Aging as a Mitochondria-Mediated Atavistic Program

Can Aging Be Switched Off?

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ABSTRACT: Programmed death phenomena have been demonstrated on subcellular (mitoptosis), cellular (apoptosis), and supracellular (collective apoptosis) levels. There are numerous examples of suicide mechanisms at the organismal level (phenoptosis). In yeast, it was recently shown that the death of aging cells is programmed. Many of the steps of programmed cell death are shown to be common for yeast and animals, including mammals. In particular, generation of the mitochondrial reactive oxygen species (ROS) is involved in the suicide programs. Aging of higher animals is accompanied by an increase in damage induced by mitochondrial ROS. Perhaps prevention of such damage by scavenging of mitochondrial ROS might slow down or even switch off the aging programs.

KEYWORDS: programmed death; mitochondria; reactive oxygen species

Since the 19th century, two alternative concepts of aging—optimistic and pessimistic—have been discussed. The former suggests that aging is the final step of an ontogenetic program and, hence, can be prevented by switching the step off. The latter assumes that this process is an inevitable result of life, being a consequence of the accumulation of errors and injuries in biomolecules, the exhaustion of vital forces, and the malfunctioning of some genes that are useful in the beginning of life but become harmful at its end. If the second view is correct, there is no chance to greatly increase the span of life, since such a complicated system as an organism must of necessity break at some point, like an old car. Comfort once mentioned that it is difficult to believe that a horse ages in the same way as a cart.[1] However, the optimistic concept of aging never dominated the discussion. Only quite recently, significant observations have been made that strongly favor the programmed aging hypothesis.

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BRIEF HISTORY

It was Alfred Russel Wallace, the codiscoverer of the natural selection law, who first mentioned that death can be programmed. He wrote in one of his letters (around 1865–1870): "… when one or more individuals have provided a sufficient number of successors, they themselves, as consumers of nourishment in a constantly increasing degree, are an injury to those successors. Natural selection therefore weeds them out, and in many cases favors such races as die almost immediately after they have successors."[2] This principle was later developed by August Weismann: "Work-out individuals are not only valueless to the species but they are even harmful, for they take the place of those, which are sound … I consider that death is not a primary necessity but it has been secondarily acquired as an adaptation." Weismann was immediately attacked as an anti-Darwinist even though Darwin wrote: "There can be no doubt that a tribe including many members who … were always ready to aid one another, and to scarify themselves for the common good would be victorious over most other tribes; and this would be natural selection"[3] (for discussion see Ref. 112). Recently this aspect was comprehensively analyzed by Goldsmith.[4]

Today Weismann critics are usually cite Medawar,[5] who assumed that aging could not be invented by biological evolution. He stressed that, under natural conditions, the majority of organisms die before they become old. Such an assumption is hardly correct since the aging starts long before it appears to be an immediate cause of death.[4,6–8] Indirectly, age-dependent weakening of an organism can be the reason of death due, for example, to attack by predators, pathogens, etc. [4,6–9] Recently Loison and colleagues[10] and Bonduriansky and Brassil[11] clearly showed that both long-lived ungulates (roe deer, bighorn sheep, izards) and the short-lived antler fly suffer senescence under natural conditions. This is not surprising because a decrease in, for example, skeletal muscle strength starts, as a rule, rather early, that is, at the age when organism stops growing.

THE PRECEDENT OF THE PROGRAMMED DEATH MECHANISM: APOPTOSIS

The phenomenon of the programmed death was directly proved in the cell. We mean discovery of apoptosis. In 1972, Kerr, Wyllie, and Currie published their famous paper "Apoptosis: a basic biological phenomenon with wide-ranging implication in tissue kinetics."[12] Later, numerous observations clearly demonstrated that apoptosis is involved in ontogenic development, anticancer defense, immune response, etc. In 1996–1997, the role of mitochondrial proteins, such as apoptosis-inducing factor (AIF)[13] and cytochrome *c*,[14–16] as well as of mitochondrial ROS,[17] in amplification of apoptotic stimuli was elucidated. In 2002, a Nobel Prize in Physiology and Medicine was given to Brenner, Horvitz, and Sulston for studies on *C. elegans*, including identification of genes of an apoptotic program responsible for elimination of about 6% of cells during ontogenesis of the worm.[18,19]

MITOPTOSIS, COLLECTIVE APOPTOSIS, AND ORGANOPTOSIS

During the last decade, programmed death mechanisms were shown to operate at both sub- and supracellular levels. In 1992, Zorov and colleagues[20] suggested that animal mitochondria possess a mechanism of self-elimination. This function was attributed to so-called permeability transition pore (PTP), a large, unspecific channel in the inner mitochondrial membrane. Normally PTP is closed but under certain (as a rule, pathological) conditions it opens causing dissipation of mitochondrial transmembrane electric potential difference ($\Delta\Psi_m$). It is known that $\Delta\Psi_m$ is required for the import of cytoplasmic precursors of mitochondrial proteins and for proper arrangement of mitochondria-synthesized proteins in the inner membrane. Thus, repair of the PTP-bearing mitochondrion ceases, and such an organelle perishes.[21,22] Reactive oxygen species (ROS) are one of the most powerful PTP-opening stimuli. If ROS are overproduced by a mitochondrion, it will be killed by these ROS due to the PTP opening. This mechanism was suggested to rid the intracellular mitochondrial population of those that have become dangerous to the cell because their ROS generation exceeded their ROS scavenging capacity. Such a process of self-elimination of malfunctioning mitochondria was termed *mitoptosis*.[23] Recently Lemasters and coworkers[24] have shown that glucagon added to hepatocyte cell culture causes mitoptosis followed by autophagia of dead mitochondria. The effect was arrested by cyclosporin A, the PTP inhibitor. It was found that a similar process accompanies apoptosis induced by the tumor necrosis factor.[25] Tolkovsky and coworkers[26,27] have demonstrated disappearance of all the mitochondria in the cell in response to apoptotic stimuli provided that apoptosis was blocked downstream of mitochondria. A type of progeria (accelerated aging) accompanied by mitoptosis was described by Jazwinski and coworkers in yeast.[28,29] The authors showed that a point mutation in the *ATP2* gene coding for β-subunit of mitochondrial H^+-ATP-synthase resulted in a situation where the daughter yeast cell is no longer born young and instead possesses the age of its mother. Such a defect was manifested only when glycolysis was the sole energy source, which suggests that, under conditions used, H^+-ATP-synthase operated as H^+-ATPase being required to form $\Delta\Psi_m$ at the expense of hydrolysis of glycolytic ATP while respiratory phosphorylation was impossible. In line with the concept of mitoptosis, Jarwinski and colleagues observed progressing decline in $\Delta\Psi_m$ and reduction of number of mitochondria in the mutant. The net result was the generation of cells totally lacking mitochondria, which become dominant cell type as yeast clones became extinct.[28,29]

Mitoptosis exemplifies a self-elimination program operating at a subcellular level. As to supracellular level, several cases were described when apoptotic cells formed clusters in tissues *in vivo* or in cell cultures *in vitro* (so-called death of bystander cells[8,30,31]). It was suggested that H_2O_2 produced in an apoptotic cell serves as an apoptogenic signal to the bystander cells surrounding the apoptotic cell. In this way, a tissue antiviral defense may be organized, assuming that propagation of H_2O_2 is faster than that of a virus. As a result, a "dead area" may be organized around the infected cell, as observed when a leaf of the so-called hypersensitive strain of tobacco plant is infected by the tobacco mosaic virus.[32]

The hypothesis of an H_2O_2-mediated bystander killing was confirmed by Bakalkin and coworkers.[30] The authors reported that serum deprivation resulted in formation of clusters of cultured apoptotic osteosarcoma cells. The clustering was

abolished by adding catalase, the H_2O_2 scavenger. The long-distance transmission of an apoptotic signal was quite recently demonstrated by Pletjushkina and colleagues.[31] The human cervical carcinoma cells of a HeLa line was grown on two glass coverslips. One of them was treated with an apoptogen (TNF, staurosporine, or H_2O_2). Then this coverslip was removed from the apoptogen-containing medium, washed by a medium without apoptogen, and put side by side with the second coverslip (which was not treated by the apoptogen). It was found that numerous apoptotic cells appear on the second (nontreated) coverslip, the effect being sensitive to catalase. The cells on the treated coverslip were shown to produce some H_2O_2. Apoptosis induced by added H_2O_2 or by HeLa cell–produced H_2O_2 proved to be abolished by the mitochondria-addressed antioxidant MitoQ, whereas apoptoses caused by TNF or staurosporine were not.

It is obvious that massive apoptosis of cells composing an organ that should be eliminated during ontogenesis can be a mechanism of such an elimination, being defined as *organoptosis*.[33] Consider the disappearance of the tail of a tadpole when it converts to a frog. Addition of thyroxine (the hormone known to cause regression of the tadpole tail) to severed tails surviving in a special medium was shown to cause shortening of the tails, occurring on the time scale of hours. The following chain of events was elucidated:[34]

$$\text{thyroxine} \rightarrow \text{NO synthase induction} \rightarrow [NO\cdot]\uparrow \rightarrow [H_2O_2]\uparrow \rightarrow \text{apoptosis} \rightarrow \text{organoptosis}$$

The mechanism of the $NO\cdot$-induced H_2O_2 increase may consist of inhibition by $NO\cdot$ of the main H_2O_2 scavengers, catalase and glutathione peroxidase,[35] as well as in the NO-induced arrest of the respiratory chain in the cyanide and antimycin A–sensitive sites.[8]

PROGRAMMED DEATH OF ORGANISM: PHENOPTOSIS

Bacteria and Unicellular Eukaryotes

Mechanisms of self-elimination are clearly operative at subcellular, cellular, and supracellular levels. Life seems to follow a Samurai principle: "It is better to die than to be wrong."[35] This principle can be stated as: "Any complex biological system is equipped with programs of self-elimination that are actuated when the system in question appears to be dangerous or unnecessary for the system(s) of higher position in biological hierarchy."[36]

If such a principle operates at an organismal level, we may speak about the programmed death of organism. Such a phenomenon can be defined it as *phenoptosis*, by analogy with mitoptosis and organoptosis.[33] Existence of phenoptosis, if it were experimentally proved, would mean that an organism, at least in some cases, would control its own death. This might be a consequence of operation of one more biological principle: "Living creatures try to avoid spontaneous process controlling all events occurring in their bodies."[8] Within the framework of such a concept, it is not surprising that death, the most dramatic event in the life history of any individual, is also under its control.

In bacteria, a suicide mechanism may represent the last line of defense of the population gene pool, assuming that a cell that cannot guarantee maintenance of intactness of its genome—hence, if survives, can generate antisocial monsters among its progeny—commits suicide.[8,33] Such a process of self-elimination should be referred to phenoptosis since we deal here with a unicellular organism (as compared to apoptosis, which is the self-elimination of a cell in multicellular organism). A scheme of phenoptosis induced by damage to bacterial DNA was considered by Lewis,[37] who followed the path of signal transduction from damaged DNA to autolysin-mediated death of a bacterium.

Moreover, these events may be referred to bacterial phenoptoses: (1) active lysis of the mother cell of *B. subtilis* and *Streptomyces* during sporulation, which is required to release spores; (2) development of bacteroids in *Rhizobium*; (3) lysis of some cells of *S. pneumoniae* to release DNA, which is picked up by other cells that did not lyse; (4) toxin/antitoxin systems killing bacterium when protein synthesis is inhibited; (5) lysis of the colicin forming *E. coli* to release a colicin killing bacteria of other strains; (6) suicide of a phage-infected *E. coli* cell to prevent propagation of the phage infection (in the latter case, three different suicide mechanisms, all resulting in arrest of the protein synthesis, were described).[8,37] In these events, the suicide mechanisms proved to be quite different from those in the animal cells.

Mechanisms of self-elimination in unicellular eukaryotes are studied mainly in the yeast *Saccharomyces cerevisiae*. Here it was found that pro- or anti-apoptotic mammalian proteins, such as Bax or Bcl-2 expressed in the yeast, induce the cell death or rescue the cells, respectively. Certain mutations in the *S. cerevisiae* genome entail death showing features of the mammalian cell apoptosis.[8, 38–40] Harsh treatments (H_2O_2, acetic acid, etc.) proved to be inducers of the yeast death also resembling apoptosis.[41,42] Indications were published that in *S. cerevisiae* there are proteases involved in the apoptotic cascade, namely, a caspase[43] and Omi.[44] As was shown by Allis and coworkers,[45] the H_2O_2-induced programmed death of yeast is accompanied by phosphorylation of histone H2B at serine 10, catalyzed by Ste20 protein kinase. This process proved to be responsible for chromatin condensation, a typical trait of the programmed cell death both in animals and yeast. In humans, the same histone is phosphorylated at serine 14 by Mst1 kinase, a mammalian homolog of yeast Ste20. This fact strongly points to mechanistic similarity of animal apoptosis and yeast phenoptosis.

In 2001, Narasimhan and colleagues[46] showed that plant antibiotic osmotin caused an apoptosis-like death of yeast. Surprisingly, components of the pheromone cascade were reported to be involved in the osmotin killing.[46,47] The reason for this became clear when Severin and Hyman[48] revealed that killing *S. cerevisiae* by high concentration of pheromone (α-factor) is programmed. In fact, this observation created the precedent of a signal compound produced by a unicellular eukaryote, which induces death of cells of the same species. Analysis of the mechanism of this effect showed[48,49] that it includes the following consecutive components: (1) pheromone receptor, (2) Ste20 kinase, (3) synthesis of some protein(s), (4) an increase in cytosolic [Ca^{2+}], (5) stimulation of mitochondrial respiration and an increase in energy coupling, (6) a $\Delta\Psi_m$ elevation, (7) a burst in the ROS production in the middle span of the mitochondrial respiratory chain, (8) decomposition of mitochondrial filaments to small roundish mitochondria, and (9) $\Delta\Psi_m$ collapse and cytochrome *c* release from mitochondria to cytosol (FIG. 1).

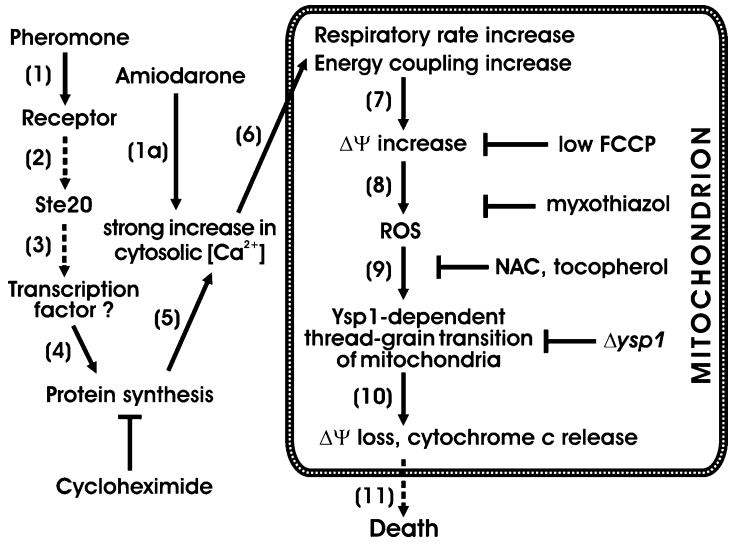

FIGURE 1. Mechanism of programmed death of yeast (phenoptosis), initiated by pheromone or amiodarone. Pheromone (α-factor) combines with a receptor on the outer surface of the plasma membrane (step 1). The binding initiates a cascade of events (MAPK cascade) including activation of the Ste20 kinase (step 2). Ste20 activates (apparently via a transcription factor, step 3), synthesis of protein(s) (step 4), inducing a strong increase in cytosolic Ca^{2+} due to the Ca^{2+} release from the intracellular depot (step 5). Amiodarone can substitute for these protein(s) (step 1a). Cytosolic Ca^{2+} activates respiration and increases its coupling to generation of mitochondrial membrane potential ($\Delta\Psi$) (step 6). This in turn increases $\Delta\Psi$ (step 7), an effect elevating ROS production by the respiratory chain (step 8). ROS initiate fission of mitochondrial filaments to small, roundish mitochondria (thread-grain transition), which requires *ysp1* protein (step 9). Then a $\Delta\Psi$ collapse and release to cytosol of proteins hidden in the mitochondrial intermembrane space (e.g., cytochrome *c*) take place (step 10), events involved with the cell death (step 11). (Reproduced from Pozniakovsky *et al.*[49] with permission.)

Multicellular Organisms That Reproduce Only Once

As was noticed by Wallace and Weismann, some of organisms of this kind are constructed in a way predetermining death shortly after reproduction. For instance, imagos of mayflies die within a few days since they cannot eat due to lack of functional mouth and their intestines are filled with air.[2] In the mite *Adactilidium*, the young hatch inside the mother's body and eat their way out.[50] The male of some squids dies just after transferring his spermatophore to a female.[51] The female octopus stops eating when her children are hatched. This does not occur if her optical glands are removed. Such an operation results in a four-fold increase in life span of the animal.[52] Bamboo can live for 15–20 years reproducing vegetatively but then, in the year of flowering, dies at the height of the summer time immediately after the ripening of the seeds (see Skulachev[8] for discussion).

Striking observations were made in studies of salmon. The Pacific salmon was shown to die immediately after spawning as a result of accelerated aging (progeria),

which develops when the fish leaves the ocean and swims along a river to its upper reaches. The traditional explanation of this kind of death was that the animal spends too much energy when swimming in the river for a long distance against current. However, this point of view proved to be wrong since (1) aging and death did not occur if gonads or adrenal glands were removed[53] and (2) progeria was observed even when the river was very short and current was weak. In the Far East of Russia, two populations of salmon were compared, one spawning in the upper reaches of the Amur river (thousands of kilometers long) and another spawning in a very small river on the Sakhalin island (only 0.2 km long). In both cases, the spawning fish showed typical traits of aging that resulted in death. A signal for progeria proved to be change from the sea to fresh water. In this example, a biological function of suicide seems to be that the remains of the old fish become food for river invertebrates who, in turn, are food for the young fish.[54]

The Atlantic salmon, in contrast to its Pacific relative, after spawning in a river returns from river to ocean. If it is the summer generation of the fish, it often dies in the fall. A Russian ichthyologist V.V. Ziuganov has recently studied larvae of a mollusk (pearl mussel *Margaritifera margaritifera*) that develops in gills of the Atlantic salmon. He found that larvae can somehow switch off the fish's "death program" so the larvae-infected fish live at least one season more than the majority of non-infected salmon (some of infected salmon live up to 13 years). An increase in the host's life span is needed for the larvae to complete their own development. It was shown that the infected fish had fewer tumors and were more resistant to wounds and burns.[54] Leng and colleagues[55] reported that a peptide from another mollusk related to the mussel, a *Mercenaria meretrix*, activates superoxide dismutase but inhibits tyrosinase and proliferation of carcinoma cells. Earlier it was shown that a *Mercenaria* extract possesses anticancer activity and decreases the blood sugar and fat.[55]

AGING AS AN EVOLUTION-ACCELERATING MECHANISM

The Case of the Hare versus the Fox

It is a time to revisit the old Wallace-Weismann idea concerning aging as a program.[2] Two questions should be addressed: What is the physiological function(s) of aging if it represents a particular case of slow phenoptosis? (2) How is the mechanism of the aging program constructed to avoid substitutions of non-aging phenotype for an aging one by means of natural selection?

The answer to the first question is that it seems reasonable to assume that slow aging serves as a specialized mechanism to accelerate evolution. Let us consider an example. Two young hares differing "intellectually" have almost equal chances to escape from a fox since both of them run much faster than a fox. However, with age the clever hare acquires some advantage, which is of crucial importance as the older hare slows down and runs as fast as the fox. Now the clever hare has a better chance to escape and, hence, to produce leverets, than the stupid hare. This is turn will be favorable for selection of clever hares.[8]

This answer presumes that muscles weaken with age when reproduction is still possible. This is apparently the case at least for humans. Here the age-dependent atrophy of muscles seems to begin around 25 years.[56] Initially, this process is slow but

it is activated in an age-dependent fashion. Nowadays, measurable loss of muscles strength in Swedish men was shown to start at age 50[57] and in Saudi Arabian men in the fourth decade of life.[58] On the other hand, at the beginning of nineteenth century the decline of this parameter started just after 25 years (in a study of Belgian men[57]). Such a dynamics may be explained by improvement of living conditions during studied period of time. In any case, as is noticed by Goldsmith, "because even a relative minute deterioration will cause a statistically significant increase in death rate, one suspects that the evolutionary effects of aging in wild animals begin at relatively young ages."[4] As Loison and colleagues noticed, "observed death rates in wild animals increase beginning at puberty."[10]

In line with the above reasoning, the better adapted creatures live, as a rule, longer. Species were described that do not age at all. The pearl mussel lives up to 200 years without any traits of aging. Its weight and reproductive ability continue to increase for its entire life. The mollusc suddenly dies when the shell weight appears to be too great for the muscles of the mollusc to maintain a vertical position at the bottom of a river.[59] Pike has no natural enemies and lives for a century without losing the ability to reproduce. This is also true for some big ocean fish.[59] Some big ocean birds live about 50 years and then suddenly die.[6] Among birds, there are examples of very long-lived species with constant (or even increasing with age) ability to reproduce, showing no age-dependent increase in the death rate.[59] Bat lives 17 times longer than shrew of the same weight with the same diet of insects.[60] It seems that species occupying a new area (like the air or the ocean) can stop paying a price for a fast evolution—aging and a short life span that results in a high rate of the change of generations.[8]

CHRONOLOGICAL AGING OF YEAST

In yeast, two kinds of aging are described, a replicative and a chronological. The first one means that each yeast cell can form a limited number of daughter cells (for *S. cerevisiae* the number is about 30). The second type means that the yeast can survive for a limited amount of time. In both types, death shows many typical apoptotic markers (see Breitenbach and colleagues[40] for review). For replicative aging, the physiological role of cell suicide is not obvious, since old mother cells compose an insignificant part of population. In this case, phenoptosis may be the result of the aforementioned "Samurai" law.

As for chronological aging, it proved to be precedent for the adaptive (evolution-accelerating) role of aging recently directly demonstrated. It was found that yeast strains isolated from grapes[61] and from laboratory strains[61,62] undergo an age-dependent death that has features of animal cell apoptosis. Moreover, it was shown that such a death was prevented by maintaining of the medium pH at the initial (6.5–7) level (normally, pH lowered to 3.5–4 in the stationary phase at day 12 of cultivation) (FIG. 2A) or by exclusion of nutrients from the medium (FIG. 2B).[61] In rich medium, acidification resulted in the death of 90–99% of the population despite the fact that nutrients are still available. Then a small subpopulation showed a regrowth (FIG. 2C).[61,62] The regrowth is accompanied by a strong increase in the mutation rate, being stimulated by deletions in the superoxide dismutase (SOD1) and catalase genes and inhibited by overexpression these ROS-scavenging enzymes.[61] Disruption

of the death program through deletion of yeast caspase YCA1 initially resulted in better survival of aged cultures. However, surviving cells have lost regrowth capacity.[62]

A four-step explanation of these data is: (1) Long-term cultivation of yeast results in lowering of pH due to accumulation of acidic endproducts of metabolism. (2) The pH decrease entails protonation of the primary ROS, the superoxide anion ($O_2^{-\cdot}$), so that much more aggressive $HO_2^{\cdot}$ is formed[63] that easily penetrating through membranes (the pK value for the $O_2^{-\cdot}$ protonation is 4.8). (3) $HO_2^{\cdot}$ attacks (i) DNA, resulting in an increase of mutagenesis, and (ii) mitochondria that react to ROS by

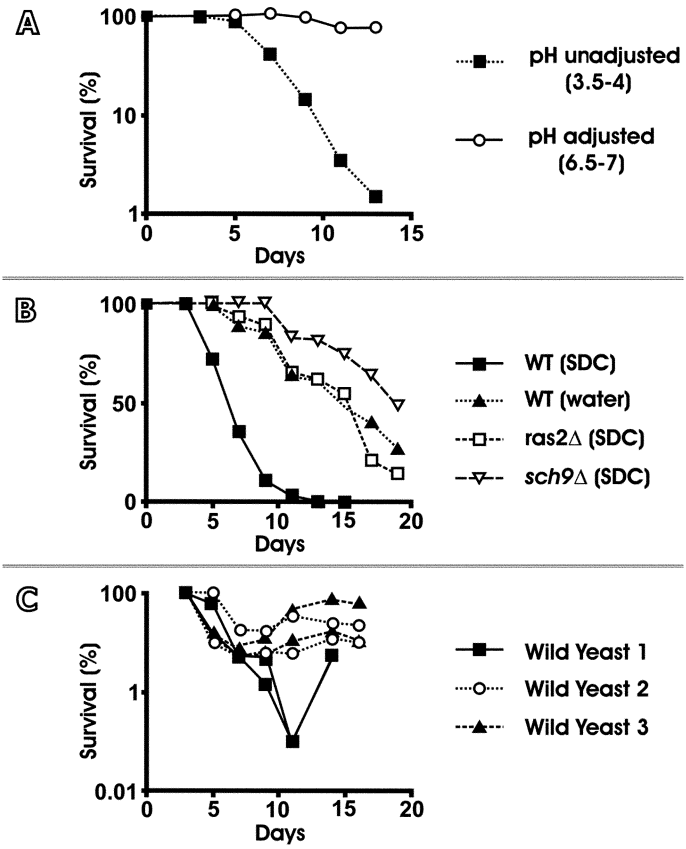

FIGURE 2. Chronological aging of yeast *Saccharomyces cerevisiae*. (**A**) Death is delayed when the pH value in the medium, normally lowering to 3.5–4 on day 12, is adjusted at 6.5–7. The growth medium contained 2% synthetic dextrose (SDC). (**B**) Death is delayed when water substitutes for a rich (SDC) medium or genes encoding regulatory proteins (*ras2* or *sch9*) are mutated. (**C**) The regrowth phenomenon: a small fraction of old yeast starts regrowth on days 10–15 in the SDC medium. Three samples of wild yeast isolated from grapes were studied. (Reprinted from Fabrizio *et al.*[61] with permission.)

committing suicide (mitoptosis). (4) Massive mitoptosis initiates cell suicide. However, a very small fraction of cells proved to be resistant to massive mitoptosis due to the increased mutagenesis that gave rise to the disruption of a gene involved in the cell suicide mechanism. If the ambient conditions improve, the wild type genetic pattern is recovered in certain mutant cells by formation of a diploid from two haploids differing in mutated proteins involved in the phenoptotic cascade.

This four-step explanation may describe how suicide mechanisms appear as a result of biological evolution. It is obvious that such systems as SOD–catalase or mitoptotic cascade are favorable for cell survival under more or less normal conditions. In the above experiments, viability of *SOD1* deletant in the young culture was about ten time lower than that of the wild type—that is, 7 versus 70 cells $\times 10^6$/mL. However, at day 12 of cultivation, the ratio of these values was reversed—that is, 7×10^{-2} versus 1×10^{-5} cells $\times 10^6$/mL, respectively.[61]

It should be stressed that such a scheme can operate only if the death is programmed to occur *before* the conditions are worsened so strongly that *all* the cells in populations die in a non-controlled fashion. As a result, a chance appears that, due to increased mutagenesis, the cell suicide system is damaged, allowing a mutant to survive. In the same study,[61] some mutations preventing the programmed death were identified. One of them is localized in the gene encoding the Sch9 protein (analog of animal Akt, or protein kinase B). Knock-out of this gene was shown to result in an increase in SOD1 level.[64] Another mutation inactivated the gene encoding Ras2 protein (analog of the oncogen Ras of mammals). Both mutations markedly lengthen the life span of yeast (FIG. 1B).[61,64,65]

THE p53 PARADOX

It was recently demonstrated in mammals that the same mechanism can help a young organism survive but also shortens the life of an old one. In mice, Donehower and coworkers found that a mutation giving rise to an increase in activity of a protein, p53, resulted in disappearance of cancer from causes of death. Normally, almost 50% of mice die from cancer so one could expect the mutation in question to prolong life. Surprisingly, the life span of the mutants was shortened by 20%.[66] Later Garcia-Cao and colleagues, who apparently obtained lower level of the p53 activation than Donehower's group (the death from cancer decreased not to zero but to 17%) did not observed any statistically significant effect on the life span.[67] Such a result, however, is in line with Donehower's observation because a big decrease in cancer death rate would result in an increase in the life span if p53 did not have an effect on aging. Quite recently, Scrable and colleagues[68] have activated p53 in one more fashion that seems to result in the highest level of its activity so the life span decreased by factor 3. In both Donehower's and Scrable's experiments, the mutant mice died from progeria.[66,68]

The p53 protein is known as a "guard of genome." It is involved in stimulation of DNA repair, cell cycle arrest, and mitochondria-mediated apoptosis, occurring in response to the increasing DNA damage. Moreover, p53 able to stimulate aging (as is clear from the above-cited works). This means that for p53 deletants, where presumably aging should be absent, it is impossible to win competition with the wild-type animals since all of them die from DNA damage–linked pathologies, most of all to

cancer. In fact, p53 deletants were found to have much shorter life spans and died exclusively from cancer.[66] Thus, today we can answer the question posed more than a century by Weismann: "There cannot be the least doubt that the higher organisms, as they are now constructed, contain within themselves the germs of death … The question arises as to how this has come to pass."[2]

MUTATIONS PROLONGING LIFE

Examples of the aging program discussed illustrate possibilities of how the mechanism of aging could have evolved. Mitoptosis in yeast, which is employed to remove mitochondria that overproduce ROS, ultimately kills the cell when ROS become much more aggressive due to acidification of cytoplasm so that too many mitochondria commit suicide and release proapoptotic proteins to cytosol.[49,69] The p53 protein, which initiates apoptosis in malignant cells with damaged DNA, initiates suicide of cells damaged only slightly when is activated too much. This creates difficulties in selecting mutant organisms who are immortal. However, in certain mutants an interplay of these factors results in a prolongation of life span, sometimes very significantly.

In an ascomycete, filamentous fungus *Podospora anserina*, mutation in a cytochrome oxidase subunit arrests activity of this enzyme, which entails induction of the alternative cyanide-resistant oxidase, an event strongly lowering the rate of ROS formation by the respiratory chain (FIG. 3). This results in a strong decrease in the ROS level and, by some unknown mechanism, in a switch from sexual to vegetative reproduction. Such a switch is accompanied by disappearance of typical morphological age-dependent changes in the fungus. Death due to aging, normally occurring on day 25 since germination of spore, disappears so the mutant survives for years. The same result can be achieved by any other mutation (or by adding inhibitors) making operation of the canonical respiratory chain impossible.[70,71]

Hekimi and his colleagues succeeded in extending by a factor of 5.5 the life span of *Caenorhabditis elegans*, if two genes were inactivated—those coding for an insulin-receptor-like protein DAF-2 and for an enzyme catalyzing a final step of CoQ synthesis.[72–74]

In mice, Pelicci and coworkers[75–77] have reported that animals lacking a particular 66 kDa protein (p66Shc) live 30% longer and are less sensitive to paraquat-induced oxidative stress, and that fibroblasts derived from these mice did not respond more to an H_2O_2 addition by initiating apoptosis. The following chain of events seems to be responsible for these effects:

$$\text{ROS} \rightarrow \text{DNA damage} \rightarrow \text{p53} \rightarrow \text{stabilization of p66Shc} \rightarrow [\text{p66Shc}]\uparrow \rightarrow [\text{ROS}]\uparrow \rightarrow \text{PTP opening in mitochondria} \rightarrow \text{massive mitoptosis} \rightarrow \text{apoptosis.}$$

In vivo knock-out of the p66Shc gene resulted in a decrease in oxidative damage to both mitochondrial and nuclear DNA in lung, liver, spleen, skin, skeletal muscles, and kidney but not in brain and heart. This correlated with the p66Shc content in various organs, which is lowest in brain and heart.[75–77]

Quite recently, Rabinovich and coworkers[78] have demonstrated an increase in the medium (by 5 months) and maximal (by 5.5 months) life span of mice overexpress-

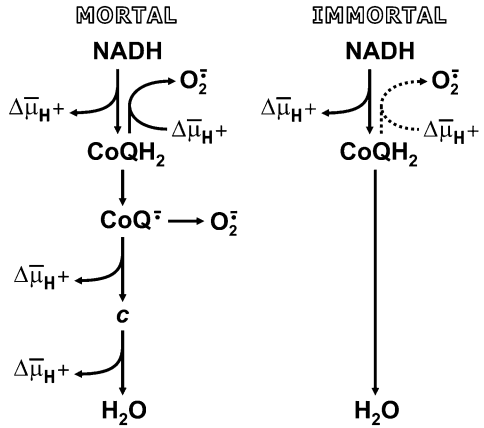

FIGURE 3. The respiratory chain inherent in the mortal and immortal modes of life of fungus *Podospora anserina*. **(Left)** The mortal mode. "Canonical" respiratory chain forms the transmembrane electrochemical H^+ potential difference ($\Delta\bar{\mu}_H+$) in three energy coupling sites. Superoxide is generated by reverse electron transfer in the first coupling site and by oxidation of semiubiquinone ($CoQ^{-\cdot}$) in the second site. **(Right)** The immortal mode. Cytochrome *c* oxidase operating in the third coupling site is replaced by noncoupled alternative $CoQH_2$ oxidase without the second and third coupling sites being involved. The second place of the $O_2^{-\cdot}$ generation in the middle span of the chain is omitted, whereas that in the initial span is partially inhibited due to the lower rate of $\Delta\bar{\mu}_{H^+}$ production. As a result, the rate of mitochondrial $O_2^{-\cdot}$ generation is strongly decreased.

ing catalase targeted to the mitochondrial matrix, which normally contains no catalase. Cardiac pathologies and cataract development were delayed, oxidative damage of DNA was reduced, H_2O_2 production and H_2O_2-induced aconitase inactivation were attenuated, and age-related accumulation of mitochondrial DNA deletions was decelerated. Targeting of catalase to nucleus or peroxisome was of smaller or negligible effects.

HORMONAL CONTROL OF LIFE SPAN

Programmed aging needs a time-measuring device. In higher animals, such a biological clock might be localized in the same place as other biotimers responsible for initiation and maintenance of periodic processes in their bodies, such as circadian rhythm, menstrual cycle, and season changes. In all the cases listed, the pineal gland hormone melatonin is involved. The pineal gland is primarily responsible for generation of the circadian rhythm signals in birds and is the second step in transmission of these signals in mammals, with suprachiasmatic nucleus of hypothalamus being the signal generator.

In 1971, Dilman postulated that aging is under hormonal control and involves hormones of hypothalamus, pineal gland, hypophysis and pancreas.[79–81] Later, this

idea was confirmed by studies carried out on eukaryotes of various taxonomic positions. In 1974, Denckla showed that pituitary ablation prevents the age-dependent decline of O_2 consumption by mice and of sensitivity of O_2 consumption to thyroid hormones.[82] Later, Pierpaoli succeeded in demonstrating that (1) substitution of pineal gland in old mice by that from the young mice prolongs life whereas the old-to-young substitution has the opposite effect, (2) transplantation of the old pineal gland to thymus of a young mouse shortens the life span, (3) removal of pineal gland on the fourteenth month of the life has an effect similar to substitution of old pineal gland in young mice, (4) the nocturnal treatment of old mice with melatonin is favorable for longevity (see Pierpaoli, this volume).[83–88]

Blüher and colleagues[89] reported that knockout of the insulin receptor in adipocytes resulted in an 18% increase in the mouse life span. Independently, Holzenberger and colleagues[90] showed that heterozygote *Igf1r*$^{+/-}$ mice (Igf1r is for insulin-like growth factor receptor 1) lived 26% longer than the wild type. This was accompanied by 50% decrease in p66Shc (for reviews on other participants of the hormonal life-shortening cascade, see Bowles,[6,7] Longo and Finch,[91] and Anisimov, this volume).

In flies, indications of involvement of an insulin-type hormone in shortening the life span were reported.[92] In *C. elegans*, a single mutation in the *daf-2* gene coding for an insulin receptor prolonged life by two to three times without arresting development of the worm at the dauer stage.[93,94] In the same animal, NAD^+-dependent histone deacetylase (Sir2.1) was shown to downregulate the insulin pathway and to increase the life span.[8] High doses of gene *SIR2* in yeast were shown to cause some rise in duration of life.[8] Also in yeast, Fabrizio and colleagues[65] showed that deletion in genes encoding RAS2 protein or a Akt/PKB-like protein kinase entailed a two- to threefold increase in the life span (see above).

WHY ARE ROS AN OBLIGATORY COMPONENT OF THE AGING PROGRAM?

There are numerous pieces of evidence that intramitochondrial ROS are specifically involved in the life-shortening cascade responsible for aging of both yeast and animals.[8,61,64] We already mentioned that targeting of catalase to the mitochondrial matrix lowers the rate of accumulation of the mitochondrial DNA deletions and is favorable for longevity. Opposite effect on the life span was shown to be inherent in when the probability of accumulation of mistakes in the mitochondrial DNA increases. Recently, Larsson and colleagues[95] and Zassenhaus and coworkers[96] independently reported that expression of a proof reading-deficient mitochondrial DNA polymerase results in (1) a strong increase in mutations in mitochondrial DNA, especially in the region of cytochrome *b* of the respiratory chain complex III (inhibition of this cytochrome by antimycin A is known to strongly increase ROS production), (2) a strong decrease in the mouse life span, and (3) the appearance of many features typical for aging. Zasselhaus and coworkers,[96] who modified the polymerase only in the heart, succeeded in preventing of such changes by adding cyclosporin A, an inhibitor of the mitochondrial PTP and related ROS formation.

It is obvious that destructive role of ROS is still the great problem for any aerobe. This is apparently why aging as a specialized mechanism of evolution is arranged in such a way that it is favorable for improvement of the multifaceted antioxidant system of organism. To some degree, ROS operate like the fox in our hare-versus-fox case, so evolution is always directed toward more robust antioxidant defense. This appears to be a consequence of the fact that execution of the aging signal results in lowering of the antioxidant defense in organelles, cells, tissues, and organs.[8] Such a lowering could be a consequence of an increase in ROS generation and/or a decrease in the amount of ROS scavengers. The above relationships explain why longevity correlates with low level of ROS and high resistance to the oxidative stress, This correlation was revealed at levels of the cell cultures[97] and isolated mitochondria. In the latter case, it was shown that mitochondria from long-lived birds produce less ROS than from short-lived mammals of the same body weight.[98–99] Similar effect was found to be inherent in mammals i.e. flying (bat) and non-flying (shrew).[60] It is noteworthy that both longevity and low mitochondrial ROS production correlated with slower rate of accumulation of oxidized proteins and damaged DNA in tissues of aging animals.[98]

PRESENT STATE OF THE ART AND SOME PERSPECTIVES

In 2005, Thomas Kirkwood, one of the most prominent modern gerontologists, treated the programmed aging concept as a mistaken view.[100] In favor of such reasoning, Kirkwood noticed three major items. (1) *Aging cannot contribute significantly to the mortality in natural populations since most animals die comparatively young*. However, as was discussed above, aging seems to start at puberty and aging-induced weakness of the middle-aged organisms can well contribute to death caused by predators, infections, etc. (2) *Any mutant in whom the aging process was inactivated would enjoy an advantage and the mutant genotype would spread.* This item ignores at least three essential facts. First, the death of many organisms sexually reproducing only once occurs immediately after the reproduction process is over (e.g., bamboo or octopus). Undoubtedly, this takes place due to execution of a suicide (phenoptotic) program that could be lost by a mutation but evolution somehow preserved such a program. Second, aging of unicellular eukaryotic organisms such as yeast is shown to be programmed. This program cannot be eliminated by a mutation since such a mutation should be lethal (e.g., mutation in the mitoptotic program). Third, the latter is also valid for mammals. Here aging program includes, e.g., p53 which is also involved in the anticancer defense so mutation in p53, instead of resulting in immortality due to arrest of aging, gives rise to a strong decline of the life span because of stimulation of carcinogenesis. (3) *If there is a program of aging, why are immortal organisms lacking this program not described?* All the vegetatively reproducing organisms are, in fact, immortal. *Podospora anserina* is an example when immortality accompanying the sexual-to-vegetative reproduction switch is experimentally shown (see above). Moreover, switching off the death program does not necessarily mean immortality. It seems quite possible that organisms lacking the programmed death systems will live longer but after some period of time die because of non-programmed aging.

Thus, at present there are no unequivocal arguments excluding the possibility that aging is programmed. On the other hand, there is a precedent for programmed aging in yeast[61,62] and numerous pieces of indirect evidence that this may also be the case for organisms other than unicellular fungi.

More and more scientists now recognize that the generally accepted ideas concerning aging are insufficient to explain this phenomenon. Nemoto and Finkel,[101] when analyzing the present state of the Harman's hypothesis on aging as a simple result of accumulation of the ROS-induced injuries,[102] wrote: "If ageing represents the legacy of the combustible mixture of food and oxygen in our mitochondria, then oxidant-related damage should be, to some degree, cumulative. But when fruitflies were caloric-restricted for just two days, their mortality rate became equivalent to that of flies that spent their life hungry."[103] Within the framework of the phenoptotic concept of aging, caloric restriction can prolong the life of animals by such a change in hormonal balance, which inhibits execution of the aging program to some degree. Perhaps that practice of religious fasts was empirically invented as a tool to prolong the lives of the believers, provided that mechanisms of caloric restriction effect are similar in fly and man.

Few years ago, modern proponents of the Wallace-Weismann concept were very rare.[4,6–8,82,88,104–108] However, today specialists from various fields of research and different countries are likely to accept such a point of view. Curiously, some of them apologize for attacking the paradigm of "non-adaptive aging" still accepted by the great majority of gerontologists, by saying that their observations deal with some specific conditions only. For example, Hekimi (the world champion in prolonging the life of *C. elegans*) and Guarente wrote in 2003: "life span, therefore, appears to be regulated in these situations in spite of the fact that it is not the feature shaped adaptively by natural selection."[73] Certainly, at present we cannot guarantee that all the age-related events in all the living creatures should be regarded as programmed death. On the other hand, as soon as such a possibility seems rather probable at least in some cases, it must be taken into account in consideration of strategy of the twenty-first century medicine. The phenoptotic concept of aging opens the possibility of a dramatic increase in the human life span whereas the opposite point of view, still traditional for gerontologists, excludes such a perspective (if aging is an inevitable breakage in a complicated system, its improvement will be followed by a next breakage).

As a perspective on the longevity studies within the framework of our concept, we can point to the necessity for further analysis of major constituents of the aging programs. When identified, these constituents or the products of their activity should be arrested by specific small molecule ligands to interrupt execution of the phenoptotic program. Among them, mitochondria-targeted antioxidants look promising. Being constructed from a penetrating cation[109] and an antioxidant (e.g., ubiquinol[110]), such compounds can accumulate in mitochondria at concentrations up to 1000 times higher than outside mitochondria, strongly increasing the antioxidant capacity of these organelles responsible for apoptosis-inducing ROS formation. They were found to completely arrest the H_2O_2-induced apoptosis[84,110,111] like deletion of the gene encoding p66Shc, which entails prolongation the life span of a mammal.[75]

As was mentioned above, the larvae of the pearl mussel succeed in switching off the aging program in Atlantic salmon. Perhaps now is the right time to consider the possibility of doing the same in man.

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