identical.[70)] Surprisingly, in contrast with the traditional view of a bacterium as an unicellular organism, cell differentiation occurs within single bacterial colonies, just like the cells in multicellular organisms.[71),72)] Because a certain cell type, once specified, is usually remembered through subsequent cell generations, there must be an inherited pattern of gene regulation, called *cell memory*,[22)] in each cell lineage. How are different cell types generated and maintained for the life-span of organisms, even if the cells divide? It is a self-regulatory network of gene expression mediated by their own products called *gene regulatory proteins* that is not only inherited upon cell division but also subject to variations for cell differentiation. In this sense, cell memory is considered as a

dynamic process of "reinterpretation" of gene instructions. (Because DNA methylation in vertebrates is associated with gene inactivation, it may play an additional role in generating different cell types.[73),74)]) Besides remembering the internal state of a cell as cell memory, any cell continually senses its environment such as signaling molecules secreted by its neighbors and adjust its behavior to suit the circumstances: in fact, the fate of cells to divide, differentiate or die is externally determined by cell-cell interactions known as inhibition, induction or competition.[26)] Consequently, cellular behavior is to varying degrees removed from the immediate gene instructions.

The final evidence is provided by brain function.[17),27),28),75)] It is clear that structural variability within the nervous system (e.g., the plastic changes that occur at synapses as a consequence of activity) is not always genetically coded, but represents the result of epigenetic regulatory processes acting during its development and ongoing operation. Such structural variability can additionally give rise to dynamic variability at the level of neural networks. The resultant combination of both the structural variability and the dynamic variability may contribute to the construction and destruction of motor actions, perceptual and conceptual categories, associative memory and many other brain functions in response to internal as well as external stimuli. Like 'cell memory' stored in the cell lineage, 'memory' in the neural network is viewed as a dynamic process of "recategorization" rather than a replicative recall from storage of discrete data.[28)]

All the above evidence strongly suggests that the genetic information in the form of DNA base sequences is merely part of the information necessary for not only *building up* but also *breaking down* constituents, ranging from hierarchically lower organization like molecules to hierarchically higher organization such as intracellular organelles, unicellular organisms, cells of multicellular organisms, and even the 'structure and function' of neural networks in the brain. It is the epigenetic information that plays an important role in any process of biological organization at various levels and scales.

4.2. *Dual effects: cell memory versus epigenetic cancer*[76)]

As discussed in § 3, the genetic inheritance systems cause dual effects: on the one hand, as in the immune system, they provide a large repertoire of preexisting variant cells, from which adaptive cells recognizing particular antigens are selectively proliferating; on the other hand, as in the development of cancer, they provide dividing cells with a great opportunity of being selfish at the expense of their neighbors. Along this line, we can imagine how the epigenetic inheritance systems have dual effects as well.

To understand this dilemma, let us consider, for example, how gene regulatory proteins underlie cell differentiation. In general, most gene regulatory proteins are shared with different cell types. Such multiple gene regulatory proteins can, thus, act in combination to regulate gene expression.[77)~79)] Because a specific set of gene regulatory proteins must be accumulated in each cell lineage, a cell being committed to a particular developmental fate by a critical gene regulatory protein, referred to as *determination*,[80)] does not spontaneously express its specialized character, referred to as cell differentiation,[80)] until the requisite combination of gene regulatory proteins is

completed by adding its final member. With this combinatorial control, a single regulatory protein does not necessarily have a predetermined function; instead, its function depends on the final combination of all the gene regulatory proteins. It is now clear that different cell types reflect different 'interpretations' of gene instructions or epigenetic variations in the combination of gene products.

Of course, the individual proteins are the products of genes. It is, however, out of genetic control how to form the final combination from individual proteins. Here, we are faced with the dilemma: just as the genetic inheritance systems give rise to selfish cancer as well as a large repertoire of variant lymphocytes through genetic variations and natural selection, the epigenetic inheritance systems have similarly conflicting effects through epigenetic variations and natural selection.

On the one hand, the epigenetic inheritance systems can increase a potential variety of cell memory, and thus enhance the adaptability of the cells during both the ontogeny and the phylogeny of multicellular organisms, firstly because gene regulatory proteins are not dedicated to a particular predetermined combination, secondly because a slight change in a few regulatory proteins can cause a substantial change in cell differentiation by affecting the expression pattern of many genes, and finally because a potential diversity of combinations will enhance a capacity to vary regardless of whether the environment changes or not. On the other hand, the epigenetic inheritance systems create selfish cell states: for example, they allow the dividing cells to give rise to selfish cancer possibly through successive accumulation of gene regulatory proteins that can stimulate cell-division cycles. It is the *teratocarcinoma* — one rare type of cancer — that is thought to have an epigenetic origin.[81]

As long as genetic or epigenetic variations are present — though they have important implications for both the evolution and the development of multicellular organisms — dividing cells potentially harbor cancer and hence endanger the multicellular organisms through natural selection. Along this line, now is the time to understand how nondividing cells undergo selective cell death. This is the central problem in neurodegenerative disorders as discussed in the next section.

§ 5. Theory of aging

First of all, the present theory accounts for the origins, causes and effects of the 'selfish' behavior of denatured proteins —characteristic of neurodegenerative diseases — in the context of the clonal evolution of cancer, with strong emphasis on the scale-invariant principles of natural selection and self-organization. Then, predictions of the theory are detailed. In short, aging is a concerted process of natural selection and self-organization; thus, aging reflects a capacity of individual; and so, aging is viewed as an 'evolutionary' process; as a result, aging is not essentially different from other living phenomena such as brain function.

5.1. *Structural variability*

Concerning the structural variability of proteins, there arise three problems: the first is how normal proteins become denatured, the second is how self-organization alters the denatured proteins, and the third is how natural selection operates at the

molecular level without the aid of genes.

To attack the first problem, it is useful to understand the origins of prion proteins (PrP^{SC}).[7),8)] In infectious prion diseases, denatured proteins (PrP^{SC}) arise when exogenous prions containing PrP^{SC} would trigger the conversion of normal isoforms (PrP^{C}) into abnormal ones (PrP^{SC}) without mutations in PrP gene.[23)] In some — but not all — of sporadic prion diseases, fluctuated increase in the concentration of endogenous abnormal isoforms (PrP^{SC}) would cause the same effects because they would stimulate the formation of themselves through a positive feedback loop.[23)]

There are, in contrast, inherited and some sporadic prion diseases, in which endogenous abnormal proteins arise from either inherited mutations or somatic mutations in PrP genes.[23)] Indeed, several different mutations in the PrP gene have been found associated with familial prion diseases such as Creutzfeldt-Jakob disease (CJD) and Gerstmann-Sträussler-Scheinker syndrome (GSS).[7)] These abnormal proteins are not genetically identical to PrP^{SC}, but characteristically similar to PrP^{SC} in such a way that they would increase the likelihood of self-aggregation of themselves. Defective proteins thus arise not only from genetic mutations as in the case of inherited and some sporadic prion diseases, but also from post-translational modifications or epigenetic mutations as in the case of infectious and other sporadic prion diseases. There appear to be many ways in which similar denatured proteins arise through a combination of genetic and epigenetic mutations. Note that denatured proteins occur randomly. As a result, there must arise a diversity of denatured proteins. While some of them are degraded by proteases, others undergo further alteration or self-organization.

To attack the second problem of how self-organization alters the denatured proteins, it is important to consider the self-aggregating process of denatured proteins such as β-amyloid, PHF and PrP^{SC}. We now know that noncovalent interactions are essential to the self-templating nature of DNA molecules, because they are individually weak but collectively strong. For the same reason, noncovalent interactions allow any denatured protein to act as either a template directing the self-assembly of itself, as in the accumulation of prions,[7)] or a seed of the polymerization, as in the deposits of β-amyloid.[24),82)]

Although each of these insoluble proteins consists of quite different amino acids, all of them commonly have a high content of β-sheet. Regardless of their amino acid contents, the predominance of β-sheet structures suggests that much of the protein is exposed to its environment, ready to interact with other molecules. The way for the denatured proteins to interact is by the contact of one rigid surface with that of another. Such surface-surface interactions are highly specific, allowing the denatured proteins to select their partner proteins from a vast diversity of proteins. Since these interactions are very tight and hence resistive to proteases, they will provide the selective advantage over the other proteins. Then, the selected proteins begin to be irreversibly connected with one another. In this sense, self-organization as well as random mutations (genetic and/or epigenetic mutations) can provide a new class of variations among proteins within the cell, upon which natural selection can operate.

Now consider the third problem of how natural selection operates at the level of molecules lacking the intrinsic nature of self-reproduction. Actually, we have too

much familiar with the adaptive evolution of self-reproducing entities such as self-reproducing molecules,[42),83),84)] dividing cells[15),16),18),19)] and living organisms.[10)] However, this is not always the case. Indeed, any molecule devoid of the ability of self-reproduction can be a direct target of variations and natural selection when its environment — concentrated by a variety of many proteins — is self-sustaining, where most of newly synthesized proteins are continually degraded by proteases (see § 5.2) In such a dynamic environment, any protein can be 'selfish' if it acquires the protease-resistive nature and thus becomes 'long-lived' one. This is a Copernician revolution in the modern biology, as I mentioned earlier in this paper.

Consequently, the accumulation of denatured proteins can be explained by three steps.

(i) Proteins are randomly exposed to genetic and/or epigenetic mutations. As a result, there arise a wide variety of denatured proteins.

(ii) Some of the denatured proteins, which are resistive to proteases, undergo self-organization or self-aggregation through surface-surface interactions. Then, there may arise a new class of variations among proteins within the cell.

(iii) Natural selection operates on such a preexisting diversity of the denatured proteins to favor more and more accumulation of the denatured proteins that have the protease-resistive nature.

It is cumulative effects of random variations, self-organization and natural selection that favor the accumulation of denatured proteins. What is worse, because of many ways in producing denatured proteins, most abnormal assemblies are highly heterogeneous in many respects[82)] and capable of evolving when subject to new selection pressures such as the enhanced activity of proteases and the exposure to drugs.

5.2. *Dynamic variability*

To understand the dynamic nature of the intracellular society, it is useful to consider supramolecular structures such as enzymes, ribosomes, cytoplasmic filaments and membranes. They are not made as single molecules connected by covalent bonds, but formed into hierarchical organization by the noncovalent assembly of many subunits. Such macromolecules are not permanent structures; they undergo constant *disassembly* into small subunits and *reassembly* into large macromolecules.

Let us begin with considering dynamic processes of intracellular protein metabolism. Some of newly synthesized proteins contain within their amino acid sequences the information necessary for proper folding, though others require interactions with sophisticated enzymes referred to as *molecular chaperons*.[85),86)] Most of the properly folded proteins act as subunits in the subsequent formation of macromolecules. This process of *self-assembly* occurs on the basis of information contained in the subunits themselves.[87)]

It is true that all the information necessary for folding and subsequent self-assembly seems to be traced to the 'genetic' information in the form of DNA sequences. However, when interactions with different kinds of proteins are taken into account, both the fate of subunits and the final conformation of macromolecules

are no longer immediately controlled by gene instructions, just as the fate of cells and the final form of body are not totally controlled by immediate gene instructions, but generally determined by cell-cell interactions known as inhibition, induction or competition. In this sense, the 'epigenetic' information is thought to reside not only in proteins themselves but also in noncovalent protein-protein interactions.

Unlike the covalent bonds which connect subunits of fixed structure, the noncovalent protein-protein interactions between the subunits can reversibly form and break, and so the function of multisubunit assemblies can be easily controlled by adding or removing some of different subunits. For this reason, a single protein does not necessarily have a predetermined function. Instead, its function emerges only when the final combination of all of the individual components is completed, as in the case of gene regulatory proteins (see § 4.1). New function arises through a slight change in the combination of multiple subunits out of the limited pool of molecules. Therefore, this conbinatorial control is of great advantage to both the evolution and the development of complex living organisms.

The dynamic nature of intracellular molecules is more than this. In fact, the self-assembled macromolecules are frequently recognized by other proteins to form much more complex assemblies, and these complex assemblies then serve as new recognition targets by separate proteins, and so on. Actually, such protein-protein interactions are essential to the biogenesis of plasma membranes,[88] lysosomes and endosomes,[89] the secretion of molecules by exocytosis,[90] the uptake of external molecules by endocytosis,[39] the catalytic activity of enzymes,[33] the formation of gene regulatory proteins,[77)~79] the budding and fusion of transporting vesicles,[91] the dimerization[92] or oligomerization[93] of cell surface receptors in signal transduction, the viral envelope assembly and budding process[94] and many other biological functions.[95),96]

Many thousands of different molecules are thus organized into a complex network of molecular metabolism. As one progresses from small subunits to large macromolecules in this hierarchical organization, the functions that the large macromolecules can perform become more remote from the immediate gene instructions and hence more elaborate and flexible. We thus see that adaptive cellular behavior emerges out of the coordinated activity of the complex network of molecular metabolism. As long as this complex network operates well, the cell — independent of whether it is dividing or differentiated — can continually adapt to its environmental changes. However, at the same time, this complex network is always subject to a threat of collapse to a short-circuit 'catastrophe' leading to selfish behavior, as in the case of hypercycle,[58),97),98] because it is impossible to avoid any dynamic variability. Of course, structural variability can give rise to additional variability in the resulting dynamics. Even without any structural variability, however, dynamic variability can still arise.

Just as structural components or 'elements' are exposed to random variations, self-organization and natural selection, dynamic processes or 'elementary processes' may proceed in the similar steps independent of whether structural variability is present or not. To understand this possibility, let us consider membrane-bound proteins such as PrP^c and the amyloid precursor protein (APP) of β-amyloid. Like

other macromolecules formed from many subunits, plasma membrane is not a permanent structure; it is continually removed and added by *endocytic-exocytic cycles*,[39),40)] by which PrP^C and APP are recycled back to the plasma membrane. Some of PrP^C and APP further undergo the *endosomal-lysosomal pathway* and end up in lysosomes where they are degraded by proteases for the recycling of amino acids.[7),8),99),100)]

Although such recycling not only maintains a steady-state distribution of membrane components essential to the homeostasis of a cell, but also coordinates the cellular processes responsible for the cell's adaptability to its changing environment, it potentially provides a short cut to the selfish dynamics with *no* structural variability. The selfish dynamics, in turn, may trigger structural variability or the formation of denatured proteins. Then, denatured proteins begin to accumulate because the dynamic processes, like structural components, are further subject to random variations, self-organization and natural selection. Consequently, there are different ways in which structural and dynamic variability arise, as suggested by studies of defects in intracellular protein trafficking.[101)]

5.3. *Comparison of neurodegenerative disorders with cancers*

(1) Lessons from clonal evolution of cancers

In order to understand the origins of cancers, it is very important to consider two aspects of life: ontogeny and phylogeny.

Ontogeny: In contrast with the traditional view of the ontogeny of multicellular organisms as a cooperative enterprise, competition seems more likely to be the rule.[26)] Even though a multicellular organism is originally developed as a clone from a single cell, it will be populated by normal and abnormal cells later on due to unavoidable genetic mutations. (Roles of epigenetic mutations will be discussed later.) Actually, conflicts often arise through competition not only between different cell lineages, but also between different hierarchical levels. The effects of natural selection upon genetic mutations are, therefore, a double-edged sword: on the one hand, dividing cells such as lymphoid cells continually sense their environment and adjust their behavior to suit the circumstances, as in the immune system;[15)] they, on the other, potentially give rise to cancers (for example, lymphoma) or autoimmune disorders[102)] at the expense of the whole body through essentially the same process.[18),19)] As a consequence, though individual organisms at the higher level of organization are the *units of selection*[22),25)] in a population of organisms, they themselves are subject to a threat of disintegration or self-*dis*organization or *de*differentiation when dividing cells become selfish through lower level cell selection. In this sense, it is much more important to consider both the 'creative' aspects and the 'non-creative' aspects of biological phenomena in the global context, rather than considering them separately.

Phylogeny: It is true that variant components arising from genetic mutations endanger the integrity of the whole individual at a higher organizational level. Nevertheless, life has hierarchically organized (i.e., new levels of individuality[26)] have successfully emerged) since it developed sophisticated systems such as DNA repair systems and epigenetic inheritance systems.

DNA repair systems provide a variety of mechanisms for repairing many acciden-

tal lesions that occur continually in DNA.[103] Suppose that DNA is the sole unit of heredity at all levels. Then, it is possible to resolve the emerging conflicts between different levels of organization through changes in DNA due to repair mechanisms.[22] However, such DNA repair systems also have dual effects: on the one hand, they increase genetic stability of individuals and therefore help the individual to survive in a hazardous world; on the other hand, they become the new targets of variations and natural selection. In *Bloom's syndrome*, an inherited human disease, individuals are particularly defective in DNA repair systems and thus they have a dramatically increased incidence of cancer.[104]

If, in addition to DNA (i.e., the genetic inheritance systems), there are other inheritance systems which can transmit additional information, it is also possible to resolve the conflicts between levels of organization. It is the epigenetic inheritance systems that have played an important role in the evolution of complex organisms and of developmental strategies, which can protect the higher individual from disintegrating into its lower component parts. However, such epigenetic inheritance systems are again a double-edged sword: on the one hand, cell memory, for example, is remembered through subsequent cell generations, which may be essential for the transition from unicellularity to multicellularity;[105] it is, on the other hand, exposed to variations and natural selection leading to epigenetic cancer.[76]

We are now faced with a serious dilemma. Indeed, living organisms have evolved some sophisticated systems to protect the genetic inheritance systems from variations and natural selection. Such sophisticated systems play an important role in the transition to multicellularity and the evolution of complex organisms, for they can ensure the cohesiveness of the whole individual at a higher organizational level. However, they themselves are the new targets of variations and natural selection, as in the case of cancers, which suggests the *devolution*[106] of complex organisms through the disintegration into their component parts. Even though nature would have evolved quite new systems that protect the individual from disintegrating into its component parts, the new systems could be in turn the additional targets of variations and natural selection. This dilemma will continue for ever. Actually, nature has evolved nondividing cells such as neurons and muscle cells for the purpose of division of labor among the cells of the multicellular organism. However, they have been the new targets of cell death, which will be discussed next.

(2) Subcellular 'cancer' and neuronal cell death

Unlike dividing cells, our nerve cells and our muscle cells supplied during fetal development must last us for an entire lifetime. These nondividing or long-lived cells, even damaged, are not competitively outgrown like cancer. Nevertheless, we are faced with a similar dilemma: in fact, these nondividing cells are essential for our thinking and our motion as a division of labor among cells of our body; but they become the new targets of negative selection leading to cell death due to the accumulation of variant proteins. To understand how variant proteins accumulate, we must consider the cellular process with respect to the degradation of proteins. Indeed, most proteins are constantly being degraded not only to prevent the accumulation of defective proteins, but also to facilitate the recycling of amino acids. Let us consider

two protein-degradation systems.

Two protein-degradation systems: One protein-degradation system operates in lysosomes and serves to recycle membrane-bound proteins (e.g., receptors), extracellular proteins, and long-lived proteins.[65] Lysosomes are found in all eukaryotic cells and are extraordinarily diverse in their morphology, which reflects the wide variety of digestive functions mediated by lysosomal proteases. In general, cells undergo the endocytic-exocytic cycles, by which they cannot only recycle their own plasma membranes, but also take up extracellular macromolecules and even other cells such as senescent and damaged cells as in the specialized case of macrophages. The ingested materials are delivered to lysosomes via the endosomal-lysosomal pathway[99),100)] for degradation. Indigestible substances therefore remain in lysosomes and form *residual bodies*.

The other is an ATP-dependent degradation system, by which defective proteins, incompletely assembled proteins, and short-lived proteins are degraded in the cytosol.[33] Most of proteins that are degraded in the cytosol are specifically marked for destruction by the covalent attachment at a small protein called *ubiquitin*.[107] Such ubiquinated proteins are delivered to large protein complexes called *proteasomes*,[33] where they are rapidly degraded not only to facilitate the recycling of amino acids, but also to change their concentrations promptly with alternation in the state of a cell. Thus the protein degradation plays an important role in the adaptability of a cell to a changing environment.

Accumulation of a variety of denatured proteins: Because a variety of proteins and other molecules are organized into the complex network of metabolic reactions, they are all candidates for progressive accumulation in a cell. One of the oldest known characteristics of senescent cells is the progressive accumulation of a heterogeneous group of pigment granules referred to as *lipofuscin*.[52] Because it appears to be chemically unreactive, lipofuscin does not seem to interfere with the normal functioning of the cell. Nevertheless, the cellular processes underlying lipofuscin accumulation would provide clues to the understanding of the etiology of AD and other neurodegenerative disorders. The evidence that inhibitors of lysosomal enzymes cause a rapid and massive increase in lipofuscin-like dense bodies in the brain cells[108] suggests that lipofuscins and some of deposits are derived from lysosomes due to impairments in proteases. Alternatively, if lipofuscins and related pigment granules acquire the protease-resistive nature, they will progressively accumulate in lysosomes.

In the light of some evidence of lipofuscin accumulation, it is now possible to understand the origins and causes of plaques in the brain. Consider prion proteins.[7),8)] In cell cultures, the conversion of normal PrP^{C} to the abnormal PrP^{SC} occurs inside neurons, after which PrP^{SC} accumulates in lysosomes via the endosomal-lysosomal pathway. In the brain, such filled lysosomes could conceivably burst and damage cells. In AD, the source of extracellular β-amyloid deposition is still unclear. It is, however, likely that at least a proportion of β-amyloid has intracellular origins possibly via the endosomal-lysosomal pathway. The accumulation of PHF, another hallmark of AD, could occur in the cytosol due to a defective ATP-dependent protease

or to an unusual resistance of PHF to the protease, because ubiquitin, usually involved in the cytosolic proteolysis of abnormal or short-lived proteins, is covalently linked to PHF.[109]

The general principles of natural selection and self-organization: Because there are a wide variety of accumulating proteins, it may be difficult to understand simple principles underlying the pathogenesis of neurodegenerative disorders. However, lessons learned from cancers provide us with important insights into understanding of the general principles regardless of the details of diseases. Actually, just as any dividing or short-lived cell is potentially capable of selfish cancer and in the end destroys the whole cellular society, so any short-lived protein is likely to gain selfish nature (i.e., self-aggregating nature) and finally attack the whole intracellular society like subcellular 'cancer'. In this sense, cancers and neurodegenerative disorders, all fatal, share common features at different levels of organization, though they apparently differ in pathology. During aging all the cells of our body are potentially subject to a threat of accumulation of a variety of denatured proteins. Among different cell types, nondividing cells are particularly vulnerable to accumulating erroneous proteins, because most proteins are turned over rapidly in relation to the cell's life-span, upon which natural selection and self-organization can operate with cumulative effects. It is now clear that the general principles operate at different hierarchical levels and scales of biological organization.

5.4. *Predictions*

The present theory of aging strongly suggests that the scale-invariant principles of natural selection and self-organization operate at different hierarchical levels of biological organization. If so, the theory will predict the etiologies and the pathogenic mechanisms of many other age-related diseases. For this purpose, the arguments of the present theory — though most attention has been paid on proteins — are now extended to other constituents of the cells such as sugars and lipids.

(1) Diseases associated with defects in short-lived molecules

Parkinson's disease and Amyotrophic lateral sclerosis (*ALS*): Besides AD and prion diseases, there are many other neurodegenerative disorders such as Parkinson's disease and ALS. Although the details of their pathologies are different from one another, there are marked similarities in all these disorders.[5),7),52)]

(i) All are diseases of middle to later age in humans, most of which occur sporadically but sometimes appear in families.

(ii) Protein and other molecular deposits accumulate as plaques, though different plaques occur in different diseases.

(iii) Neurons degenerate resulting in the enlargement of *glial cells*.[110]

(iv) White blood cells — known as warriors of the immune system — have not been detected in all the cases. This suggests that viral infection does not occur, for if a virus were involved in these illnesses, white blood cells would be expected to appear.

(v) Depending on diseases, different neurons show a high degree of selectivity with respect to neuronal cell death as well as changes in neuronal morphology.

The present theory of aging argues that any short-lived molecule is potentially capable of a target of natural selection leading to the self-aggregation within nondividing cells. Because cellular characteristics are determined by the complex network of molecular metabolism involving cell-type specific gene regulatory proteins, different molecules can be the candidates for accumulation in different neurons. Therefore, it is not surprising that different neurons show a high degree of selectivity with respect to neuronal cell death in different diseases. Despite these complex phenomena, the present theory predicts that the common principles of natural selection and self-organization operate at the molecular level as well.

Age-related atrophy of muscles: Like nerve cells, muscle cells also are nondividing or long-lived cells. The present theory, therefore, predicts that denatured proteins and other molecules may also play an important role in the age-related atrophy of muscle cells. This prediction seems to be supported by the surprising evidence that the overexpression of normal PrP gene causes the destruction of both muscles and peripheral nerves.[7)]

Indeed, the skeletal muscle shows the remarkable adaptability to environmental influences: while exercise stimulates the growth of skeletal muscle, disuse or denervation bring about an atrophy in the muscle structure. Thus, it is very difficult to determine the causal relationship in the atrophy of muscle fibers. Nevertheless, it is worth investigating that selfish behavior of denatured proteins and other molecules would trigger illnesses that attack the heart muscle as well as the skeletal muscle.

(2) Diseases associated with defects in long-lived molecules

We have now understood the common features among cancers and neurodegenerative disorders: just as dividing or short-lived cells can be selfish to attack the whole individual, short-lived proteins can be selfish to accumulate as long-lived deposits and finally attack the nondividing or long-lived cell. As the present theory stresses the scale-invariant principles, it can predict the possible roles of long-lived proteins to age-related dysfunctions as well. Because short-lived proteins are transformed into long-lived deposits, which in turn cause the degeneration of neurons, the presence of intact long-lived proteins itself poses another serious dilemma.

Consider the most abundant protein in our body: *collagen*. This long-lived protein is a major constituent of blood vessels, tendons, skins and many other connective tissues,[111)] for it provides strength and stability to them. However, as collagen has a very long life-span, it is a target of natural selection and self-organization. Studies of *diabetes*, a disease characterized by elevated blood-glucose levels,[112)] give insights into the roles of long-lived proteins in age-related diseases, for diabetic individuals accelerate aging changes: the early onset of atherosclerosis and senile cataracts, which are disorders generally develop in the elderly.[5),113)] Another clue to the aging process has been provided by studies of food: the nonenzymatic reactions between glucose and proteins, known as the *Maillard* or *browning reaction*.[5),113)]

From studies of diabetes and food, it is clear that sugar glucose in high concentrations is toxic to cells and tissues, for the nonenzymatic *glycosylation* of long-lived proteins (the chemical attachment of glucose to long-lived proteins) in our body triggers a series of chemical reactions to form endproducts, collectively called

advanced glycosylation endproducts (AGE's).[5),113)] Most AGE's can irreversibly cross-link adjacent proteins and eventually accumulate in our body. Whereas enzymatic glycosylation occurs at a specific site on a specific molecule for a specific purpose, nonenzymatic glycosylation occurs randomly at any of several sites along any available peptide chain. The resulting cross-linked proteins derived from AGE's, therefore, become more and more resistive to any disposal mechanism such as macrophages, though they attempt to ingest some of them.

In the light of this brief overview, we can now understand typical age-related diseases such as atherosclerosis, senile cataract and autoimmune diseases.

Atherosclerosis: Atherosclerosis, a disease characterized by the accumulation of cholesterol in artery walls,[114),115)] forms plaques that inhibit the flow of blood. As these atherosclerotic plaques grow, they often trigger the formation of clots that eventually block the flow of blood and cause a heart attack or a stroke. Like collagen, elastin — another constituent of the artery walls — has a long life-span. Both of the long-lived proteins are the targets of natural selection and self-organization leading to the formation of AGE's. When the inner lining of a blood vessel is accidentally damaged, blood glucose, short-lived *plasma proteins* and *low-density lipoproteins* (*LDL*) leak into the artery wall, where glucose first targets the long-lived proteins such as collagen and elastin to form AGE's and then AGE's on these long-lived proteins trap LDL as well as a variety of plasma proteins to develop bulky plaques.[113)] At the same time, macrophages and smooth-muscle cells ingest and degrade the LDL and become *foam cells*. As a result, *Cholesterol*, derived from the LDL, progressively accumulate in and among the form cells.

No one has yet understood the exact processes leading to atherosclerosis. This is probably because there are highly complex phenomena associated with atherosclerosis and there are different ways in which atherosclerosis occurs. However, the present theory strongly suggests that a wide diversity of observed phenomena must reflect the operation of the general principles of natural selection and self-organization at any level of organization.

Senile cataract: As we get old, lenses of our eyes turn brown and cloudy. Such age-related changes in the eyes are known as senile cataract.[113)] The *crystallin* proteins with characteristically long half-lives are major constituents of the lens,[116)] so that they are the targets of nonenzymatic glycosylation. Because nonenzymatic glycosylation causes AGE's on crystallin proteins and AGE-derived cross-links between them, it could help to explain why lenses turn brown and cloudy during cataract formation. In addition to this nonenzymatic glycosylation, there are other types of variations such as *racemization*.[5)] Although all the newly synthesized proteins are L-amino acids, D-amino acids accumulate very slowly in lens crystallins over our life-span. This racemization may also contribute to cataract formation. Because there are a diversity of these time-dependent degenerating changes in lens crystallins, responsible for senile cataracts, such time-dependent changes must be the result of the general principles of natural selection and self-organization.

Autoimmune diseases: There are many other long-lived molecules in living organisms: myelin, histon, hemoglobin and even DNA.[113)] When these molecules are

exposed to natural selection and self-organization at the level of molecules, they are entirely different from the original ones. The modified molecules, though originate from the 'self', could be recognized as the 'non-self' by the immune system. Then, the immune system would begin to attack the modified 'self', leading to autoimmune diseases.

Myelin, for example, is the complex mixture of long-lived proteins that forms a sheath around neurons. If myelin is subject to natural selection and self-organization, it is gradually altered and is eventually distinct from intact one. The immune system can now attack the altered 'myelin', resulting in *multiple sclerosis*,[117] a type of autoimmune diseases attacking the white matter of the brain and spinal cord. Because there are many long-lived molecules to provide strength and stability at individual tissues and cells, some — but not all — autoimmune diseases may be associated with defects in these molecules. The general principles of natural selection and self-organization may also underlie the mechanisms of autoimmune diseases.

(3) Aging as a reflection of the capacity of complex organisms

We have discussed many of age-related diseases such as cancers, neurodegenerative disorders, atherosclerosis and so on. The present theory of aging not only suggests that each age-related disease can be viewed as a concerted process of natural selection and self-organization operating at various levels and scales of biological organization, but also predicts that such a diversity of diseases, as a whole, reflect the capacity of complex organisms. In this sense, I believe that there is no essential difference between 'non-creative' aspects of life such as age-related diseases and 'creative' aspects such as the development and evolution of life, and brain function (see also § 6). To understand how such 'dual' aspects emerge out of the same individual and thus how aging reflects the capacity of living organisms, let us now consider two important features: hierarchy of duality and cascade of variations.

Hierarchy of duality: We have discussed different kinds of duality in life: development versus cancer (§ 3.3), immune recognition versus cancer (§ 3.4), cell memory versus epigenetic cancer (§ 4.2), DNA repair systems versus cancer (§ 5.3). Since biological organization is hierarchical, there must be many other kinds of duality at different levels leading to a hierarchy of duality.

To understand the hierarchy of duality, let us consider newly synthesized proteins in a long-lived cell. Since the cell is the environment extremely concentrated by normal and abnormal proteins, the folding of newly synthesized proteins must be a highly competitive process between *on-pathway reactions* leading to the properly folded state and *off-pathway reactions* leading to aggregation.[86] Such a competitive process reflects the duality of the noncovalent protein-protein interactions: on the one hand, the noncovalent interactions facilitate the proper folding of themselves; on the other hand, they give rise to intermolecular aggregation if similar unfolded proteins are present at high concentrations. Even without variations at the level of amino acid sequences, therefore, newly synthesized proteins would be always subject to a threat of aggregation.

Nature has thus evolved molecular chaperons. Indeed, the molecular chaperons play an important role in reducing the risk of aggregation. In this sense, the

molecular chaperons are considered as 'helpers' or 'fail-safe' systems. For example, the molecular chaperons, *Hsp 70* and its relatives, can bind to the exposed hydrophobic regions of the newly synthesized proteins and thus keep the partially folded proteins from aggregating by blocking the hydrophobic regions. Now, there arises another type of duality: although the binding of molecular chaperons can prevent the partially folded proteins from aggregating, it will also block normal folding because the hydrophobic regions must be buried in the normally folded proteins.

Furthermore, the molecular chaperons themselves become new targets of random variations due to genetic mutations and/or post-translational modifications (or epigenetic mutations). As a result, the molecular chaperons are potentially capable of possessing new functions independent of whether or not the new functions are favorable to the 'social behavior' of proteins. This frames a serious dilemma: on the one hand, the molecular chaperons can serve as 'helpers'; on the other hand, they will serve as 'warriors'. This duality is strongly analogous to that of proto-oncogenes: indeed, the proto-oncogenes are favorable early in life but have cumulative bad effects later on when converted into oncogenes by mutations.

Along this line, I hold that 'prions' may be the variant forms of molecular chaperons for they would act as templates or specific 'enzymes' to promote the conversion from normal isoforms to abnormal ones without the aid of genetic instructions. Furthermore, I predict that we will discover new types of prions in the future, for cells contain a variety of molecular chaperons and many other specific enzymes that would be candidates for the new types of prions.

Since the molecular chaperons themselves have dual effects, there arise many kinds of denatured proteins in the cell. To prevent the buildup of abnormal proteins, therefore, nature has evolved two other fail-safe systems: protein repair systems and protein-degradation systems. Unfortunately, these fail-safe systems have dual effects as well: they can repair or remove the denatured proteins, whereas they themselves are the new targets of random variations. As we get old, therefore, the long-lived cell is sooner or later vulnerable to a large amount of denatured proteins and at last the cell will die. Then, there remains the cellular debris.

The resultant cellular debris as well as senescent and damaged cells can be now taken up by scavenger cells or macrophages through endocytosis or phagocytosis. Like many other cells, these scavenger cells also undergo endocytic-exocytic cycles. The endocyosed and phagocytosed materials are thereafter delivered to lysosomes, where they are degraded. But, indigestible or protease-resistive substances will remain in lysosomes, forming residual bodies. Here, the scavenger cells also have dual effects: indeed, they can take up and remove the cellular debris from the external medium; however, they will intensively accumulate the residual bodies in lysosomes. If the residual bodies have the self-aggregating nature like prions, PHF and β-amyloid, the scavenger cells will be damaged or die.

Now we should notice a huge recycling pathway, though it also has dual effects. Starting from the newly synthesized proteins in the long-lived cell, we have reached the residual bodies in the scavenger cells. Even though some of these scavenger cells are damaged or die, others will take over the cellular debris by phagocytosis. There arises a huge recycling pathway. Of course, some of the digested substances can be

reutilized by the cells in this pathway. However, once certain proteins acquire the protease-resistive nature, like prions, PHF and β-amyloid, they will remain in this pathway for ever.

It is now clear that there are many types of fail-safe systems at different hierarchical levels and scales of biological organization, and that each of them has always dual, seemingly conflicting, effects.

Cascade of variations: It seems that aging takes place at all levels of biological organization ranging from molecules up to a whole body. Indeed, random variations occur at all the components of organization such as DNA molecules, short- and long-lived proteins, many other molecules, short- and long-lived cells, tissues and so on. All the variations are, however, not completely independent of each other, but rather fairly interdependent. Since the biological organization is hierarchical and all the fail-safe systems have dual effects (i.e., hierarchy of duality), there must be cascade of variations starting from molecules at a lower level up to a whole body at a higher level.

Even if initial variation is too small to be detected, it will be amplified through the cascade of variations. Since there is a huge recycling pathway in life, as mentioned above, it is hopeless for any protein to escape from this cascade of variations. Thus, the cascade of variations, along with the hierarchy of duality, implies 'dual' aspects of life that emerge out of the same individual.

From the hierarchy of duality and the cascade of variations, it seems likely that aging reflects the capacity of individual, for variability at each hierarchical level implies a capacity to vary at the highest level of individual. Therefore, a diversity of pathological hallmarks of AD (see the Abstract) will be considered to reflect this capacity.

(4) Roles of natural selection and self-organization to aging

Although I did not emphasize the roles of natural selection and self-organization to aging in the previous discussion, they are certainly of great importance. If cells are dividing, they are potentially capable of being selfish cancer through rounds of genetic mutations and natural selection. If nondividing cells contain a diversity of proteins that are recycling, the proteins themselves are the direct targets of natural selection and self-organization, as in the case of AD and prion diseases. Along this line, we have understood that there are the scale-invariant principles that govern the pathogenetic mechanisms common to cancer and neurodegenerative disorders.

Surprisingly, the living organism has much more complex network of the controls that regulate the protein behavior (e.g., the birth, assembly and death of proteins) and cell behavior (e.g., cell proliferation, differentiation and death). Therefore, the roles of natural selection and self-organization in the regulatory process must be highly complex. Suppose that there are denatured proteins that have the protease-resistive nature in the long-lived cells. The long-lived cells will sooner or later die, and scavenger cells will take up the cellular debris. However, the scavenger cells cannot digest entirely the cellular debris because they merely have proteases that are similar to those of the long-lived cells. Thus, the scavenger cells that took up the cellular debris will be damaged or die. Then, other scavenger cells will take over the