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Phenoptosis as Genetically Determined Aging Influenced by Signals from the Environment

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Abstract—Aging is a complex and not well understood process. Two opposite concepts try to explain its causes and mechanisms – programmed aging and aging of "wear and tear" (stochastic aging). To date, much evidence has been obtained that contradicts the theories of aging as being due to accumulation of various damages. For example, creation of adequate conditions for the functioning of the organism's components (appropriate microenvironment, humoral background, etc.) has been shown to cause partial or complete reversibility of signs of its aging. Programmed aging and death of an organism can be termed *phenoptosis* by analogy to the term *apoptosis* for programmed cell death (this term was first suggested by V. P. Skulachev). The necessity of this phenomenon, since A. Weismann, has been justified by the need for population renewal according to ecological and evolutionary requirements. Species-specific lifespan, age-dependent changes in expression pattern of genes, etc. are compatible with the concept of phenoptosis. However, the intraspecific rate of aging was shown to vary over of a wide range depending on living conditions. This means that the "aging program" is not set rigidly; it sensitively adjusts an individual to the specific realities of its habitat. Moreover, there are indications that in rather severe conditions of natural habitat the aging program can be completely cancelled, as the need for it disappears because of the raised mortality from external causes (high extrinsic mortality), providing fast turnover of the population.

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The root cause of aging remains unknown. Variability in the rate of age-related changes and the results of the analysis of all the accumulated data do not contradict the possibility of the existence of conditions facilitating significant retardation of aging.

However, the problem of the mystery of this process formulated over 50 years ago [1] remains unsolved. For instance, a recent review on the subject deals with this issue [2]. It starts with a classical and still relevant quote by evolutionary biologist George Williams [1] – "It is indeed remarkable that after seemingly miraculous feat of morphogenesis, a complex metazoan should be unable to perform the much simpler task of merely maintaining what is already formed".

The protracted search for a clue to this paradox is probably related to the fact that the solution is looked for at levels where one can see only the consequences and perhaps some secondary mechanisms of aging, but not its original cause. As the effects of aging are manifested at all the levels of living matter, from macromolecular to populational, researchers from various fields are engaged in gerontology – from biochemists and molecular geneticists to populational biologists and demographers. It is important to understand at what level of complexity the root cause of aging operates so as to find the best way to influence it.

The cellular level is located in the middle of the structural hierarchy of the systems of the body. Therefore, if cells are inherently capable of unlimited self-maintenance, the causes of aging should be looked for at the upper levels of the hierarchy. Otherwise, the cause of aging is hidden at the subcellular levels.

IS CELLULAR AGING THE CAUSE OR THE EFFECT OF AGING OF THE BODY?

For half a century, the majority of cytogerontologists, following L. Hayflick, have considered cellular

aging to be the primary process. As cells are not capable of unlimited self-maintenance outside the body even under ideal conditions, cytogerontologists and molecular geneticists consider them to be the root cause of aging. Then aging of the whole organism seems to be an inevitable consequence of the aging of its constituent cells. That is why aging has been mainly studied at the cellular and subcellular levels for the past half-century.

However, analysis of the accumulated data shows that the decrease and loss of proliferative potential of cultured cells (which are considered to be among the main signs of senescence) may be a natural consequence of the process of terminal differentiation, which is programmed to block the ability of cells to reproduce. The natural microenvironment of somatic stem cells, which keeps them at the stage of stem cells with unlimited mitotic potential, is inadvertently disturbed in cell cultures. This statement can be proved in at least five ways: by recreating the cellular microenvironment, eliminating differentiation stimuli from the extracellular medium, by adding inhibitors of differentiation to the cultivation medium, and by blocking the last constitutive expression of immortalizing proto-oncogenes or a telomerase gene. In all these cases, contrary to the concept of Hayflick, normal diploid cells show no signs of decrease in reproductive potential. However, when transferred into cultivating conditions that unblock the possibility of differentiation, the cells demonstrate decrease in proliferation activity. This phenomenon is always observed by numerous followers of Hayflick.

Thus, true cellular aging should be seen as a secondary process related to their functioning in an aging organism or in an inadequate culture medium. It seems interesting to note that such aging is largely reversible [3-6]. That is why even molecular biologists and cytogerontologists have started discussing the issue of moving the root cause of aging beyond the cell membrane [7-10].

IS AGING A PROGRAMMED OR STOCHASTIC PROCESS?

Despite the frequent mentioning of the hundreds of aging theories, it has been long understood that their larger part has only historical significance. It is a well-known fact that there are only two opposing approaches to the explanation of aging. According to the first, aging results from the realization of a set of genetic programs, such as telomerase inhibition in somatic cells. A set of such programs can be called phenoptosis [11, 12] (if we transpose the studied the phenomenon of apoptosis to the level of the whole organism).

The second concept assumes the absence of such special programs. It draws attention to various failures, damages, deviations, mistakes, and mismatches that inevitably arise in any complex system, including living organisms. In this case, for aging to be inevitable, it is necessary to postulate that the power of the organism's recovery systems is clearly insufficient for the complete elimination of damage. Therefore, some damage (for example that created by free radicals, mainly reactive oxygen species) should inevitably elude correction and, when accumulated, lead to aging. It is also possible to combine these two opposing concepts. Using this approach, aging is seen as a programmed process that can be enhanced by various uncontrollable events [13, 14].

FREE RADICALS AS PHYSIOLOGICAL EFFECTORS AND TELOMERE SHORTENING AS AN ACTIVELY REGULATED PROCESS

Now it has become clear that such common aging hypotheses as free radical theory and the telomere–telomerase concept may turn out to be not quite suitable for the explanation of the root cause of natural aging.

Both increased telomerase activity [15] and antioxidants [16-18] are known to slightly prolong lifespan of relatively short-lived individuals of the same species. But the free-radical and telomere theories are not suitable for explanation of *natural* aging [17, 19, 20], and the issue of its root cause remains unsolved.

Nevertheless, it must be recognized that the postulates of the free radical theory of aging have played a leading role in fundamental gerontology for almost half a century. Even an increase in the number of population doublings of cultured cells (increase in the Hayflick limit) in response to reduction of oxygen concentration in the culture medium was interpreted as retardation of cellular aging due to decrease in damage caused by reactive oxygen species [21]. However, numerous experiments have shown the physiological role of free radicals in the regulation of different vital processes. In the above example, the level of reactive oxygen species was regulated (increased or decreased) under the conditions of hypoxia created *in vitro* [22]. It is the increase that activates the processes leading to an increase in the Hayflick limit [22].

It has also been found that the geroprotective effect of certain natural and synthetic antioxidants, such as epiphysis factors [23] or dibunol [24], can be linked to their direct effects on the endocrine system. The results of recent studies have shown a long assumed (e.g. [25]) essential physiological role played by free-radical processes in the regulation of many bodily functions. For example, reactive oxygen species were shown to be a special class of secondary messengers [26, 27]. Along with adenosine monophosphate, calcium ions, and certain phospholipid metabolites, they carry out an important function in processes of signal transmission from extracellular regulatory ligands via respective receptors and subsequent cascades of intracellular biochemical reactions up to regulation of activity of transcription factors,

which control gene expression. Formation of reactive oxygen species is strictly controlled in cells; it is regulated by a set of hormones, cytokines, growth factors, etc. [26, 27]. Therefore, excessive use of antioxidants can be quite dangerous as it can suppress important cellular functions [28]. This explains why antioxidants normally have only a slight geroprotective effect, whereas in a number of pathologies connected to increased production of reactive oxygen species antioxidant therapy is very effective.

As for the telomere–telomerase concept of aging, it was found to face certain difficulties; this hypothesis certainly needs to be modified. Ten years ago the author of the concept proposed another, more valid theory of aging [17]. On the other hand, it is quite possible that telomere shortening is not just a passive phenomenon associated with steric characteristics of their structure, but a process actively regulated by positive (telomerase, tankyrase, etc.) and negative (TRF1, TRF2, etc.) factors according to the organism's needs, which proceeds under its direct control to ensure the necessary cellularity. In addition, the telomere–telomerase concept is known to have been created to explain the phenomenon of limited population doublings of normal cells *in vitro* (the so-called Hayflick limit). Again, up until recently this phenomenon has been often considered the main cause of aging. However, gradually there has been growing understanding of the fact that the Hayflick limit is probably related to the programmed suppression of the ability to divide that accompanies the cell maturation and differentiation. Therefore, increasing the potential of cell doublings by telomerase activation, which was interpreted by the supporters of the telomerase concept as overcoming of aging [29, 30], is in fact connected to inhibition of differentiation in cultured cells and their "freezing" the stage of unlimited proliferative potential of precursor cells [31-36]. A similar interpretation was proposed a decade earlier [37-39] to explain the overcoming of myc-type protooncogene in them [40].

PHENOPTOSIS AS A DYSREGULATORY PATHOLOGY. ROLE OF THE ENVIRONMENT

Analysis of accumulated experimental data suggests that the concept of inevitable accumulation of errors that lead to aging cannot be considered proved [41-46]. It is a well-known fact that living systems possess high reliability and inherent ability for self-restoration [3-6, 47-50]. Moreover, there are species with so-called negative aging. In their case, fertility and reliability continue to increase, and mortality decreases with age, even after maturity is reached [51]. In this respect they are different from the majority of other species, which are characterized by age-related decline in fertility and reliability accompanied by increased mortality. It is therefore not surprising that there are good reasons to assume the existence of active mechanisms leading to age-related functional disorders [52, 53]. The effect of these mechanisms is likely to resemble functioning of a special aging program. However, there are reasons to believe that an aging program has nothing to do with this phenomenon [41-46], and aging results from a so-called systemic imbalance and "normal" dysregulatory pathology induced by concrete habitat.

Organisms generally respond to external stimuli by systemic reactions of controlling and regulatory systems. Their survival largely depends on the adequacy of these reactions. Individuals seek to minimize any deviations from a certain objective function. This multi-parameter function has to set an adequate activity of an organism in response to a specific pressure of the environment, that is, with the current set of external influences.

Expression of many genes and activity of an individual are known to be modulated by ambient signals. These signals enter the body control systems via sensory organs, changing its parameters according to external conditions and affecting life expectancy (e.g. [54-56]). Patterns of these signals under laboratory conditions and in a natural ecological niche to which organisms have become evolutionarily adapted are very different. Therefore, the most convincing data can be obtained by studying wild animals in their natural habitat. Such data are obviously difficult to obtain. But it should be kept in mind that when captured, animals behave differently than in the wild. And many of their body parameters are different from those that we accept as the norm.

Unfortunately, in most cases when studying aging we create living conditions and corresponding body states that cannot be observed in a life cycle like that formed in the course of evolution. Therefore, the results of studies of aging processes (in humans and laboratory animals) even under strictly controlled but not natural conditions will be accurate and statistically flawless, but not extremely informative. However, study of normal aging and survival of individuals in their natural habitat is obviously difficult for researchers.

When the effects of environmental factors on the restructuring of physiological body systems were taken into account, this suggested the possibility of changing the tempo of aging over a rather wide range [41-46, 57]. The minimum rate of this process should be observed in individuals functioning in adequate life modes dictated by the combined effects of all the factors of the natural ecological niche to which individuals have adapted in the course of evolution. Banding, RFID tags, and other methods of animal and bird monitoring indicate the possibility of a near-zero aging rate in some natural populations having high "external" mortality. But civilization trends take humans farther and farther away from the activating influence of a natural habitat. This leads to acceleration of aging, increase in the number of the cases of degenerative pathologies, and mortality from these causes. However, despite the negative tendency of age-

related mortality increase, life expectancies grow due to mortality decrease at early ages.

Experiments with both genetic manipulations [58] and recreation of adequate microenvironment and humoral background [47] showed adjustability of aging, its high plasticity, and even reversibility of the process. For example, experiments were conducted in which low initial mortality level was combined with a sharp decrease in aging rate resulting from genetic changes in regulatory systems. As a result, the life span of the nematode *C. elegans* increased 10-fold [58].

Aging is perceived as an enigmatic phenomenon not because its main initiator has not yet been discovered, but because it exists despite the fact that all the components of a complex organism are theoretically capable of complete self-renewal. However, this statement does not mean automatic recognition of programmed aging.

If one goes beyond the imposed and false dichotomy ("program" versus "stochastics") and takes into account the fact that reliability of really "sustainable" systems and modes is possible only in a certain limited range of ambient conditions, then the control theory and system approach are sufficient both to discover the root cause of aging and to understand the underlying mechanisms of its implementation. Therefore, it is sometimes useful to change the approach to the problem of aging and move from analysis to synthesis, from searching for "internal aging mechanisms" to studying of "organism–environment" interaction. After all, it is when leaving the area of adequate functioning modes determined by the environment that even potentially non-aging objects such as immortalized cells [8, 9] and hydras [59] start aging "according to Gompertz" [8, 9, 59], i.e. when mortality grows exponentially, the mode typical for humans [42, 60-62].

In this context, there are good reasons to believe that individuals from many species with repeated reproduction cycles, including humans, can be potentially non-aging. They age because of functioning under conditions preventing their full self-maintenance. Comparison of age-related dependences of mortality in countries with different standards of living (e.g. [60, 61]) or in one country in a historical retrospective (e.g. [62]) shows that the development of civilization distances humans farther away from adequate "self-supporting" modes of life, accelerating aging [41-46]. But this is happening against the background of steady growth of life expectancy [63] due to decrease in mortality primarily at early ages.

There is a saying that if the question is put "correctly", it will be there forever. We consider the question "Is aging programmed or stochastic?" to belong to such a category of "correct" questions. It was this issue that was debated at the end of the penultimate Gordon Research Conference on the Biology of Aging (California, February 12-17, 2012). Three decades ago one could still propose a third option – that "aging in many species is not associated with a strictly programmed, nor a fundamentally stochastic mechanism; it rather results from their living under pessimal conditions" [64]. Similar views about the decrease and full zeroing of aging rate resulting from increased "pressure" of the environment keep appearing (e.g. [57]). This means that stochastic aging is likely to be a secondary phenomenon. But genetically determined phenoptosis apparently also does not have to be obligatorily constitutive. It may actually represent an inducible quasi-program of aging, which is triggered only when an organism leaves the area of self-sustaining modes of its functioning. In such a case aging rate should depend on the degree of deviation of life parameters from the borders of the zone of stability.

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