

Stress and Aging

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Abstract: This review focuses on the stress responses and host defense systems, which affect the aging process. Various kinds of external stresses such as heat shock, UV, X-ray, drugs, infection or internal stresses such as reactive oxygen species (ROS) generated by metabolism induce damages to nucleic acids and proteins. Several long-lived mutants have been established in *C. elegans*, all of which are resistant to environmental stresses. In humans, individuals with Werner's syndrome show early senescence. The affected gene was cloned and found to be one of DNA helicase, which may work as a repair enzyme following stress. DNA-damaging stresses such as alkylating agents, UV or radiation induce p53 protein expression. Enhanced expression of the p53 protein promotes DNA repair, cell-cycle arrest or apoptosis. The development and aging of the immune system were accelerated in p53-deficient mice. The defects of the repair system induce premature aging, and longevity may depend on the strength of the defense system. Senescent human diploid fibroblasts (HDL) contain elevated amounts of the cyclin-dependent kinase inhibitor p21^{WAF1} (p21), which has been shown to induce cell-cycle arrest. It has been shown recently that the histone deacetylase inhibitors, sodium butyrate and trichostatin A, induce a cellular senescence-like phenotype in NIH3T3 cells or HDL and enhance p21 promoter activity. We suffer from oxidative stress under normal physiological conditions. It was to counteract such oxidative stresses that mammals developed a defense system. The major enzymes to catalyze oxygen radicals are SOD, catalase and glutathion peroxidase. The expression of Mn-SOD was shown to be induced by infectious stress. We favor the hypothesis that longevity may depend on the strength of these defense systems.

INTRODUCTION

It has been suggested that various kinds of external stresses and reactive oxygen species (ROS) generated by metabolism induce damages to nucleic acids and proteins. The host has both a repair mechanism and a defense system. The response to DNA-damaging stress in mammalian cells is complex and may involve several processes, including enhanced DNA repair, cell cycle arrest and/or apoptosis. Perhaps the most prominent among the early responses induced by DNA damage is the activation of p53.

Defects in the repair system induce premature aging, and longevity may depend on the strength of the defense system. In *C. elegans*, age-1 mutants live twice as long as the wild type and are more resistant to oxidative stresses, heat shock and UV radiation. In human's individuals with Werner's

syndrome show a very similar phenotype to that of a normal aged person in early adult life. The affected gene was cloned and found to be one of DNA helicase, which may work as a repair enzyme against DNA-damaging stress. Patients who have defective repair responses to UV are grouped into Xeroderma pigmentosum (XP), Cockayne syndrome (CS) and trichothiodystrophy (TTD). All three diseases have defects in nucleotide excision repair (NER) with CS patients exhibiting a short life span.

Some investigators have suggested the relationship between stresses and aging. Johnson et al. extensively studied the mechanism of aging by *C. elegans*. They proposed that oxidative damage produced from various stresses is the main factor in aging and that long-lived strains are relatively resistant to different environmental stress [1]. Harman first proposed the oxidative stress theory of an aging mechanism. A recent review [2] also proposed the oxidative stress theory of aging from the viewpoints of molecular biology. Another recent review discussed the more general aging mechanism based on genetics [3].

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In this review, we will discuss the mechanism of aging from the perspective of stresses and the host defense system. First, we will review the recent progress in studies of the aging of *C. elegans*. Long-lived *C. elegans* are resistant to various environmental stresses. The different kinds of human premature syndrome are the result of defects in repair genes. We first discuss aging and the DNA repair system, then genotoxic stress signaling in mammals, especially concerning p53 protein and p21^{WAF1} (p21), both of which are elevated in senescent fibroblast. Cell cycles are arrested (G0/G1 arrest) by the induction of p21 protein. During cell cycle arrest, broken DNA is repaired by host repair systems. Finally, oxidative stress and host defense system will be discussed as they relate to aging.

1. Longevity of *C. elegans* and Stress Responses

Caenorhabditis elegans (*C. elegans*) is a nematode species widely used to study development, and recently, aging. Isolation of long-lived mutants is critical for identifying genes specifying key processes in a lifespan. Several long-lived mutants, has been established. Among the long-lived mutants, those related to dauer formation have a pronounced ability to live longer. The dauer larva is an alternative larval stage in *C. elegans*, which allows animals to survive through periods of low food availability. Well-fed worms live for about three weeks, but dauer larvae can live for at least two months without affecting post-dauer lifespan. Age-1 and daf-2 are major long-lived mutants, which produce a dauer constitutive phenotype. Age-1 encodes a homologue of mammalian phosphatidylinositol-3-OH kinase (PI(3)K) catalytic subunits. Lack of both maternal and zygotic age-1 activity causes dauer formation, whereas animals with maternal but not zygotic age-1 activity develop as non-daughters that live more than twice as long as normal [4]. Daf-2 encodes an insulin receptor family member, and also lives more than twice as long [5]. The life-span extension caused by DAF-2 mutations requires the activity of the gene DAF-16, which appears to play a unique role in life-span regulation and encodes a member of the hepatocyte nuclear factor 3 (HNF-3)/forkhead family of transcriptional regulators [6] Fig.(1). The pathway in the nematode is similar to mammalian insulin signaling. In mammals, activation of the insulin receptor leads to activation of other signaling molecules such as phosphoinositide-3-OH kinase (PI3K) and Ras as

well as their downstream effectors, such as Akt/PKB and MAP kinase cascade [7]. Molecules that have been identified downstream of the DAF-2 (insulin/IGF-1 receptor) and are positively regulated by DAF-2 in *C. elegans* include the Age-1(PI3K), the Akt/ PKB homologue Akt-1 and Akt-2 [8]. From these results it has been suggested that the long-lived mutants related to dauer formation were similar to mammalian insulin- dependent diabetes. One of the kinases that phosphorylates Akt/PKB and is required for its activation is 3-phosphoinositide-dependent kinase (PDK1). The *C. elegans* PDK1 homologue is required to prevent developmental arrest at the dauer larval stage. Loss-of-function mutations in *pdk-1* cause a dauer constitutive phenotype and life span increase that are suppressed by loss-of-function mutations in the DAF-16 transcription factor [9]. Are there any relations between these mutations and stress resistance? The mutant of IGF-1 receptor (*daf-2*) acquired longevity. The defect in IGF-1 receptor causes the decrease of cellular metabolism, which produces smaller amount of ROS. There exist the possibility that SOD or other genes related to the host defense system might be the target genes of DAF-16(forkhead family of transcriptional regulators). Recently, it was found that catalase activity decreases as *C. elegans* adults age, but the decrease is minimized in *age-1* mutants. Furthermore, the *ctl-1* mutant, which encodes an unusual cytosolic catalase, reduces adult lifespan in otherwise wild animals [10]. By analyzing short lived mutants, Ishii et al. found that oxidative stresses dramatically decrease the life span of *C. elegans*. A *mev-1(kn1)* mutant of *C. elegans* has been found to be hypersensitive to raised oxygen concentrations. The gene, which encodes the meV-1 mutant, was found to be succinate dehydrogenase cytochrome b [11]. As mentioned above, several kinds of genes affect the longevity of nematode. However, almost all long-lived mutants are resistant to various kind of external stresses or internal ROS. As a result of further studies of *C. elegans*, other mutants have been reported. The *clk-1* mutation extends the lifespan of *C. elegans* by slowing its metabolism [12]. It has been proposed that the *clk-1* mutation, directly or indirectly, induces *ctl-1* (catalase) which, in turn, extends life span by protecting cells from oxidative damage [10]. Another mutant is related to the reproductive system. If the cells that give rise to the germ line are killed with a laser microbeam, the lifespan of the animal is extended. It was found that, in order for germline ablation to extend lifespan, DAF-16 is required as well as a putative nuclear hormone receptor, DAF-12 [13]. Another mutant is related to a neurosecretory signal [14]. To explore what kind

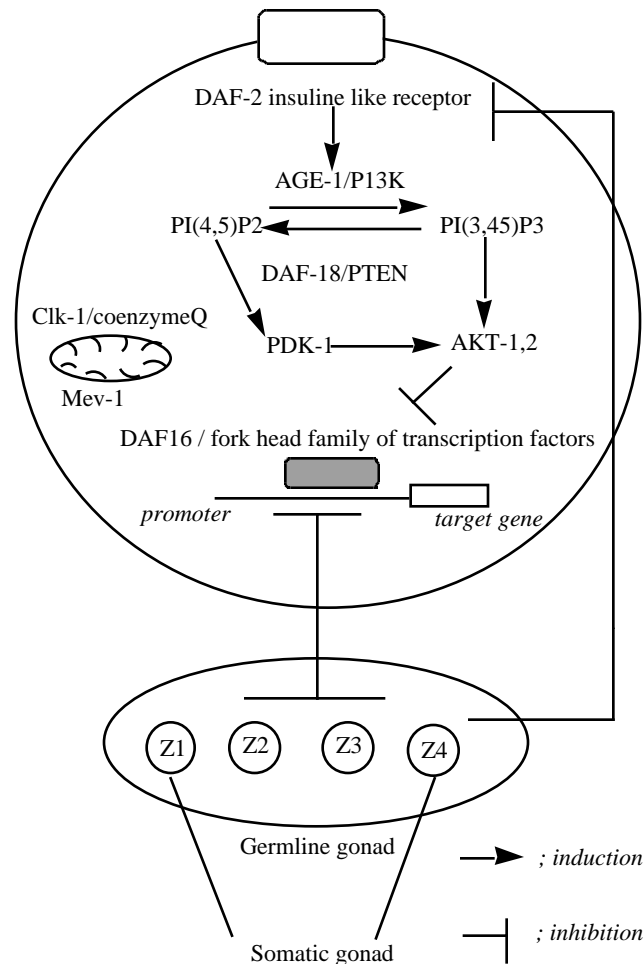


Fig. (1). Genetic pathway of *C. elegans* longevity.

Model of regulation of DAF-2 insulin receptor-like signaling pathway and relation to reproductive pathway. Clk-1 and Mev-1 are related to ROS. PI(4,5)P₂; phosphatidylinositol 4,5-bisphosphate, PI(3,4,5)P₃; phosphatidylinositol 3,4,5-triphosphate. The gonad consists of four cells. Z1 and Z4 give rise to the somatic gonad, and Z2 and Z3 give rise to the germ line of the adult. The human homologous gene is written in right side of a backslash.

of defense systems are involved that enable long-lived nematodes to survive in a stressful environment, is one target of future studies.

2. Human Premature Syndromes and DNA Damage and Repair

Several kinds of human premature syndrome have been reported, which are related to DNA damages and repair. Among them Werner's syndrome (WS) is associated with a large number of symptoms displayed in normal aging, including osteoporosis, atherosclerosis, diabetes mellitus, cataracts, wrinkled skin, gray hair and cancer developing in early adulthood. Most patients affected with WS die before age 50. The defective gene in WS, designated *WRN*, has been cloned

which encodes a protein of 1432 amino acid [15]. *WRN* gene product belongs to the RecQ superfamily of DNA helicase that includes *RecQ* (*E. coli*), *Sgs1* (*S. cerevisiae*) and *BLM* (human bloom syndrome). Recently, another human helicase gene *RECQL4* has been shown to cause a subset of cases of Rothmund-Thomson syndrome (RTS; also known as poikiloderma congenital), which is a rare, autosomal recessive genetic disorder characterized by abnormalities in the skin and skeleton, juvenile cataracts, premature aging, and a predisposition to neoplasm [16]. The *WRN* gene product, a DNA helicase, has been previously shown to unwind short DNA duplexes (≤ 53 base pairs) in a reaction stimulated by single-stranded DNA-binding proteins. *WRN* protein may be involved in DNA repair, since other DNA helicase such as *XPB* or *XPD* have been shown to be very important proteins involved in DNA excision repair (described

in this section), although the actual function of WRN helicase has not yet been elucidated. The function of *Sgs1* (homologue of WRN protein) in *S. cerevisiae* has been studied extensively. In the budding yeast *S. cerevisiae*, cell division is asymmetric; the mother cell gives rise to a small daughter cell at each division. Aging yeast mother cells showed a progressive enlargement and fragmentation of the nucleolus. These nucleolar changes are likely due to the accumulation of extrachromosomal rDNA circles (ERCs) in old cells, and, in fact, ERCs cause aging. Mutants for *sgs1* accumulate ERCs more rapidly, leading to premature aging and a shorter life span [17].

Three kinds of genetic disorders caused by defects of the NER pathway are XP, CS and TTD. XP is a rare autosomal recessive disease characterized by hypersensitivity to sunlight, abnormal pigmentation, and predisposition to skin cancers. The genes encoding XPA, XPB, XPC, XPD, XPF and XPG proteins have been cloned. CS is characterized by a postnatal failure of growth, a

limited life span, and progressive neurological dysfunction. The genes encoding CSA and CSB proteins were cloned. Recently, it has been shown that XPB, XPD and XPG also show characteristic features of CS. XPB and XPD have been shown to encode DNA helicases that are subunits of TFIIH, a multisubunit complex engaged not only in NER but also in basal transcription [18]. TFIIH contains nine subunits including XPB, XPD, Cdk7(MO15), cyclin H, p44, p34 and another two DNA helicases with DNA-dependent ATPase activities. Therefore, it is not surprising that the mutations in TFIIH helicases (XPB and XPD) are related to XP, CS and TTD. At present in human nucleotide excision repair, 14 polypeptides in six repair factors act in concert to excise DNA damage in the form of 24-32-nucleotide long oligomers. The first step is the damage recognition, in which XPA, RPA and XPC have been implicated. Then, TFIIH, including XPB and XPD, opens the damaged double-stranded DNA. Finally, the damaged DNA is incised by XPF (5') and XPG (3') Fig.(2). The mechanism of gap filling has not yet been elucidated. It has

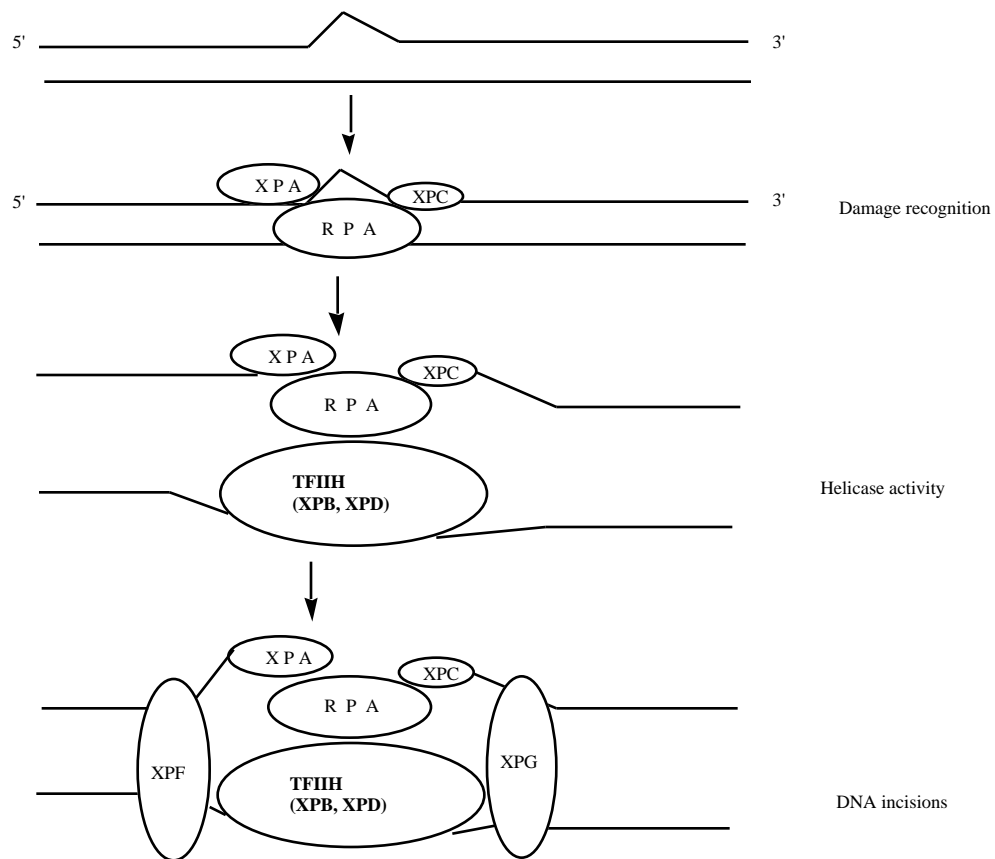


Fig. (2). Nucleotide excision repair and human premature syndromes.

RPA; replication protein A, XPA, XPB, XPC, XPD, XPG and XPF are described in the text.

been shown recently that XPG knockout mice exhibited postnatal growth failure, short life span, and early onset of cellular senescence, which are similar to the phenotype of CS patient [19].

3. Cellular Stress Response and Aging

Before discussing stress and aging, we will briefly summarize cellular-stress signaling in mammals Fig.(3). Various kinds of environmental stresses induce important signaling pathways in cells. In particular, DNA damaging stresses such as alkylating agents, UV or radiation induces p53 protein expression. Enhanced expression of the p53 protein promotes DNA repair, cell cycle arrest (G1 or G2) or programmed cell death (apoptosis) [20]. Normal cells have limited proliferative potential in culture [21]. The expression and transcriptional activity of p53 were reported to be increased in senescent fibroblasts [22,23]. Several reports suggest that the mammalian aging process is governed by p53 protein. p53 binds to the WRN protein in vivo and in vitro through its carboxyl

terminus. p53-mediated apoptosis is attenuated in Werner's syndrome cells [24]. The XPB and XPD helicases have also been shown to be components of the p53-mediated apoptosis pathway, and they bind to the carboxyl terminus of p53 protein [25]. One critical reason of the mortality of normal cells comes from the facts that telomeres shorten at each division. Maintenance of telomere length and function requires telomerase, a specialized reverse transcriptase, as well as a complex of telomere-associated proteins [26]. Most somatic human tissues and primary cells possess low or undetectable telomerase activity, and telomeres shorten with each cell division in vivo and in vitro. Chin L. et al. examined the relation between telomere shortening and p53. They hypothesized that shortened telomeres, exposed chromosomal termini, and/or chromosomal fusion-bridge-breakage events might be recognized as DNA-damage signals, thus activating p53 and its associated checkpoint functions. They used cells and organs of late-generation telomerase-deficient mice, and found that coincident with severe telomere shortening and associated genomic

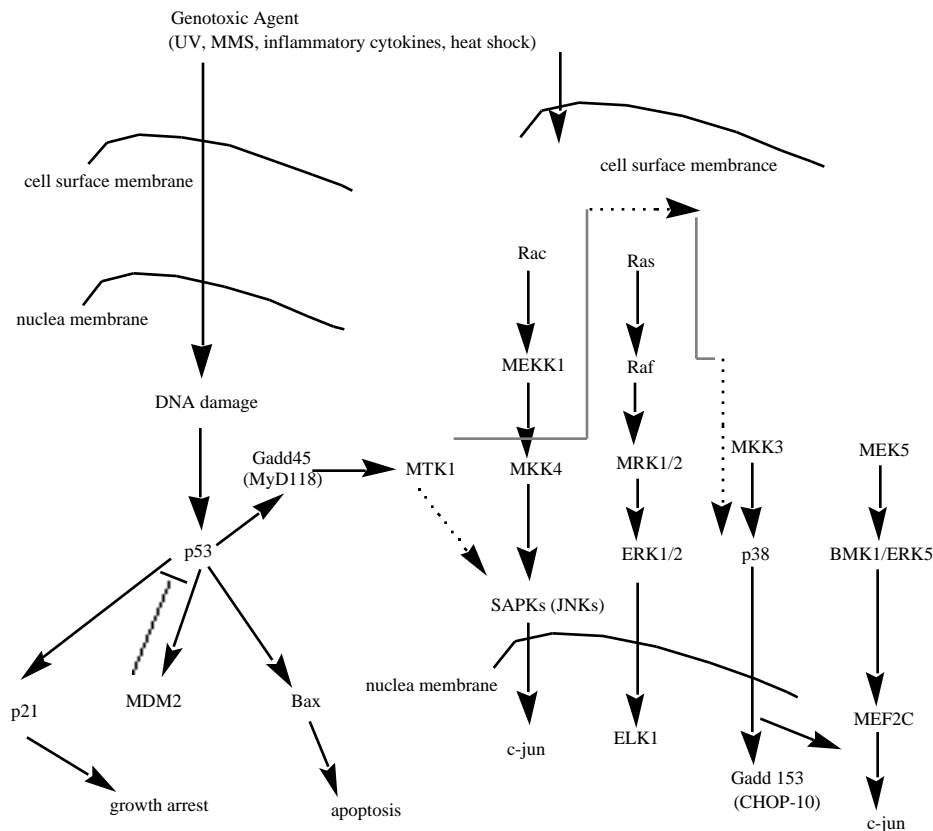


Fig. (3). Stress signaling.

Genotoxic stress signaling. Left side; p53 dependent signaling. Right side; MAP kinase signaling.

instability, p53 is activated, leading to growth arrest and/or apoptosis [27]. Rudolph *et al.* examined a link with organism aging processes and cellular aging by using telomerase-deficient mice. Sixth-generation mice (G6) were infertile, had a decreased proliferative capacity of splenocytes and bone marrow cells, and showed reduced longevity. They examined the stress response in aged telomerase-deficient mice, and found that G3 or G6 mice showed impaired stress responses in wound healing and blood cell depletion [28].

We designed the *in vivo* experiments in p53 deficient mice to detect functional senescence by observing their immune system. It has been reported that immune responses decline with aging in mammals [29-33]. In particular, much of the age-related decline in protective immune responses is thought to be induced by changes in T-cell composition. The accumulation of memory-phenotype T cells in the place of naive-phenotype T cells [30,31] has been linked to the reduced proliferative response [32] and decreased production of IL-2 by T cells with aging [33]. We found that the development and aging of the immune system were accelerated in p53-deficient mice; moreover, the accumulation of memory T cells spontaneously accelerated, and a strong T-cell-dependent antibody response and T helper type 2 (Th2) cytokine expression (IL-4, IL-6 and IL-10) were induced by antigen stimulation in young p53-deficient mice in the developmental stage. The high T-cell proliferative response in young mice rapidly progressed to a depressed proliferative response in adult mice. It was suggested that the loss of regulation of the cell cycle, DNA repair, and apoptosis by p53 deficiency potentially lead to immunosenescence with the accumulation of memory T cells [34].

From the above description, p53 seems to play a major role in the mammalian aging process, especially from the standpoint of DNA damaging stresses and the host defense system. The activation of p53 protein by DNA damaging stresses induces the elimination of the damaged cells by apoptosis and DNA repair during cell-cycle arrest. However, p53 independent pathways may also exist. Senescent human diploid fibroblasts (HDL) contain elevated amounts of the ubiquitously acting cyclin-dependent kinase inhibitor p21^{WAF1} (p21) [35]. p21 was first cloned and characterized as an important effector that acts to inhibit cyclin-dependent kinase activity in p53-mediated cell cycle arrest induced by DNA damage [36,37]. Subsequently, in various differentiation systems, the induction of p21 occurs by p53-independent mechanisms [38,39]. We have

shown recently that sodium butyrate induces a cellular senescence-like phenotype in NIH3T3 cells and enhances p21 promoter activity in this cell line. Furthermore, we have shown that this sodium butyrate-induced p21 promoter activity is p53 independent by using a p53 deletion-mutant promoter and a p53-deficient fibroblast cell line [40]. Histone deacetylase inhibitor-induced phenotype changes were also observed in human fibroblast. Ogrzyzko *et al.* showed that two histone deacetylase inhibitors, sodium butyrate and trichostatin A, dramatically reduced the HDF proliferative life span in a manner that is dependent on one or more cell doublings in the presence of these agents [41]. Furthermore, we showed that trichostatin A induced a senescence-like phenotype in NIH3T3 cells and enhanced p21 promoter activity. We decided that the minimal region of TSA-responsive p21 promoter is 100bp from transcription start site. One GC-rich cis element was critical for p21 promoter activity, and both Sp1 and Sp3 were the main transcriptional factors for p21 promoter [42].

In mammalian cells, the GADD (growth arrest and DNA damage-inducible) genes were originally isolated on the basis of rapid induction by UV radiation in Chinese hamster ovary (CHO) cells, but have subsequently been found to be induced by a variety of DNA-damaging agents and certain other growth arrest treatments [43]. Among GADD family genes, GADD45 has been shown to activate the p38/JNK pathway [44]. Furthermore, GADD45 has been activated not only by p53 but also by BRCA1, another tumor suppressor gene [45]. We have cloned GADD34, which binds to BFCOL1, a zinc finger transcription factor, which in turn binds to the proximal pro 2(I)-collagen promoter [46]. The function and signal transduction of GADD34 has been poorly understood. BFCOL1 could also bind to this GC-box of the p21 promoter [47]. We also found that the expression of GADD34 was independent of p53. Future will elucidate the p53 independent pathway of stress responses and its relation to aging.

4. Oxidative Stresses and Host-Defense System

We suffer from oxidative stress under normal physiological conditions. It is estimated that 2-to 3% of the oxygen consumed by aerobic cells is diverted to the generation of O₂⁻ and H₂O₂ [48]. One theory of aging proposes that ROS, which are generated by metabolism, cause cumulative damage

over a lifetime [49]. DNA is highly susceptible to reactive oxygen species *in vivo*, especially hydroxyl radicals that are generated by lipid peroxidation, cellular respiration, products of inflammation low-level ionizing radiation, and near-ultraviolet (320-380 nm). The major mutagenic base lesion in DNA caused by exposure to ROS is 8-hydroxyguanine (8-oxo-7,8-dihydroguanine). Polymerases incorporate A instead of C opposite to 8-oxo G, and in the absence of a repair enzyme (8-hydroxy-guanine-DNA glycosylase), a misincorporation leads to G-T transversions [50].

To cope with these oxidative stresses, mammals developed a defense system. The major enzymes to catalyze oxygen radicals are SOD, catalase and glutathione peroxidase Fig.(4). Transgenic flies carrying three copies of each of these genes exhibited as much as a one-third extension of life-span, a longer mortality rate doubling time, a lower amount of protein oxidative damage, and a delayed loss in physical performance [51]. Moreover, overexpression of a single gene, Cu/ZnSOD (SOD1), in a single cell type, the motor neuron, extends normal lifespan by up to 40% and rescues the lifespan of a short-lived Sod null mutant [52]. Mice knocked out for SOD1 gene develop normally and show no overt motor deficits by 6 months of age. Histological examination of the spinal cord reveals no signs of pathology in animals 4 months of age. However, Cu/Zn SOD-deficient mice exhibit marked vulnerability to motor neuron loss after axonal injury [53].

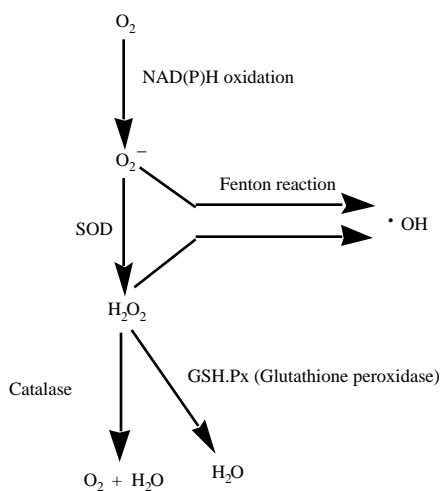


Fig. (4). Production and removal of ROS.

An NAD(P)H oxidase converts O_2 to superoxide anion (O_2^-), which is converted to hydrogen peroxide anion (H_2O_2) by superoxide dismutase (SOD).

Infectious stresses caused by bacterial or viral infection also induce ROS and nitric oxide (NO). Others and we showed that expression of the manganese superoxide dismutase (Mn-SOD) is induced by pro-inflammatory cytokine such as tumor necrosis factor- (TNF^-), interleukin-1 (IL-1) and interferon- (IFN^-). Bacterial products such as lipopolysaccharide (LPS) also induce the expression of Mn-SOD. We identified the TNF-responsive cis-acting element in the second intron of Mn-SOD gene. Also, we showed that NF κ B and c/EBP transcription factors bound to this element by gel shift analysis [54]. Our results indicate that activation of NF- κ B, which translocates into the nucleus by various immunological or other environmental stimuli, induce Mn-SOD expression. Mn-SOD works as defense system to dismutate ROS. The elucidation of a signaling pathway to induce Mn-SOD expression will help to provide the means to combat to oxidative damage.

5. Perspective

There are several hypotheses of aging. Based on natural selection, individuals after the production of progeny must be eliminated. The caloric-restriction theory may be explained by oxidative stress, because the metabolic level will be low and produce less ROS by caloric restriction. Cultured normal cells have a definite life span as mentioned by Hayflick. However, ES cells divide indefinitely with some factors. They divide *in vivo* and differentiate into various stem cells in tissue organs such as hematopoietic stem cells and neuronal stem cells, and continued to divide and differentiate Fig.(5). However, differentiation at some stage will be reversible. This was shown by experiments with cloned animals, whose somatic cells have the ability to produce a whole animal [55,56]. It is surprising that DNA of tissue cells has this ability. For the studies of stress signaling, especially in DNA-damaging stresses and repair systems, we have to discriminate between differentiated and undifferentiated stages of target cells. Almost all neuronal cells are terminally differentiated and non-dividing after birth. They survive for 100 years *in vivo*. For non-dividing cells, stresses caused by metabolism (ROS) may play a major role. Other stresses such as UV, radiation and infection also produce ROS. From that point of view, an understanding of the host defense system, which dismutates ROS, will be interesting to study for the general understanding of aging within the central nervous system of complex vertebrates.

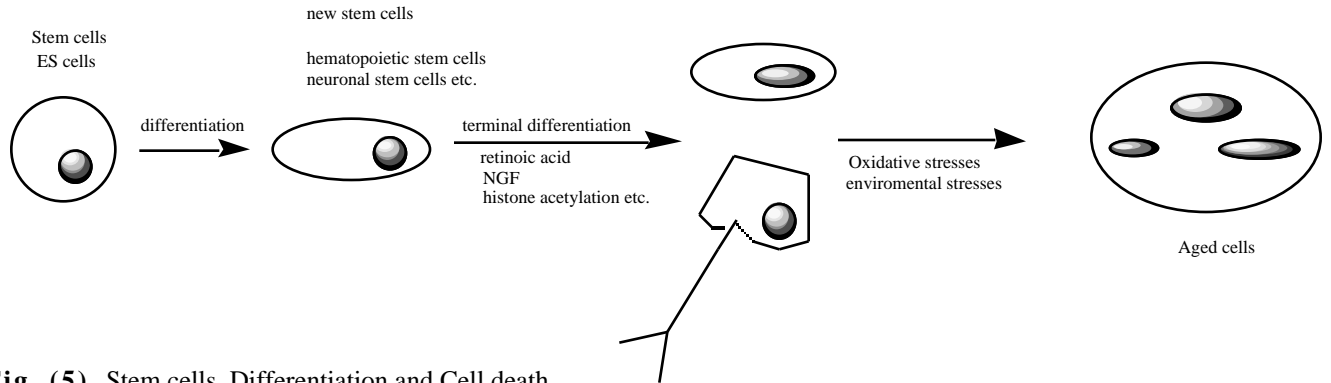


Fig. (5). Stem cells, Differentiation and Cell death.

This figure shows the differentiation of stem cells. Various kinds of tissue stem cells are derived from ES cells. In particular organ, tissue stem cells differentiate. Although terminally differentiated cells can not divide, they continue to alive longer time in vivo.

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NOTE

In *C. elegans*, *Italic means a strain of animal and regular means a gene such as daf-2*, DAF-2. In human premature syndrome *italic means a gene and regular means a protein.*

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