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Review

Signaling and Damaging Functions of Free Radicals in Aging—Free Radical Theory, Hormesis, and TOR

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ABSTRACT: Harman's Free Radical Theory of Aging has been considered as a major theory of aging for more than 50 years. In 1956 Dr. Harman proposed that the accumulation of free radicals with the age causes the damage of biomolecules by these reactive species and the development of pathological disorders resulting in cell senescence and organismal aging. His hypothesis was supported by numerous experimental studies demonstrated an increase in free radical levels in cells and living organisms with aging. In subsequent years important discoveries of new physiological free radicals superoxide and nitric oxide have been made that led to understanding of other important functions of free radicals. It has been shown that superoxide and nitric oxide together with their diamagnetic reaction products hydrogen peroxide and peroxynitrite (all are now named reactive oxygen and nitrogen species, ROS and RNS) function as signaling species in many physiological enzymatic/gene processes. Furthermore, the disturbance of ROS and RNS physiological signaling can be an origin of various pathologies and aging. These discoveries demanded to widen original free radical theory of aging and to consider the damaging ROS signaling as an important, maybe major route to cell senescence and organismal aging. However, some experimental findings such as the extension of lifespan by calorie restriction of yeast, flies, worms, and mice, and favorable effects of physical exercises stimulated criticism of free radical theory because the expansion of lifespan accompanied in some cases by increasing oxidative stress. On these grounds such theories as Hormesis and Target of rapamycin (mTOR) theories refute the role of ROS and oxidative stress in aging. Accordingly, a major purpose of this review to show that ROS signaling is probably the most important enzyme/gene pathway responsible for the development of cell senescence and organismal aging and that ROS signaling might be considered as further development of free radical theory of aging. In spite of apparent contradictions the Hormesis or TOR theories are also describing processes of aging development regulated by ROS signaling.

Key words: ROS and RNS signaling; senescence; aging

In early chemical studies (not speaking about biology) free radicals i.e. paramagnetic species with an unpaired electron or in other words, the compounds containing a three-valent carbon atom were always considered the aggressive damaging species. Therefore, it was not surprising that biologists regarded free radicals as life damaging factors because the first free radicals identified in chemical works were reactive short-living alkyl radicals. Furthermore, in the early years biologists could not imagine free radicals to be the metabolites of normal physiological processes and believed that they were only formed due to the action of various environmental factors (contamination by toxic chemicals compounds, irradiation, etc.).

However, discovery of "physiological" radicals i.e. radicals formed in normal physiological processes, superoxide $O_2^{\cdot -}$ (McCord and Fridovich, 1968) and nitric oxide $NO^{\cdot}$ (Furchgott, Ignarro, and Murad, 1986) completely changed our views on the role of

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free radical processes in biological systems. It has been found that these species are inactive free radical agents by themselves but can be precursors of really reactive hydroxyl and peroxy free radicals, which are able to damage biomolecules.

At present free radicals and some their reactive diamagnetic products of mutual interactions are frequently named reactive oxygen species or ROS (superoxide, hydroxyl, and peroxy radicals, and hydrogen peroxide) and reactive nitrogen species or RNS (nitric oxide and peroxynitrite). (Hydrogen peroxide H_2O_2 and peroxynitrite -OONO are formed in the reactions of physiological radicals superoxide and nitric oxide by following reactions):

$$O_2^{\cdot-} + O_2^{\cdot-} + 2H+ \Rightarrow H_2O_2 + O_2$$
$$O_2^{\cdot-} + (^{\cdot})NO \Rightarrow -OONO$$

Thus mostly harmless superoxide and nitric oxide are capable of producing damaging free radicals and reactive diamagnetic molecules which might be an origin of many pathological disorders and aging.

However, a more important activity of physiological ROS and RNS is their participation in many enzymatic and gene-catalyzed processes. It has been shown that these radicals are produced by mitochondria and such enzymes as xanthine oxidase, NADPH oxidase, and nitric oxide synthase and perform important signaling functions by activating or inhibiting many other enzymes (protein kinases, MAPK kinases, phosphatases, gene-depended cascades, etc.). Therefore, the maintenance of optimal ROS and RNS levels is a critical condition for normal functioning of physiological processes.

However under certain conditions the disturbance of regulation of ROS and RNS signaling might lead to the activation of dangerous enzymatic cascades and stimulation of numerous pathological states including cardiovascular diseases, hypertension, diabetes mellitus, cancer and carcinogenesis, inflammation, and aging. Thus toxic effects of ROS and RNS signaling can depend not only on direct attack of these species on biomolecules (although it might be true for reactive hydroxyl and peroxy free radicals) but to be a consequence of disruption of their physiological levels due to enhancement or reduction of ROS or RNC formation. Below I will consider the necessity of enlargement of free radical theory of aging through incorporation of the effects of ROS and RNS signaling in senescence and aging.

Free radical signaling is a new line of work in aging studies. An important role of free radicals in aging and senescence was proposed in 1956 Dr. Harman's work "*Aging: a theory based on free radical and radiation chemistry*" [1] which opened a new era of free radical studies in biology and medicine. This hypothesis led to important practical conclusions pointing out at the possibility of aging regulation by antioxidants, capable of suppressing free radical formation. Now, a more than 50 years after Harman's proposal free radical studies in aging gather probably a hundred or more works that being a good indicator of the success of his hypothesis. Until now many good reviews on free radical theory of aging have been published. Some of them are cited here [2-5].

One of major predictions of Hartman's hypothesis was that free radical overproduction (oxidative stress) must shorten lifespan of living life forms characterized by high levels of free radicals and their reactive metabolites. However, at present there are some experimental findings which are not agreed with this early proposal (see below). But it should be stressed that previously all free radicals were considered the toxic species capable of destroying biomolecules and stimulating aging of living organisms. However we now know that ROS and RNS have important signaling functions under both physiological and pathological conditions and therefore the right route to fight aging is the regulation and not simple suppression of dangerous ROS signaling and overproduction in aging and other pathologies.

ROS overproduction in age and senescence

A lot of experimental studies demonstrate free radical and ROS overproduction in organismal aging and cellular senescence. There are some examples of earlier studies: Sawada and Carlson [6] and Sawada et al. [7] showed the enhancement of superoxide production and lipid peroxidation during lifetime of the rat. Similar data has been obtained by Schreiber et al. [8]. Chung et al. [9] found an increase in ROS formation by xanthine oxidase in aging. Hamilton et al. [10] demonstrated superoxide overproduction in hypertension and aging. Moon et al. [11] found free radical overproduction in aged mouse aortic smooth muscle cells. Chen et al. [12] showed the age-related increase in mitochondrial superoxide production in the

testosterone-producing cells of Brown Norway rat testes.

New works support these previous findings. Donato et al. [13] found that endothelial oxidative stress was developed in aged healthy men and was related with reduction in endothelium-depended dilation. In addition these authors observed that aging in humans increased the expression of NADPH oxidase and NF-κB. Choksi et al. [14] investigated the origin of resistance to oxidative stress and longevity in the Ames dwarf (DW) mice. They found that endogenous ROS production in the serum and F2-isoprostanes levels in the liver in DW mice were lower at all ages that apparently was an origin of resistance to oxidative stress. Jacobson et al. [15] showed that superoxide production by xanthine oxidase and NO synthase in mesenteric arteries from aged rats was higher than from young ones. Lener et al. [16] proposed that NADPH oxidase (Nox4) can also be responsible for superoxide overproduction in cell senescence. They showed a significant increase in replicative lifespan of human umbilical vein endothelial cells upon knockdown of Nox4. Rodriguez-Manas et al. [17] suggested that the age-dependent endothelial dysfunction in human vessels was a consequence of oxidative stress and vascular wall inflammation. Sasaki et al. [18] found that the rate of superoxide production increased with age in the mice, Wistar rats, and pigeons being inversely related to the maximum lifespan of animals. Mendoza-Nunez et al. [19] suggested that oxidative stress increased in wealthy humans older than 60 years. Miyazawa et al. [20] found that superoxide overproduction from mitochondria in mice led to premature aging. Lund et al. [21] showed that endothelial vasomotor function decreased in aged mice due to a decrease in extracellular superoxide dismutase (ecSOD). Thus ROS overproduction in aged cells and aged humans and animals is proved by numerous experimental data and must be a source of many destructive processes. It should be noted that aforementioned data show that several enzymes and other sources can increase ROS production in the age and not only mitochondria as it now widely accepted (Figure 1).

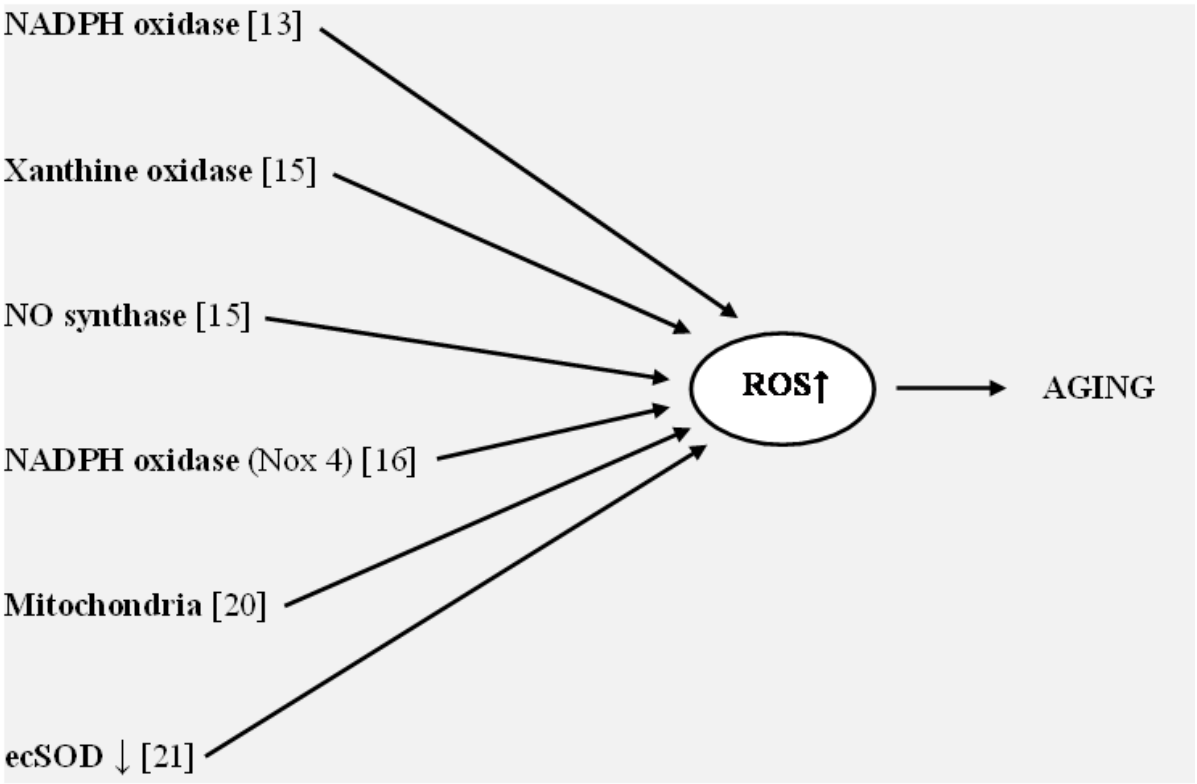

Figure 1. Sources of ROS overproduction in aging

Free radical damaging processes and ROS signaling in aging

ROS overproduction can be harmful through different damaging processes. For example, it has been shown that the overproduction of superoxide and nitric oxide is accompanied by the formation of very active species - hydroxyl radicals (through the Fenton reaction) and peroxynitrite (by combination of $O_2^{\cdot -}$ and NO). These really reactive species can destroy biomolecules directly as it has been proposed in original Harman's theory. An increase in superoxide levels can be consequence of a decrease in nitric oxide with the age [22-24] because the reduction of NO formation diminishes the inhibition by nitric oxide of mitochondrial cytochrome c oxidase that accompanied by superoxide increase [25] (Ref. 26, pp. 171-174). At the same time signaling by overproduced ROS in various enzyme/gene processes leads to cell senescence (as reviewed by Kregel and Zhang [27]). All these processes can be responsible for ROS induced damage in aging and senescence.

Damaging ROS signaling in cellular senescence and organismal aging

ROS signaling plays important role in numerous enzyme and gene catalyzed processes under normal physiological conditions [26]. However in aging these signaling processes can become damaging cascades mostly due to ROS overproduction. It might be suggested that pathological changes in aged cells and tissues initiated by ROS signaling in enzymatic/gene processes can be more important than the direct attack of reactive free radicals on biomolecules during age development. Importance of ROS signaling in aging and age-depended pathologies has already been discussed in several reviews [27-29]. Therefore excessive ROS signaling can be a new confirmation and support of free radical theory of aging.

Importance of ROS signaling functions draws attention to the question, what kind of free radicals are of great significance for aging development. It is now well known that only superoxide and nitric oxide (paramagnetic free radicals) and hydrogen peroxide (diamagnetic product of superoxide dismutation) are real signaling species. (By definition, signaling free radicals cannot be very reactive reactants in typical radical reactions of H-abstraction and the addition to double bonds because in this case they would directly destroy biomolecules without having chance to participate in enzymatic cascades. Therefore such markers of oxidative stress as lipid peroxidation or the formation of carbonyl derivatives formed in the reactions of reactive hydroxyl or peroxy free radicals or in the reaction very active peroxynitrite (the product of reaction between $O_2^{\cdot -}$ and NO) might be incorrect characteristics of free radical damage in the age).

Superoxide, nitric oxide, and hydrogen peroxide participate in enzyme/gene processes (see, below) due to their non-radical nucleophilic activity [30-32]. Owing to this they can accelerate reactions of hydrolysis, etherification, and phosphorylation catalyzed by different enzymes [26]. In addition superoxide and hydrogen peroxide can inhibit protein phosphatases by oxidation of their thiol groups and by this activate protein kinases. (It should be noted that if the mechanism of nucleophilic reactions of superoxide was thoroughly studied [33], the mechanism of hydrogen peroxide participation in heterolytic nucleophilic reactions remained uncertain. Iron-catalyzed decomposition of hydrogen peroxide to hydroxyl radicals is a well-established origin of its damaging oxidative activity, but it cannot be a mechanism of its signaling function. Some years ago I suggested that signaling functions of hydrogen peroxide might depend on conversion to superoxide by the reaction with oxidized CuZnSOD [32]).

Mechanisms of major ROS signaling pathways in the age

It has been shown that ROS signaling in the age might occur through the enzyme/gene cascades in which aging-regulating genes *p66shc*, *Sirtuin*, *FOXO3a*, and *Klotho* play critical role together with some protein kinases particularly protein kinase Akt/B. Akt kinase was frequently considered to be a surviving factor but now it has been shown that this kinase catalyzes cellular senescence in pathways with or without aging-regulating genes.

In addition to Akt kinase other mitogen activated protein kinases (MAPKs) can participate in damaging ROS signaling processes. For example Wu et al. [34] showed that compared with adult (6-month) and aged (27-month) rats, very aged rats (33-month) had higher levels of superoxide, blood glucose, phosphorylation of soleus p38-MAPK and extracellular-regulated kinase 1/2 (ERK1/2), and these changes were associated with decreased soleus Glut4 protein abundance. Chronic acetaminophen treatment (a

phenolic drag probably having antioxidant properties) diminished superoxide levels, age-associated increase in blood glucose, and reduced p38-MAPK and ERK1/2 hyperactivation.

However, the most important factor of aging development and sell senescence is probably ROS signaling in enzyme/gene cascades. Present studies demonstrate importance of *p66shc*, *Sirtuin*, *FOXO3a*, and *Klotho* genes in the development of various pathologies such as diabetes mellitus, cancer, and aging. Their role in aging has already been widely discussed [25-31]. These genes are able to stimulate or inhibit aging processes. It has been shown [35] that the deletion of *p66shc* gene induced stress resistance and prolonged lifespan in experimental animals. This study stimulated numerous investigations in which a role of *p66shc* gene in various pathologies and aging has been studied.

ROS signaling is an important factor of these enzyme/gene cascades. Trinei et al. [36] recently suggested that p66shc is able to stimulate directly ROS overproduction and aging. However, previous studies pointed out that the regulation of lifespan by p66shc depended on the stimulation of apoptosis by oxidative stress [37]. Giorgio et al. [38] also suggested that p66shc initiated apoptosis by the production of hydrogen peroxide through the oxidation of mitochondrial cytochrome c. (It should be noted that electron transfer from reduced cytochrome c to p66shc seems to be thermodynamically unfavorable due to wrong potential difference). Gertz et al. [39] suggested that p66Shc generated ROS and initiated apoptosis when cellular antioxidants were unable to inhibit ROS overproduction.

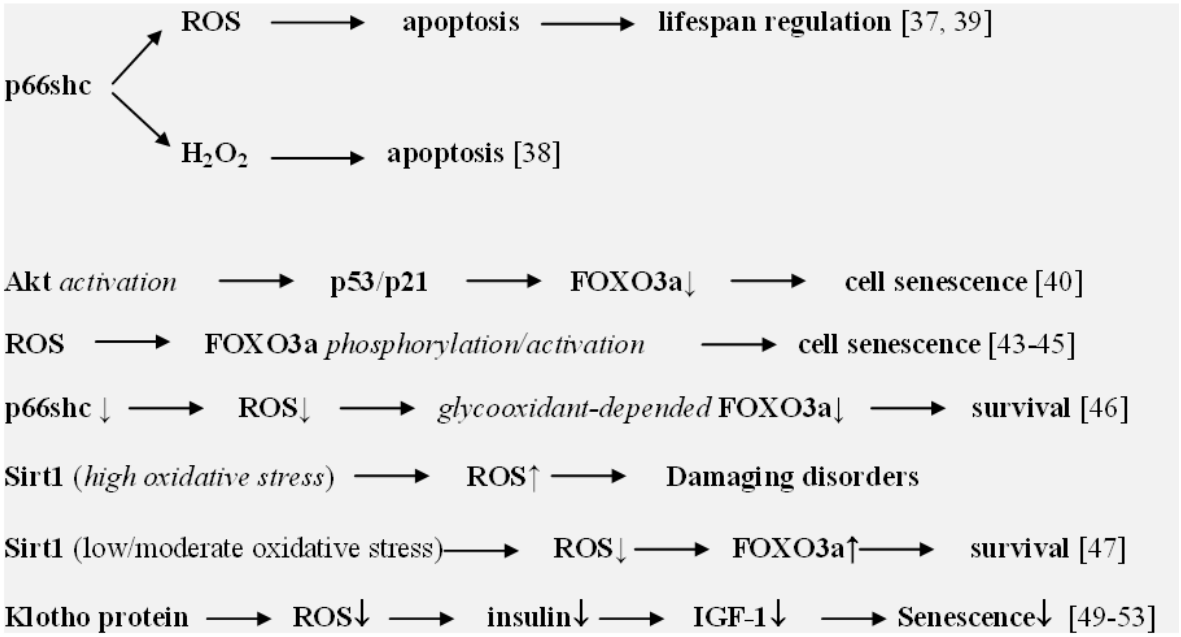

Figure 2. ROS signaling in enzyme/gene processes in aging and cell senescence

FOXO3a belong to the O subclass of the forkhead family of transcription factors. There are various examples of ROS signaling in aging processes with participation of FOXO3a. Thus Miyauchi et al. [40] showed that the activation of Akt kinase in human endothelial cells promoted senescence-like arrest of cell growth through a p53/p21-dependent pathway and the inhibition of forkhead transcription factor FOXO3a. FOXO3a influenced p53 activity by the regulation of ROS formation. As it has earlier been shown, superoxide and hydrogen peroxide are the activators of Akt kinase [41, 42]. Therefore ROS can apparently be an initiator and a mediator of enzyme/gene cascades stimulating cell senescence. Several authors also demonstrated that ROS-stimulated phosphorylation/activation of FOXO3a resulted in cellular senescence [43-45]. p66shcA and FOXO3a can mutually interact during aging

processes. Thus Chintapalli et al. [46] showed that the inhibition of p66shcA in mesangial cells prevented glycooxidant-dependent FOXO3a regulation and promoted the survival phenotype.

Upregulation of silent information regulator Sirtuin (human Sirt1 and Sirt3) suppresses age-dependent cardiac hypertrophy, apoptosis, cardiac dysfunction, and expression of senescence markers. Although a high level of Sirt1 enhanced damaging disorders, moderate overexpression of Sirt1 protected from ROS overproduction through the FOXO3a-dependent mechanism. Alcendor et al. [47] showed that Sirt1 was significantly upregulated in response to low/moderate oxidative stress in adult mouse hearts. It was suggested that Sirt3 and FOXO1 can comprise a mitochondrial signaling survival cascade.

Klotho gene is another gene which regulates ROS-depended aging processes. Yamamoto et al. [48] showed that cell surface-bound Klotho inhibited FOXO3a phosphorylation and promoted its nuclear translocation. The nuclear FOXO3a then bound to the MnSOD promoter and suppressed ROS formation. It has been shown that the Klotho protein can suppress aging through both the inhibition of insulin-like signaling and an increase in resistance to oxidative stress. Other studies have also demonstrated the suppression of aging processes by Klotho through the inhibition of ROS formation [49-53]. Some pathways of aging-regulated enzyme/gene processes are shown in Figure 2.

Effects of calorie restriction

It has been demonstrated that calorie restriction (CR) exhibits favorable effects on the lifespan of living organisms even though its effects is accompanied by an increase in oxidative stress. Nonetheless numerous data show that CR activity depends also on its antioxidative action. For example, Castello et al. [54] have studied the effect of CR on oxidative damage and its relationship with fibrosis during aging. They found CR suppressed an increase of oxidative stress and fibrosis parameters in the aortae from aged vs. young rats. Ungvari et al. [55] suggested that CR induced pathways responsible for increasing cellular oxidative stress resistance. CR can increase bioavailability of nitric oxide, decrease vascular ROS generation, and activate the Nrf2/ARE antioxidative pathway. These effects of CR might lead to the suppression of vascular disease that accompanies aging. It has also been shown that the transcription factor Nrf2 that binds to the antioxidant response element (ARE) of target genes in response to oxidative stress could be an origin of CR longevity effects [56]. Thus the mechanisms of CR longevity must certainly include an antioxidant component. In recent review Fontana et al. [57] discussed major mechanisms of CR effects on aging and longevity. They showed that in worm, flies, and mammals' dietary (calorie) restriction influenced various signal pathways through the reduction of insulin-like growth factor I (IGF-1). It was also suggested that antioxidant enzymes SOD and catalase are active ingredients of CR activity. Authors pointed out that the inhibition of this nutrient signaling pathway depended on a decrease in superoxide production through its dismuting by SODs.

Insulin signaling in age

Undoubtedly, insulin signaling pathways are among the important factors of aging development. It has been well documented that the reduction of IGF (insulin growth factor)/insulin signaling cascades led to the extension of lifespan in worms and flies and is probably of importance in mammals [58]. At the same time there are numerous evidences indicating an important role of ROS in insulin signaling processes. For example Koya and King [59] assumed that glucose adverse effects in diabetes and hyperglycemia might depend on ROS-stimulated activation of diacylglycerol (DAG)/PKC enzymatic pathway because the treatment with α-tocopherol prevented glucose-induced vascular dysfunctions and inhibited DAG/PKC activation. Geolotto et al. [60] studied the effect of insulin on ROS formation and the stimulation of PKC-dependent enzymatic cascade in cultured skin fibroblasts from human volunteers. They showed that insulin induced translocation of the p47 (phox) subunit of NADPH oxidase from the cytosol to the membrane and the generation of ROS through a PKC-δ-dependent mechanism. Similar data have been received in many other works (see, for example [61-65].

However damaging effects of ROS in aging and other pathologies can depend on their concentrations. Droge [66] pointed out that the activity of basal insulin receptor tyrosine kinase was strongly increased by small concentrations of hydrogen peroxide or by the oxidation of the intracellular sulfhydryl residues. However, prolonged exposure to hydrogen peroxide inhibited insulin action suggesting that insulin

signaling was enhanced only by moderate oxidative conditions and inhibited by excessive exposure to hydrogen peroxide. Goldstein et al. [67] also demonstrated that the weak ROS production can enhance insulin activity through the inhibition of protein tyrosine phosphatases and the activation of protein kinases. Intensification of ROS formation led to the damage of normal enzymatic cascades. Thus levels of ROS production could be critical parameters in favorable or damaging ROS signaling under pathological conditions.

It has been also shown that calorie restriction (CR) can influence insulin signaling in aging in different ways. Thus CR improved insulin sensitivity and increased lifespan in normal mice [68] through an increase in the phosphorylation of cardiac Akt without elevation of Akt protein. This effect might be cardioprotective and thus contribute to increased longevity in response to CR. However insulin signaling cascade was unaffected by CR in the heart of long-lived growth hormone-resistant knockout (GHRKO) mice.

Modifications of free radical theory of aging

Free radical theory of aging is widely discussed in contemporary studies both supporting and criticizing her major conclusions. A main criticism of this theory is directed at the suggestion that free radicals are responsible for the damage of biomolecules which must be a major reason for cell senescence and organismal aging. This proposal is indeed questionable and been criticized by modern contradictory theories such as the Hormesis theory and the TOR (target of rapamycin) theory (see, below). However, as it has been shown above, free radical activity in aging must not be limited just to ROS damaging effects but should include more important ROS signaling functions. Below we will discuss some interpretations and modifications developed for improvement of free radical theory of aging as well as contradictory theories.

Dioxygen consumption and ROS production

In 2007 Barja [69] discussed an increase in longevity during dietary restriction that can occur together with the enhancement of dioxygen consumption. He concluded that although this phenomenon is frequently interpreted as a contradictory one to the mitochondrial free radical theory of aging, it is erroneous assumption because increasing dioxygen consumption should not always associate with an increase in the rate of mitochondrial oxygen radical generation. Indeed, mitochondrial ROS production must depend not only on mitochondrial respiration but it can be regulated independently of dioxygen consumption in many different physiologic situations, tissues, and animal species.

Mitochondrial 'vicious cycle' theory of aging

Mitochondrial "vicious cycle" theory of aging proposes that accumulation of mitochondrial DNA (mtDNA) mutations may lead to the enhanced mitochondrial ROS production and to subsequent increase in oxidative stress in aging. This theory was considered to be a further development of original Harman's theory of aging. It has been suggested that the mutated mtDNA formed during the ROS attack on mtDNA are able by themselves to generate ROS (apparently by the reduction of dioxygen to superoxide) and by this to enhance oxidative stress (vicious cycle!).

This hypothesis was widely discussed in literature [70-73]. For example Vermulst et al. [71] and Hiona and Leeuwenburgh [72] founded no confirmations that mitochondrial mutations can limit the natural lifespan of animals. On the other hand Poovathingal et al. [73] suggested that improved experimental methods might help to get reliable data.

Unfortunately, it is not clear whatsoever, why mutated mtDNA or their fragments must produced ROS. Opposite case (inhibition of ROS) seems to be equally possible. We also do not know how ROS can induce mtDNA mutations. One of possible directions of ROS attack has been suggested by Sarkar and his co-workers [74]: they proposed that due to the close proximity of the zinc finger to DNA, iron-substituted zinc fingers may generate free radicals formed through the reaction of hydrogen peroxide with ferric ions and by that damage DNA molecules. Thus the source of development of "vicious cycle" in mitochondria remains completely unclear.

Recently Perez et al. [75] showed that among 18 genes coding for antioxidant enzymes only Sod1 gene deletion affected longevity of mice. I believe that it is important fact showing that lifespan depends on the Sod1 gene and correspondingly on the activity of CuZnSOD enzyme performing dismutation of superoxide, a main signaling free radical in enzyme/gene cascades in aging).

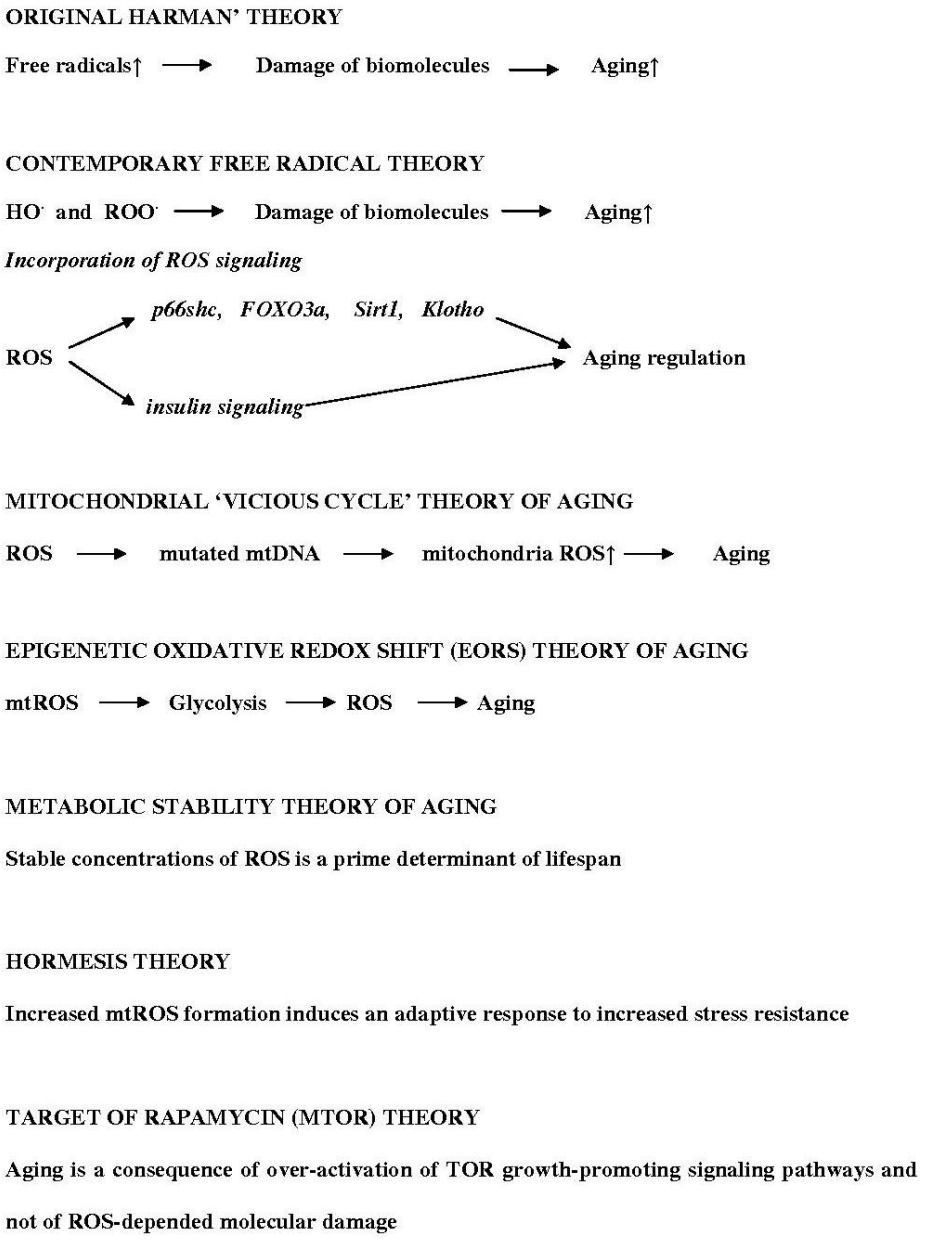

Figure 3. Free radical theories of aging

Epigenetic oxidative redox shift (EORS) theory of aging

For improvement of free radical theory Brewer [76] proposed to unify free radical theory with the effects of insulin signaling in aging giving this hypothesis the title - the epigenetic oxidative redox shift (EORS) theory of aging. According to EORS, sedentary behavior associated with age triggers an oxidized redox shift and impairs mitochondrial function. (I should comment that the name "oxidized redox shift" is very unfortunate because it means "oxidative reduction/oxidation shift"). Author believes that in contrast to the 2% inefficiency of mitochondrial reduction of dioxygen (to form superoxide) "oxidized redox shift" upregulates aerobic glycolysis and enhances ROS production by 100%. I am uncertain how to estimate an amount of real mitochondrial ROS formation in aging or cell senescence. Even a 2% leak from mitochondrial respiratory chain which must be

responsible for superoxide formation is probably seriously overestimated. The same is more true for ROS formation under glycolic conditions. However, aforementioned data show that insulin signaling cascades in aging also regulated by ROS; therefore there is probably no need to add new hypotheses to free radical theory of aging.

Metabolic stability theory of aging

In accord with the metabolic stability theory of aging [77] the ability of cells to maintain the stable concentrations of ROS is a prime determinant of lifespan. The discussion of this theory is too far from the principal topics of this review. However, it is of interest that Brink et al. [77] criticizes Harman's theory on the basis that it ignores the fact that ROS are not only damaging species but specific signaling molecules which are necessary for maintaining normal cell functions. It is of course true and ROS damaging signaling under pathological conditions including aging is widely discussed in present review.

Theories opposing the significance of free radicals in aging development

Hormesis theory

Despite numerous findings showing ROS overproduction and damaging ROS signaling in cell senescence and organismal ageing some contemporary studies raise objections to importance of these factors in age development. It has been shown that calorie restriction which was proven to result in enhanced lifespan in yeast, worms, flies, and even mice caused simultaneously the enhancement of mitochondrial ROS formation. Similar effect might have physical exercise which is good for healthy life but also accompanied by ROS overproduction. In recent review Ristow and Zarse [78] discussed the effects of calorie restriction and reduced glucose metabolism on the longevity of S. cerevisiae, D. melanogaster, C. elegans, and mice. They suggested that increased ROS formation within the mitochondria caused an adaptive response to increased stress resistance. This type of retrograde response was named mitochondrial hormesis or mitohormesis.

One of findings supporting hormesis theory is an unfavorable effect of antioxidants on aging development when they applied simultaneously with calorie restriction or during physical exercise. In addition, it has been demonstrated [79] that in some case for example in the fungal aging model Podospora anserina the overexpression of antioxidative enzyme MnSOD led to lifespan shortening. Goto and Radak [80] suggested that antioxidant supplementations attenuated beneficial effects of exercise and that hormesis can be caused by ROS in animals. It has been also shown that long term vitamin C supplementation diminished the adaptive response of exercise to oxidants in human lymphocytes [81]. Ristow et al. [82] concluded that exercise increased insulin sensitivity in humans only in the absence of antioxidants in both previously untrained and pretrained individuals. This fact was paralleled by increasing ROS generation.

Do these findings contradict free radicals theory of aging? I believe that there is no contradiction. As it has been proposed above, ascorbic acid (vitamin C) is a free radical scavenger which reacts with reactive free radicals such as $HO^{\cdot}$ or $ROO^{\cdot}$. Exercise also enhanced the formation of these radicals which were formed, for example during lipid peroxidation. Thus, if aging is regulated by ROS signaling, then antioxidants such as ascorbic acid will not decrease the level of superoxide, a major ROS signaling species. It should be mentioned that ascorbic acid can exhibit both antioxidant and prooxidant properties in *in vitro* systems and at supplementation to humans [83, 87]. Prooxidant effects of ascorbic acid could depend on iron overloading in various biological systems and can be an origin of free radical overproduction at the supplementation of vitamin C.

To some degree the suggestion about the positive effects of additional antioxidants presented in surrogate vitamin C was supported by data obtained by Thaler et al. [84, 85]. These authors showed that the supplementation of vitamin C to 86 nondiabetic subjects at increased risk for diabetes type 2 had positive effect in lifestyle intervention. However Bluher et al. [86] demonstrated the opposite effect of vitamin C. Therefore it is quite possible this difference might be a consequence of the application of pharmacological vitamin C by Bluher et al. [86] and the surrogate of a healthy lifestyle by Thaler et al. [84, 85]. Surrogate of vitamin C probably contained other antioxidants such flavonoids and natural quinones, which in contrast to ascorbic acid can readily react with superoxide (see, for example Table 29.3, 29.4 in Ref. 87). If we accept this point of view, we might suggest that the use of vitamin C could not affect aging development, while the scavengers of superoxide (flavonoids and quinones) can.

Target of rapamycin (mTOR) theory

The mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase that regulates cell growth, cell proliferation, cell survival, and other cellular functions. It has been recently suggested that aging is a consequence of over-activation of growth-promoting signaling pathways such as the TOR or mTOR and not the consequence of ROS-depended molecular damages [88]. Surprisingly in this paper Blagosklonny presumes that although ROS can induce molecular damage but it is not life-limiting "because the TOR-driven aging terminates life first."

There is no doubt that the reduction of TOR signaling can result in an extension of lifespan of living organisms. Mechanisms of TOR signaling in aging is not fully understood, although Pan and Shadel [89] documented that the Sch9p kinase is a key downstream effector of oxidative phosphorylation, ROS, and yeast chronological lifespan in the TOR-mitochondria pathway. Thus despite all criticism (see below) many TOR signaling pathways are mediated by ROS. Contemporary free radical theories of aging are summarized in Figure 3.

Discussion

As is well known, Dr. Harman developed his free radical theory of aging in 1956. It is interesting to compare our knowledge about free radicals in biological systems there and now. In 1956 Dr. Harman could not see any difference between various free radicals – all of them should seem to him the criminals, the destroyers of living organisms. His theory was really a new leap forward understanding of mechanism of aging.

However, at present, more than 50 years after his discovery our knowledge of free radical-mediated processes in cells and tissues is completely different. We know that major reactive oxygen and nitrogen species (ROS and RNS) physiological free radicals superoxide and nitric oxide and the products of their reactions hydrogen peroxide and peroxynitrite are the obligatory mediators and promoters of numerous enzyme/gene pathways under both physiological and pathophysiological conditions. Yes, of course, reactive free radicals such as hydroxyl and peroxy radicals can damage biomolecules and their activities can be suppressed by some antioxidants (free radical scavengers) such as α-tocopherol (vitamin E) and ascorbic acid (vitamin C). However, ROS and RNS probably play a much more important role as signaling species. Enlargement out knowledge about free radicals in biological systems undoubtedly must broaden and modify free radical theory of aging.

I believe that contemporary interpretation of free radicals theory in aging must incorporate all the damaging activities of ROS and RNS including damaging ROS signaling in enzyme/gene processes. Such damaging signaling probably arises from ROS overproduction – the fact strictly proved in aging. There are many sources of ROS overproduction in aging (see above), the most important source is probably the oxidizible components of diet [90]. From my point of view an increase in ROS during physical exercises or calorie restriction does not contradicted to the role of free radicals in aging because ROS signaling might result in modern oxidative stress, which is not regulated by traditional antioxidants vitamins C and E [66, 67].

Surprisingly critics of free radical theory of aging apparently criticize her at the level of the old Harman's theory as there was no development in the studies of free radicals and ROS for last fifty years. They demonstrate that scavengers of reactive oxygen radicals do not affect cell senescence and organismal aging – it is true, but they do not look at ROS signaling processes in aging. Blagosklonny's criticism [88] seems to be particularly inadequate. First of all author claims that all conclusions of free radical theory of aging can be explained by TOR theory. Yes, it could be because TOR signaling is one of the other pathways regulating aging (for example insulin signaling) where ROS participation also plays an important role. I always thought that a new interpretation of old theory is possible if it gives some new benefits. What advantages give Blagosklonny's interpretation of TOR theory if he agrees that ROS participate in TOR pathways but they are not important because "TOR will kill quicker that ROS." How can he know this? He writes that "Some opponents have called this point of view extreme, one-sided and unbalanced." Unfortunately I dare to add – a futile one.

Conclusions

Free radical theory of aging when it takes into account of new developments in free radical studies (ROS and RNS signaling) remains a reliable theory of aging.

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