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Original article

A new hypothesis about the aging process of man

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Abstract

The process of aging, essentially a natural, complex, purposeful, multi-profile, and inevitable process in the life cycle of living beings, therefore also of humans, according to today's knowledge, is most likely based on a program located in the genome. As research shows, this process consists of two closely intertwined components, normal or physiological aging, and accelerated or pathological aging related to diseases. Life is essentially a conglomeration of more or less complex physiological and biochemical processes that are constantly taking place, and whose alteration or extinction leads to life's cessation and death. An inevitable phenomenon related to a large part of these processes is the generation of the so-called reactive oxygen metabolites (ROS, reactive oxygen species). These are molecular structures characterized by two fundamental properties, aggressiveness and destructiveness of molecular body structures, and signaling effects in the form of secondary messengers, which all have an impact on the mentioned processes. Among these, the oxidative destruction of exposed molecular structures, according to some of the latest findings, are particularly important for the process of programmed aging. Within the mentioned molecullar structures, there are two large transmembrane multiligand receptors, low-density lipoprotein receptor-related protein 1 (LRP1) and the receptor for advanced glycation end products (RAGE), as well as three enzymes deoxyribonucleic acid cytosine methyltransferases (DNMTs). Among these molecules, exposed to ROS, are also two Sp1 and Sp3 transcription factors (Sp1 and Sp3 proteins), otherwise abundantly present in tissues and cells. ROS compounds by the strong activation of ERK1 (p42) and ERK2 (p44) mitogen activated protein kinases, as well as c-Jun NH2 terminal kinases, condition the transcription and expression of these two mentioned factors (Sp1 and Sp3), resulting in their strong effect on DNMTs promoters with pronounced transcription and formation of DNMTs proteins. As components of crucial importance in the epigenetics systems, DNMTs proteins condition methylation processes of DNA molecules (adding methyl group -CH_3 to the molecules), generate the formation of 5-methyl cytosine (5mC) on template DNA chains, and decrease the transcription of methylated genes while shutting down their expression. Due to specific conditions related to LRP1 and RAGE promoters, these receptors have different reactions to DNMTs-induced methylation. LRP1 promoter methylation is fast and intense, and RAGE promoter methylation is extremely slowed down and reduced. The final effects of those two genes, or their receptors, are extremely different. Biochemical and physiological processes related to LRP1 gradually slow down and dampen, and processes related to RAGE become more and more expressive. Another group of processes related to epigenetics and programmed aging includes oxidative demethylation of 5mC DNA segments via ten-eleven translocases (TET), thymine DNA glycosylase (TDG) and base excision repair enzymes (BER). This second group of events is less efficient than the first group, and methylation obviously dominates. All these processes increasingly point to the crucial importance of the process of transcription of the genes shown earlier. Regenerating transcriptions under the strong control of Sp proteins, and their programs located in the genome, influence the maximum possible life span of individuals of a species. The aim of this study is to provide explanations of the role of the mentioned receptors in the programmed aging of living beings.

***KEY WORDS*:** aging process, epigenetics, methylation and demethylation, LRP1 and RAGE receptors

Introduction

At the begining of this study the authors present the research paper written by Tohgi H *et al.*[1], in which they show the importance of the bisulfite method, polymerase chain reaction (PCR), and direct sequencing of PCR products, in this case the cytosine, in the DNA chain. Their team, analyzing the methylation status of cytosines in the promoter region of RAGE (receptor for AGE; AGE, advanced glycation endproduct) in autopsy human cortex, have found that the total number of methylcytosines significantly decreased with age in cases of ≥ 70 years old in relation to < 70 years old [1]. This can increase the expression of RAGE with implications in aging. This finding confirms the increasingly powerful

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AGE/RAGE/cytosines network during aging. In their paper they exactly explain the bisulfite method, PCR, which is based on the chemical conversion of unmodified cytosine to uracil. Cytosine that has been modified, such as methylated, is protected from this chemical conversion and remains as cytosine (in which case it is detected as "methylation"). Therefore, by this method it is possible to perform DNA methylation analysis at a resolution of one base unit. By the bisulfite method the unmodified cytosine (unmethylated) is chemically converted to uracil, so it is not detected as "methylation". The methylation of C5 (bounding the methyl group, CH_3) of cytosine on DNA strand, which occurs physiologically, can be detected by the bisulfite method. The methylated cytosine includes methylated, dimethylated, trimethylated, and also modified cytosine such as alkylation and carbonylation. In conditions of severe glycation stress such as diabetes, cytosine reacts with aldehydes to form carbonyl cytosine.

Biological or physiological aging is a general and inevitable process in the living world, *i.e.* a natural-spontaneous progressive change in the life cycle of each individual, which ends with death. The main part of this process is conditioned by genetically programmed weakening and failure of the homeostasis maintenance system. Unlike living nature, inanimate nature also ages, but in a completely different way. This involves physical and chemical events, not biochemical (*e.g.* corrosion of iron and concrete, aging of car tires, aging of mortar on house walls, aging of the tarmac on the road and highway)[2-4].

Human aging is a complex process that encompasses biological, psychological and social changes that occur in the human organism over time. It is a natural part of the life cycle and can be observed at different levels, including cellular tissue, organic level, and the level of the whole organism[2-4]. As emphasized, the aging process is an inevitable process. Unlike the hypothetical instantaneous termination of life at an optimal age (the switch system!), death during aging and in old age has a whole range of evolutionary advantages. With the gradual and continuous weakening and shutdown of a number of vital functions (essentially physiological and biochemical processes), the individual becomes more and more susceptible to the risk of injury, disease and death over time. In this way, a better adaptation and survival of certain mutants to environmental conditions is possible[1-3].

The aging process, essentially a natural, complex, inevitable, expedient and multi-profile process in the life cycle of living beings, therefore also of humans, according to today's knowledge, most likely takes place on the basis of a program located in the genome. Looking at the human being, this does not diminish the importance of dividing that process into normal or physiological aging and accelerated or pathological aging related to diseases. This study has not the aim to count and analyze the recently actual aging theories (***Fig. 1***)[5-8].

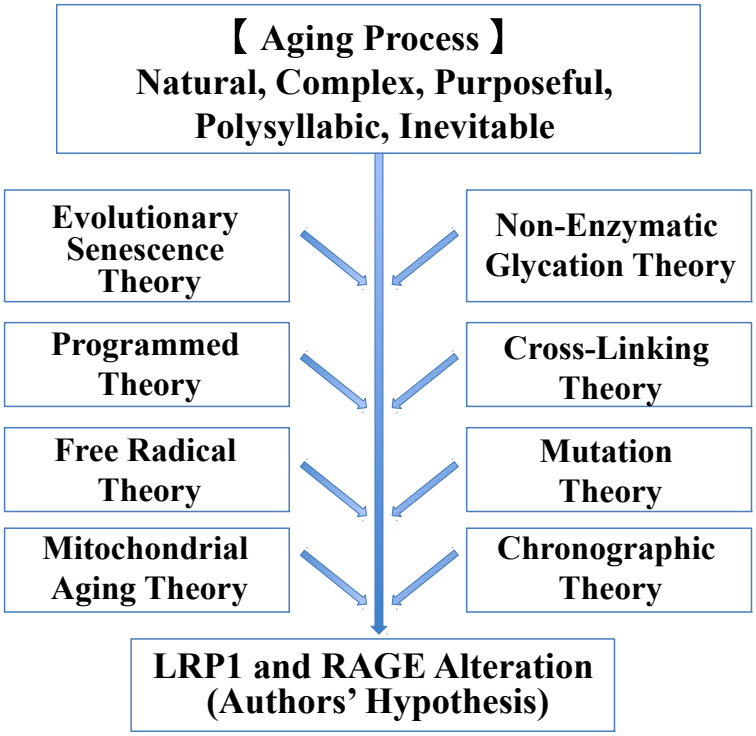

Fig. 1. **Actual theories of aging: Hypothesis.**

This schematic illustrates the principal contemporary theories of aging, emphasizing the complexity and interconnection of biological processes involved in senescence. The figure includes the evolutionary senescence theory, programmed theory, free radical and oxidative stress theories, mitochondrial aging, cross-linking of proteins and DNA, mutation theories, and the chronographic concept of time-dependent aging. In addition, it presents the authors' hypothesis concerning functional alterations of LRP1 and RAGE as potential integrative mechanisms linking metabolic, vascular, and neurodegenerative aspects of aging. Together, these theories demonstrate that aging is a natural yet multifactorial and genetically influenced process. LRP1, low-density lipoprotein receptor-related protein 1; RAGE, receptor for advanced glycation endproducts.

DNA-DSBs implications in the aging process

At the beginning of this presentation it is necessary to contemplate the role of DNA-DSBs (DNA-double strand breaks) in the aging process. DNA-DSB promptly induces the activation of the ATM kinase locally present near DNA strands (ATM kinase, Ataxia-teleangiectasis mutated kinase, a protein kinase that plays a crucial role in the cellular response to DNA damage, particularly DSBs), as well as ATM further local accumulation. The damage to DNA strands leads to rapid intermolecular autophosphorylation and transformation of ATM into monomers with their full kinase activity. The ATM signaling cascade occurs with further accumulation and phosphorylation of locally present nRAGE (nuclear rage) at Ser 376 and Ser 389. Phosphorylated and activated RAGE comes into contact with MRE11 (the enzyme, the double-strand break repair protein) the subunit of the MRN complex (MRN complex consists of MRE11, Rad50, and Nbs, and it has an important role in the initial processing of DNA-DSBs). MRE11 switches ATM kinase into ATR kinase with its signaling cascade and phosphorylation of RPA 2S4-S8 kinase and CHK15345 kinase. The ATM kinase or DNA Pkcs (c = catalytic, s = subunit enzyme that plays a crucial role in repairing DNA-DSB), induce the translation of cell surface factors Par3, MMP9, Ku, NR4A and TR4 to the site of DNA damage, where they participate in DNA-DSB repair. It is obvious that RAGE has a crucial role in the regulation of DSB repair (***Fig. 2***)[9-13].

In the above text it has already been emphasized that Sp1 and Sp3 proteins are abundantly present in tissues and cells. Local ROS (reactive oxygen species) lead to strong activation, transcription and expression of these two factors (Sp1 and Sp3). The result is their strong effect on DNMTs promoters with pronounced gene transcription and formation of DNMTs proteins (DNMT1, DNMT3A, DNMT3B)[14].

Wang J *et al.*[15] emphasize that Sp1 activity can be elevated by the fact that these stress factors (ROS) can activate the p42/p44 mitogen activated protein kinase pathway, and the c-Jun NH_2 terminal kinase-related signaling pathway, both of which may be responsible for the Sp1 transcriptor overactivation. This leads to the consequent expression of multiple Sp1 downstream genes (for example DNMTs genes).

There are several questions: 1) What molecules are oxidized by ROS? 2) What are the oxidation products as signals? 3) What is the mechanism for detecting the oxidation products? 4) By what mechanism are Sp1 and Sp2 activated? 5) Are aldehydes involved? These questions remain largely unanswered.

We put here answers to the questions:

1) What molecules are oxidized by ROS?

ROS oxidize practically all biological molecules, including DNA, proteins, lipids, and carbohydrates, leading to oxidative damage, impaired function, and disease when ROS levels are high. However, low levels of ROS also function as important signaling molecules, regulating the function of target proteins by oxidizing them. They modify key molecules in signaling pathways, thereby affecting cellular functions such as proliferation, differentiation, and apoptosis, as well as inducing gene transcription for cell protection. The molecules that induce Sp1/2 expression have not been identified. On the other hand, several important proteins bind to metals, and the loss of electrons from these metals (*e.g.*, Fe, Cu, Mn, Ni, and Se) regulates protein function. This mechanism allows superoxide dismutase (SOD) to reuse the protein, preventing it from being oxidized. Sulfur (S), which is a component of cysteine, glutathione, and lipoic acid, also plays an important role in electron transfer reactions[16-21].

2) What are the oxidation products as signals?

Nrf2 and FoxO are known to be the main transcription factors activated by ROS. Nrf2 is a primary transcription factor activated by ROS, playing a key role in the cellular stress response by initiating the production of antioxidant and cytoprotective proteins. While ROS directly activate Nrf2, they can also indirectly activate FoxO (Forkhead box O) transcription factors through pathways like SIRT1 activation. However, Nrf2 and Foxo proteins can also have complex, sometimes counteracting, interactions, with FoxO proteins sometimes attenuating Nrf2 activity by increasing the expression of Keap1, which normally inhibits Nrf2. Oxidation products are a type of waste product. Therefore, it is primarily scavenger receptors that recognize oxidative modifications as signals, and receptors that recognize AGEs include Galection-3, Scavenger Receptor Class AI & II, LOX1, Scavenger Receptor Class B CD36 & SR-BI, and FEEL-1 & 2. We speculate that oxidative modifications and scavenger receptors are not significantly involved in the induction of Sp1/2 expression. Oxidation products are new substances formed when a material undergoes an oxidation reaction, which typically involves gainingoxygen, losing hydrogen, or losing electrons. Common oxidation products include carbon dioxide, water, aldehydes, ketones, carboxylic acids, peroxides, and epoxides. The specific products depend on the starting material and the conditions of the reaction[22,23].

3) What is the mechanism for detecting the oxidation products?

The substances detected are electrophiles, not oxidation products. For example, Nrf2 and FoxO are activated in response to ROS and electrophiles, inducing the transcription of specific proteins through gene expression. Nrf2 activation is primarily mediated by post-translationally modified (oxidatively modified) electrophile sensors. The N-terminus of the Nrf2 protein is bound to the sensor Keap1 (Kelch-like ECH-associated protein 1)[24]. The requirements for a sensor of electrophiles (those with the chemical property of accepting electrons from other molecules) are: 1) the ability to sense electrophiles in the presence of several millimolar amounts of glutathione and glutathione S-transferase (GST); and 2) the ability to sense electrophiles with different chemical structures. A sensor that meets these requirements is expected to contain a cysteine with a low pKa (reactive cysteine) that is highly reactive with electrophiles. In SOD, a metal interacts with a cofactor that supplies electrons, resulting in the oxidation of the metal itself while leaving the protein itself unmodified. It acts as a kind of electrophile sensor for these transition metals[24].

4) By what mechanism are Sp1 and Sp3 activated?

Sp1 and Sp3 are activated through both changes in protein expression levels and post-translational modifications, such as phosphorylation, which can enhance their DNA-binding activity and transcriptional co-activation function.

function. The transcription factors and electrophile sensors responsible for Sp1 and Sp2 production still remain unknown.
5) Are aldehydes involved in Sp1 and Sp3 activation?

Yes, aldehydes are involved in both contexts, as some aldehydes, like malondialdehyde, can activate Sp1 and Sp3 transcription factors that bind to the promoter regions of certain genes. In particular, short-chain aldehydes are generally electrophilic substances. The carbonyl carbon atom of the aldehyde group has a δ+ (δ-positive) electron density due to the strong electron-withdrawing property of the oxygen atom, and therefore exhibits an electron-seeking property (electrophilicity). Further biochemical investigation is needed.

It is obvious that in the case of oxidative stress, the increased amount of ROS, along with the damage to receptors (LRP1 and RAGE) and to DNMTs proteins, increased level of ROS also act as a signaling pathway for the activation of Sp1 and Sp3 transcriptors. This is followed by the activation of LRP1, RAGE and DNMTs genes and their transcription. It is in fact a protective mechanism for creating new correct DNMTs proteins.

Before continuing the further analysis of the programmed aging theory of aging, it is necessary to explain the process that leads from ROS production to AGE production. ROS production can be induced from both internal and external sources. Externally, various environmental factors such as pollution, radiation, and certain drugs can induce ROS production. Internally ROS production can be induced by metabolic processes and different cellular stresses. One of the triggers for ROS production is Aβ binding to the RAGE receptor and subsequent activation of PKC and NADPH oxidase, resulting in ROS productions (O_2^*, H_2O_2, OH). It is an interesting question whether the effect of unmodified

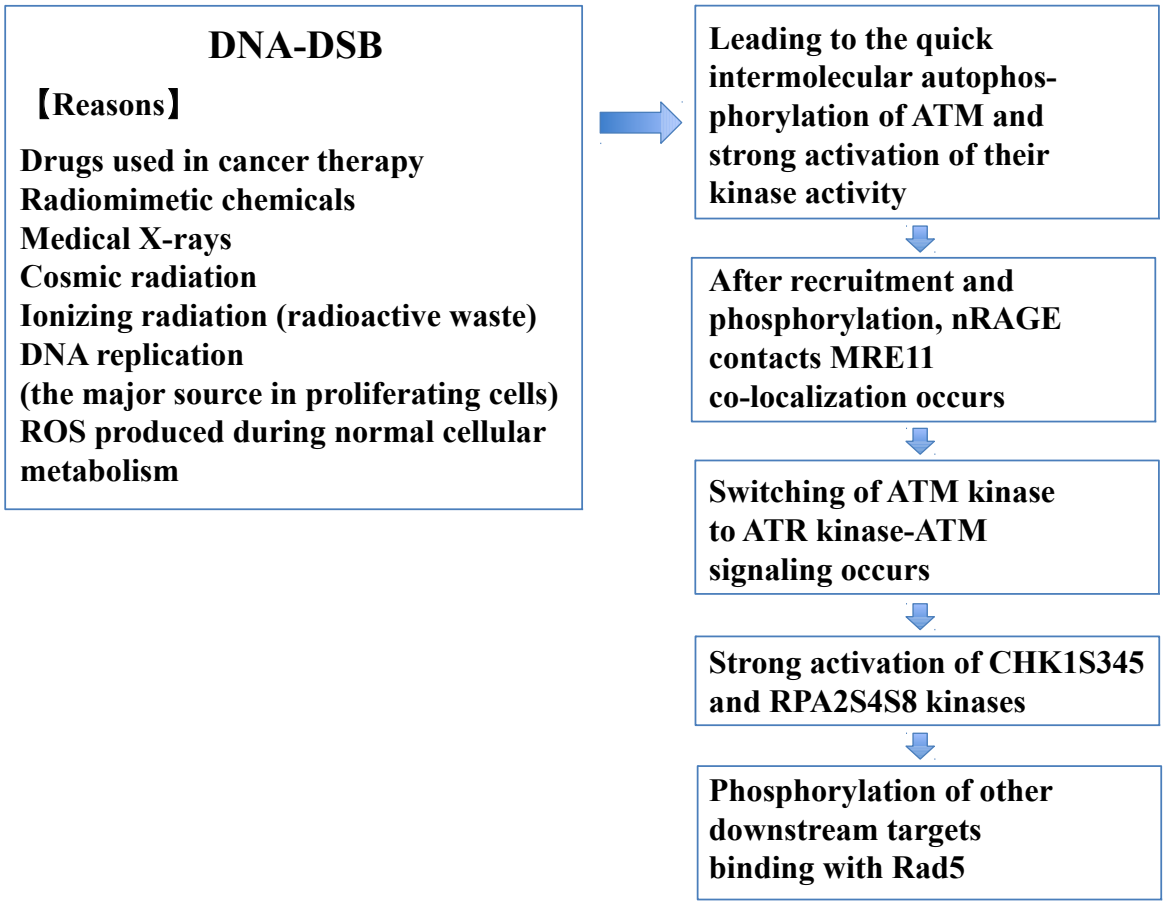

Fig. 2. **nRAGE has a central role in DNA-DSB repair.**

This figure illustrates the central role of nRAGE in the repair of DNA DSBs. DNA damage activates ATM kinase through autophosphorylation, converting dimers into active monomers. Activated ATM phosphorylates nRAGE at Ser376 and Ser389, recruiting it to the DSB site. Phosphorylated nRAGE interacts with MRE11, a component of the MRN complex, which mediates the transition from ATM to ATR kinase signaling. This leads to phosphorylation of CHK1S345 and RPA2S4S8 kinases, initiating downstream repair mechanisms. Thus, nRAGE acts as a crucial regulator connecting DNA damage recognition to effective DSB repair. nRAGE, nuclear receptor for advanced glycation endproducts; DSB, double strand break; ATM kinase, ataxia-teleangiectasis mutated kinase; ATR kinase, taxia-telangiectasia mutated (ATM) and Rad3-related kinase; MRN complex, protein complex consisting of Mre11, Rad50 and Nbs1.

Aβ or glycated Aβ is greater.The other important way for ROS production is located in the mitochondrial electronic transport chain, whose damage leads to the electrons escape, molecular oxygen reduction and O_2^{*-} (superoxide radical). ROS induces a strong oxidative cleavage of the glycation produced Amadori product, with the generation of 3-deoxyglucosone (3DG), glyoxal (GO) and methylglyoxal (MGO). The next step is the formation of AGEs, (CML, N^{ε}-(carboxymethyl)-lysine; CEL, N^{ε}-(carboxyethyl)-lysine; GOLD, glyoxal-lysine dimer; and MOLD, methyl-glyoxal-lysine dimer)[25,26].

AGE/RAGE/cytokines functional network

Before the analysis of the methylation and demethylation processes, it is necessary to explain the methylation evaluation method (bisulfite method). This method is used to detect methylation by chemically modifying cytosine bases. By this technique, unmethylated cytosines are converted into uracil, and methylated cytosines remain unchanged. This difference in conversion allows researchers to distinguish between methylated and unmethylated cytosines (base)[4].

Programmed aging, according to the authors of this study, is based on the assumption of the effects and control of two large transmembrane receptors, LRP1 (gene position 12q13.3) and RAGE (gene position 6p21.32), three DNMTs proteins (DNMT3A gene position 2p23.3, DNMT1 gene position 19p13.2, DNMT3B gene position 20q11.21) and two Sp proteins (Sp1, specificity protein 1, gene position 12q13.13, Sp3, specificity protein 3, gene position 2q31.1). The programmed aging becomes clearer after the involvement of epigenetics and ROS (O_2^{*-} superoxide radical, H_2O_2 hydrogen peroxide, *OH hydroxyl radical) in this process[9-13]. Of increasing interest to researchers is the role of epigenetics in programmed aging, especially the role of the methylation and demethylation processes of the gene promoters mentioned above. As stated in the abstract, and based on the original hypothesis of the authors of this study, the low-density lipoprotein receptor-related protein 1 (LRP1) promoter (gene) is not efficiently protected from deoxyribonucleic acid cytosine methyltransferases (DNMTs) induced methylation by means of AGE/RAGE/cytokine (TNF-α, IL-6, IL-1β) molecular network. Large molecules of DNMTs (the largest enzymes in humans, 1,620 amino acids) easily approach the LRP1 promoter and perform intense methylation. In the case of the RAGE promoter, this approach is extremely difficult due to the comprehensive network of AGE/RAGE/cytokines, and methylation is weak. This is additionally contributed by the increasing attraction of AGEs to RAGE (active form-the furin action in endplasmic reticulum [ER] is not necessary) during the life cycle, and their increasing accumulation around the RAGE promoter, especially in old age. Between AGEs and RAGE (active form) there is normally a strong mutual attraction and binding, which is not the case with LRP1. In addition, AGEs lead to a strong expression of RAGE through a positive feedback mechanism. As a result, the expression of LRP1 decreases with age, and the expression of RAGE increases. Vital processes related to the expression of LRP1 protein obtained by mRNA LRP1 translation become active by the furin action in ER. LRP1 genes become more and more mutated over time, and processes related to RAGE become more expressive. LRP1-induced processes are essential for vitality and longevity, and RAGE-related processes are pro-atherogenic in nature, blocking vitality and the possibility of longevity (***Fig. 3, 4***)[27-29].

Understanding the formation of protective anti-methylation molecular networks AGE/RAGE/cytokines around RAGE and LRP1 promoters (genes), requires an explanation of the role of RAGE at the sites of deoxyribonucleic acid-double strand breaks (DNA-double strand breaks), and especially the causes that lead to RAGE accumulation around these sites. Double strand breaks are marked at one point of continuity of both DNA strands. The cause of this phenomenon can be ionizing radiation, as well as when replication forks encounter a DNA lesion or repair intermediates. When a single strand break occurs, the undamaged strand can be used as a template to correct the damage. All living organisms are inevitably susceptible to both types of DNA strand damage. It is estimated that 10-50 DNA-DSBs occur per day per cell. The accumulation of such unrepaired damage quickly leads to cell death (***Fig. 3, 4***)[27-29].

It is obvious that in the case of oxidative stress, the increased amount of ROS, along with the damage to receptors (LRP1 and RAGE) and to DNMTs proteins, also acts as a signaling pathway for the activation of Sp1 and Sp3 transcriptors (***Fig. 5***). This is followed by the activation of LRP1, RAGE and DNMTs genes and their transcription. This is in fact a protective mechanism for creating new correct DNMTs proteins.

Most probably, all modifications of the LRP1 promoter (gene) induced by the AGE/RAGE/cytokines (TNF-α, IL-6, IL-1β) molecular network purely CH_3 modifications, are mediated by the enzymes DNMTs. The authors of the presented study have not found any evidence in the available literature that modifications of the LRP1 promoter (gene) are mediated by the enzymes DNMTs. However, it is possible that the non-enzymatic modifications have been occuring directly through oxidation or glycation. Both modifications can be judged as "methylation" by the bisulfite method, but the authors of this study consider that the primary modification (methylation) of 5th cytosine position is most probably induced by DNMTs.

It is possible that all purely CH_3 modifications of the LRP1 promoter (gene) by the AGE/RAGE/cytokines (TNF-α, IL-6, IL-1β) are not exclusively induced by the enzymes DNMTs. There is a possibility that non-enzymatic modifications occur directly through oxidation or glycation. Both modifications are judged as "methylation" by the bisulfite method. Our opinion is that when epigenetics and ROS (O_2^{*-}, H_2O_2, *OH) are involved in this process, the programmed aging becomes more evident. Formaldehyde generation associated with demethylation is enhanced by oxidative stress (excessive ROS), resulting in increased protein carbonylation and AGE production. It has also been suggested to be involved in the exacerbation of dementia. In the available literature there are no necessary data for the explanation of the above mentioned, but according to the authors of this study, the accumulation and aggregation of AGE/RAGE/cytokines are the key molecules which interfere with the approach of DNMTs[30].

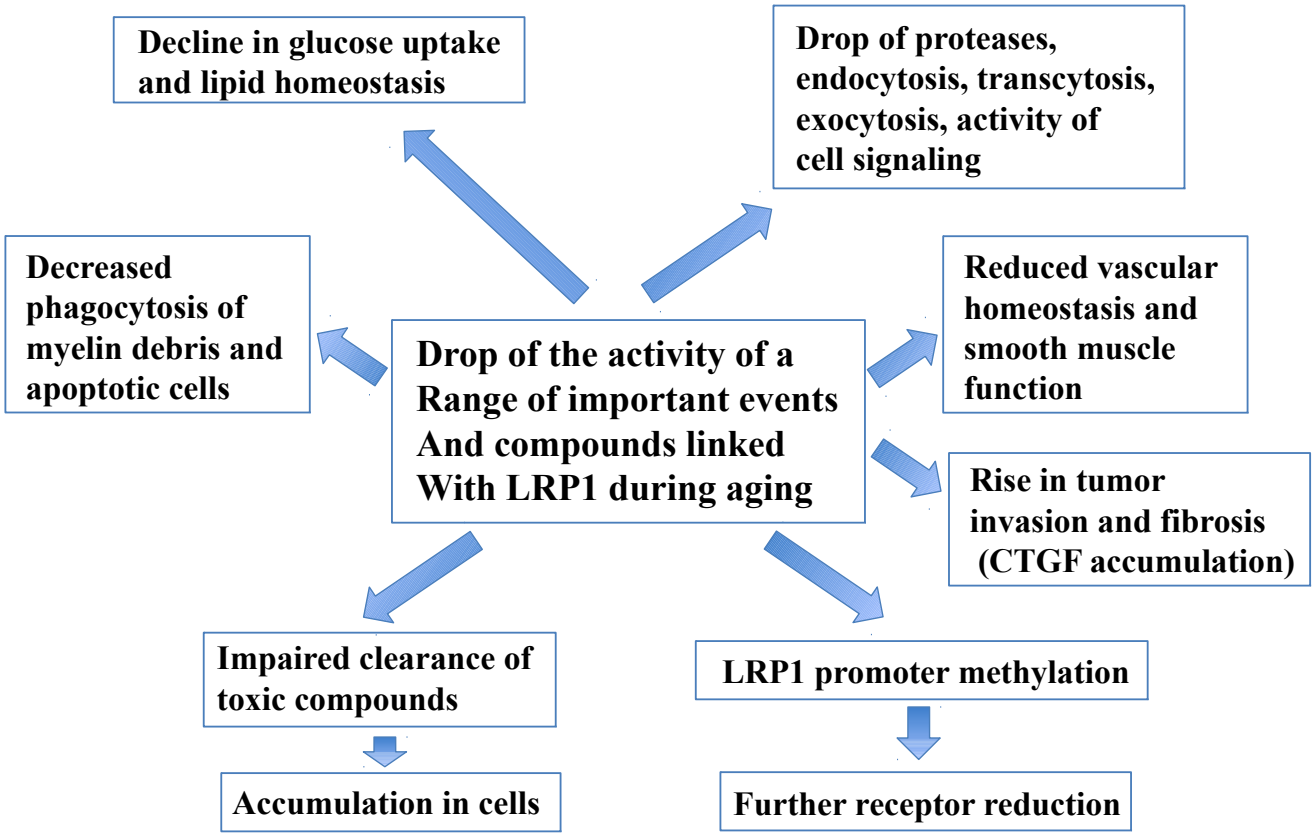

Fig. 3. **Drop of the activity of LRP1 receptor (gene) linked with ageing.**

This figure illustrates the decline of LRP1 activity associated with aging. The reduction of LRP1 function leads to decreased protease degradation and lysosomal enzyme activity, impaired endocytosis, transcytosis, and exocytosis, as well as diminished phagocytosis of myelin debris and apoptotic cells. Consequently, toxic compounds accumulate within cells, promoting inflammation, metabolic imbalance, and vascular dysfunction. LRP1 deficiency in neurons and vascular smooth muscle cells disrupts lipid and glucose homeostasis, weakens vascular wall integrity, and enhances fibrosis. These cumulative effects highlight the crucial role of LRP1 in maintaining metabolic and cellular homeostasis during aging LRP1. LRP1, low-density lipoprotein receptor-related protein 1; CTGF, connective tissue growth factor.

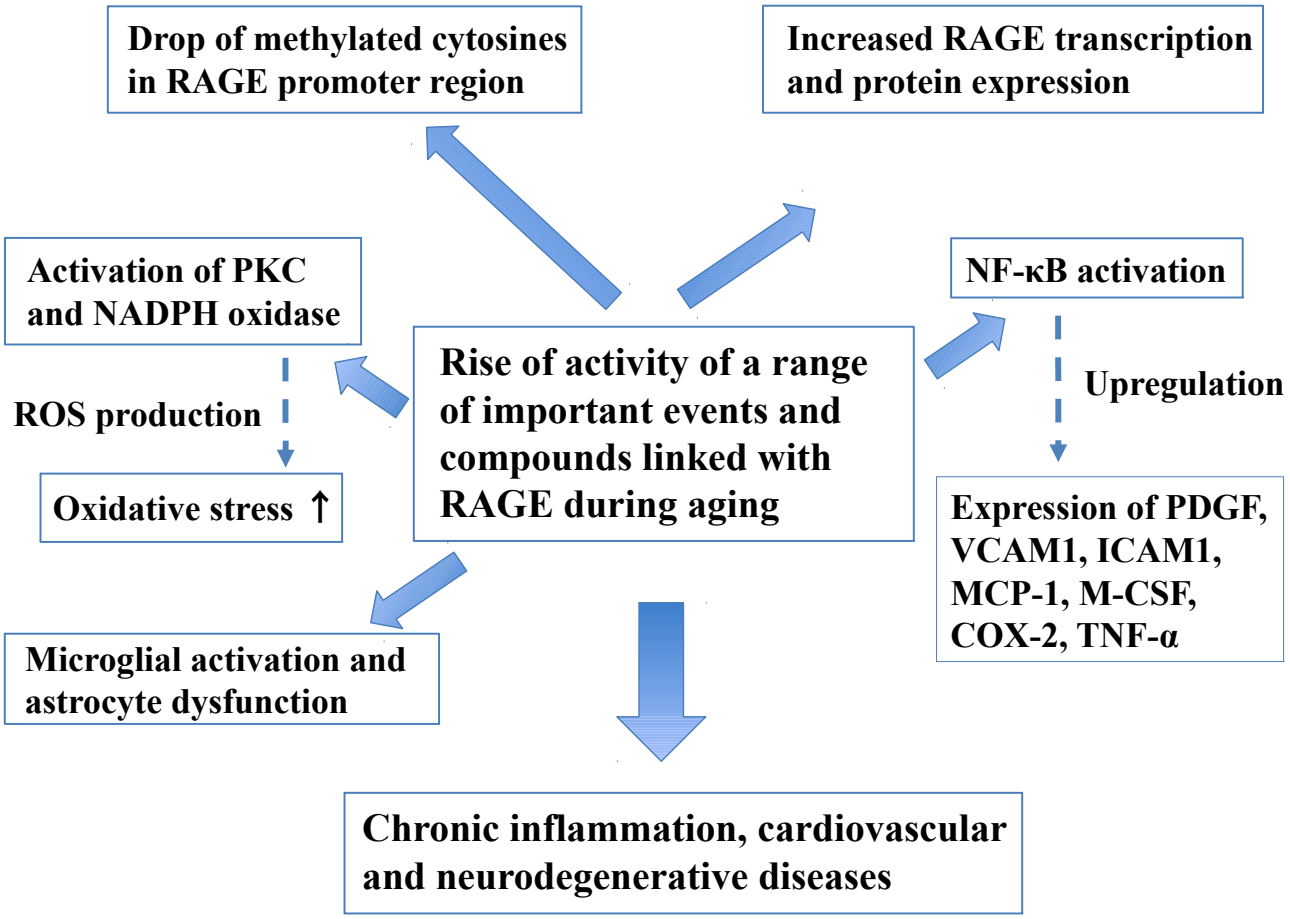

Fig. 4. **Moving of the values of a range of important parameters linked with RAGE during aging and age.** This figure illustrates age-related changes in parameters associated with RAGE. With aging, methylated cytosines in the RAGE promoter region decrease, enhancing RAGE gene expression. The resulting upregulation activates protein kinase C and NADPH oxidase, increasing ROS accumulation and oxidative stress. NF-κB signaling intensifies, inducing pro-inflammatory cytokines such as PDGF, VCAM1, ICAM1, MCP-1, and TNF-α. These molecular alterations contribute to atherogenesis, microglial activation, and astrocyte dysfunction, leading to chronic inflammation and vascular and neurodegenerative diseases, including Alzheimer's disease and diabetes mellitus. RAGE, receptor for advanced glycation endproducts; PKC, protein kinase C; NADPH, nicotinamide adenine dinucleotide phosphate; ROS, reactive oxtgen species; NF-κB, nuclear factor kappa B; PDGF, platelet-derived growth factor; VCAM1, vascular cell adhesion molecule 1; ICAM1, intercellular adhesion molecule 1; MCP-1, monocyte chemotactic protein-1; M-CSF, macrophage colony-stimulating factor; COX-2, cyclooxygenase-2; TNF-α, tumor necrosis factor-alpha.

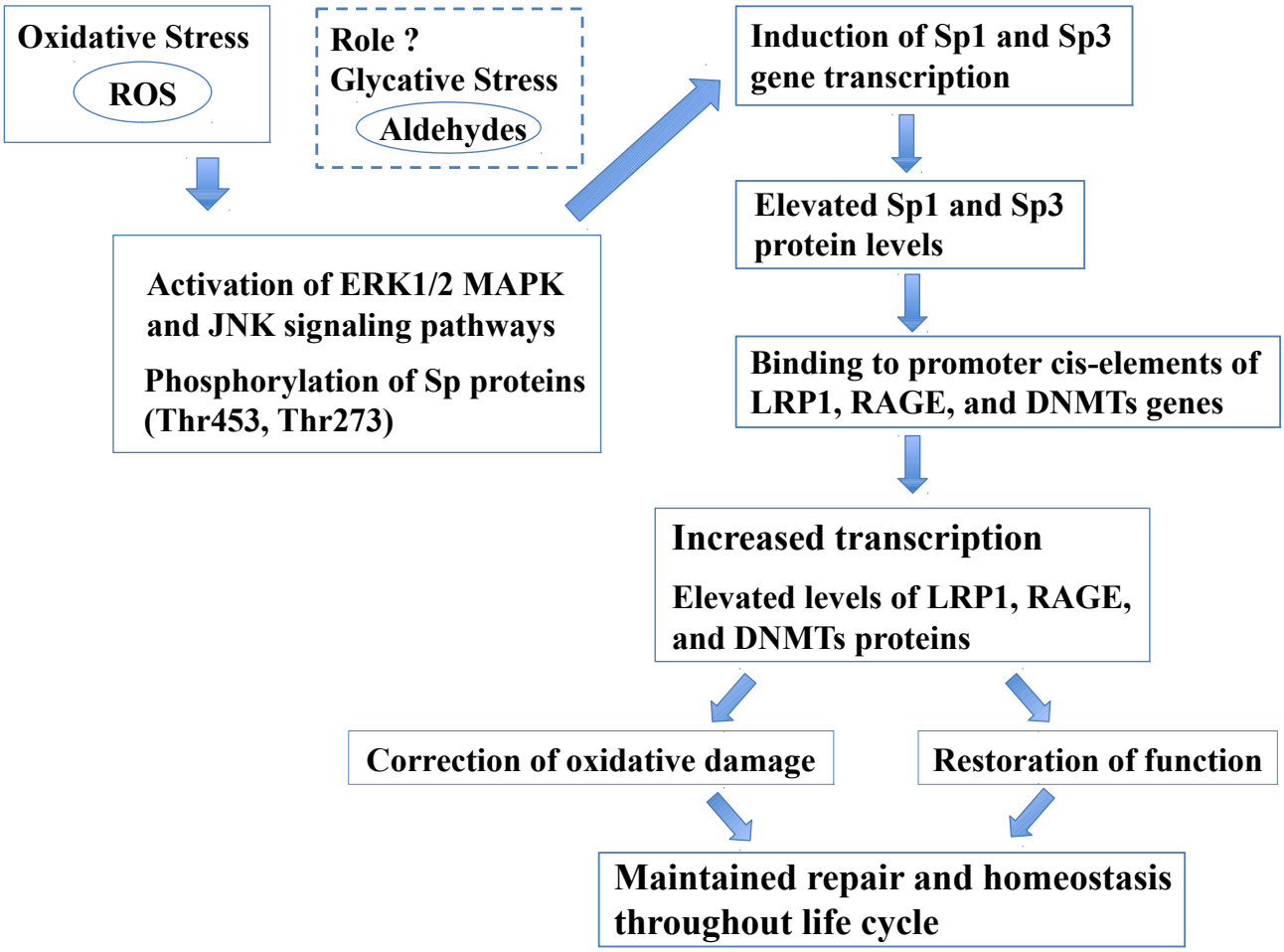

Fig. 5. **Protective role of Sp1 and Sp3.**

This figure demonstrates the protective role of transcription factors Sp1 and Sp3 under oxidative stress conditions. Elevated ROS activate ERK1/2 and JNK signaling pathways, leading to phosphorylation and activation of Sp1 and Sp3. These factors bind to the promoter regions of LRP1, RAGE, and DNMTs genes, enhancing their transcription and restoring the levels of previously damaged proteins. The resulting increase in LRP1, RAGE, and DNMTs proteins contributes to cellular repair, redox homeostasis, and resistance to oxidative injury. Thus, Sp1 and Sp3 serve as key transcriptional regulators maintaining cellular stability throughout the life cycle. The involvement of glycative stress is unknown. Sp1 and Sp3, specificity protein 1 and 3; ROS, reactive uxygen species; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; JNK, c-Jun N-terminal kinase; RAGE, receptor for advanced glycation endproducts; LRP1, low-density lipoprotein receptor-related protein 1; DNMTs, deoxyribonucleic acid cytosine methyltransferases.

Nuclear RAGE has a complex pathway

Most of the created (translated) nRAGEs in the ER return to the nucleus through the pores (nuclear pores through which RAGE mRNA entered the ER, diameter of approximately 120 nm) closest to the site of transcription, *i.e.* to the promoter (gene) RAGE. Only a small part of the generated RAGE in the ER goes via narrow ER tubes (Ø 30-100 nm) to other further pores that are close to the LRP1 promoter (gene). There are RAGE pores and LRP1 pores. Through the pores for RAGE, the turnover of RAGE is higher, and through the pores for LRP1, the turnover of RAGE is lower. This is probably why DNA-DSB repair in RAGE DNA is more powerful. Transcription is more efficient, and the safety net is just as strong. RAGE mRNA translation takes place in the ER as close as possible to the nuclear pores and nucleus (from where they exited the nucleus). That is why even when RAGE returns to the nucleus through these pores, through which they entered the ER mRNARAGE, RAGE turnover is extremely strong. RAGE entry pores (average size of soluble RAGE 12x7x4 nm) towards the LRP1 promoter are further away, and much less RAGE passes through them, so RAGE turnover is weak. With a high turnover, RAGE binds to AGE more strongly, and with a low turnover, the binding of RAGE to AGE is much weaker. This could also explain the difference in protective networks between RAGE (strong dense network-weak methylation) and LRP1 (weak sparse network-strong methylation). The difference exists in the arrival of RAGE to the RAGE promoter and RAGE to the LRP1 promoter. Part of RAGE passes through the rough endoplasmic reticulum, enters the smooth endoplasmic reticulum, and after passing through it enters the Golgi apparatus, and from there, via secretory vesicles, RAGE goes to a series of cell membranes. Part of RAGE that has returned from the ER to the nucleus can also reach the LRP1 promoter through the nucleus. It is important to emphasize that AGE compounds permanently enter into the nucleus or are generated there (***Fig. 6***)[9-21].

The analysis of the above mentioned text clearly indicates that nRAGE (nuclear RAGEs) has two main functions. One function is involved in the repair of permanently created DNA-DSBs, and the other in the formation of the AGE/RAGE/cytokines molecular network and in preventing the access of the methylation factor DNMT to promoter bases and both DNA strands (coding and template strand) during transcription. Xing P *et al.*[32] declare that the promoter region of the LRP1 gene is enriched with CpG islands and sites that govern the sensitivity of the LRP1 gene to DNA methylation. They have found that, when CPG islands are completely methylated, the transcriptional activity disappears and the expression of the LRP1 gene is silenced.

Silverberg GD *et al.*[33], in experiments with mice, have found a decreased methylation rate of the RAGE gene promoter region during the process of aging. This leads to a strong RAGE expression, PKC and NADPH oxidase activation, as well as to a significant increase of reactive oxygen metabolites (O_2^{*-}, H_2O_2, $^*OH$). The ROS elevation leads to oxidative stress and NF-κB cascade. The increased values of Aβ, AGEs, TNFα, and especially NF-κB prevent promoter methylation.

The LRP1 gene promoter is enriched with CpG islands (regions where CG sequences are concentrated) and sites that induce the sensitivity of the LRP1 gene to DNA methylation. Related to the DNA methylation level rise, the LRP1 transcriptional activity decreases, and when CpG sites are completely methylated, its transcriptional activity completely disappears and LRP1 expression ceases. As opposed to the LRP1 gene, in the process of aging and AD, there is a significant decline of DNA methylation in the promoter of the gene for the RAGE receptor. During the aging process and in AD, around this promoter there is an intensified accumulation of AGEs, cytokines and nuclear factor kB, all of which can decrease the promoter DNA methylation (blockade of the DNMT DNA methyltransferase approach to the RAGE promoter), and increase RAGE gene expression[34-35].

The binding of AGEs and RAGE requires a strong interaction between specific structural regions of AGEs and extracellular regions of RAGE. During this interaction, certain amino acids on the RAGE molecule are of particular importance. The amino acids Lys-46 and Arg-77 of RAGE form stable complexes with the corresponding regions of AGEs through hydrogen bonds and ionic interactions. Both types of chemical bonds provide a strong interaction between the two compounds[36].

By experiments with human liver cell lines, Wu X *et al.*[37] have revealed that AGE can have an impact on RAGE expression by carboxymethyl-lysine (CML) and carboxyethyl-lysine (CEL). Experiments indicate that these AGEs can promote the demethylation of RAGE promoter region. The performers of the experiments have concluded that TET1 compounds, upregulated in AGE-treated cells, may epigenetically modulate RAGE, so, AGEs can upregulate the TET1 enzyme, which could lead to increased RAGE expression. They also emphasize that AGE-RAGE activated signaling induces pathological processes such as inflammation, cell apoptosis, oxidative stress, insulin resistance, and pancreatic β-cell damage.

However, it is important to remember that formaldehyde is generated from the methyl groups releaseed by demethylation. Formaldehyde is generated almost directly by the demethylation of amino acids and N-, S-, and O-methyl compounds, and is found in the body at concentrations of 100–400 μM even in healthy individuals. This highly toxic aldehyde may be involved indegenerative tissue changes by randomly attacking surrounding proteins.

The analysis of the text above gives a detailed view of the AGE/RAGE/cytokines functional network around the RAGE promoter. This molecular network directly regulates RAGE expression, and is an important factor in the methylation intensity of the RAGE promoter. Otherwise, it is extremely compact. It should be pointed out that cytokines are an important component of this network, strongly influencing its signalling, the state of inflammation and existing diseases. The existence of this network strongly supports the hypothesis of the role of RAGE and LRP1 genes, or their receptors, in programmed human aging[4,33,35]. The negatively charged AGEs bind very successfully with positively charged V domain of RAGE (Lys52, Lys110, Arg 98). RAGE V domain also binds to some cytokines, which is important for the formation of the AGE/RAGE/cytokines network[4,33].

The presented study is not intended to present the latest results of the specific blockade of the RAGE receptor increased expression in old age, nor the targeted gene therapy

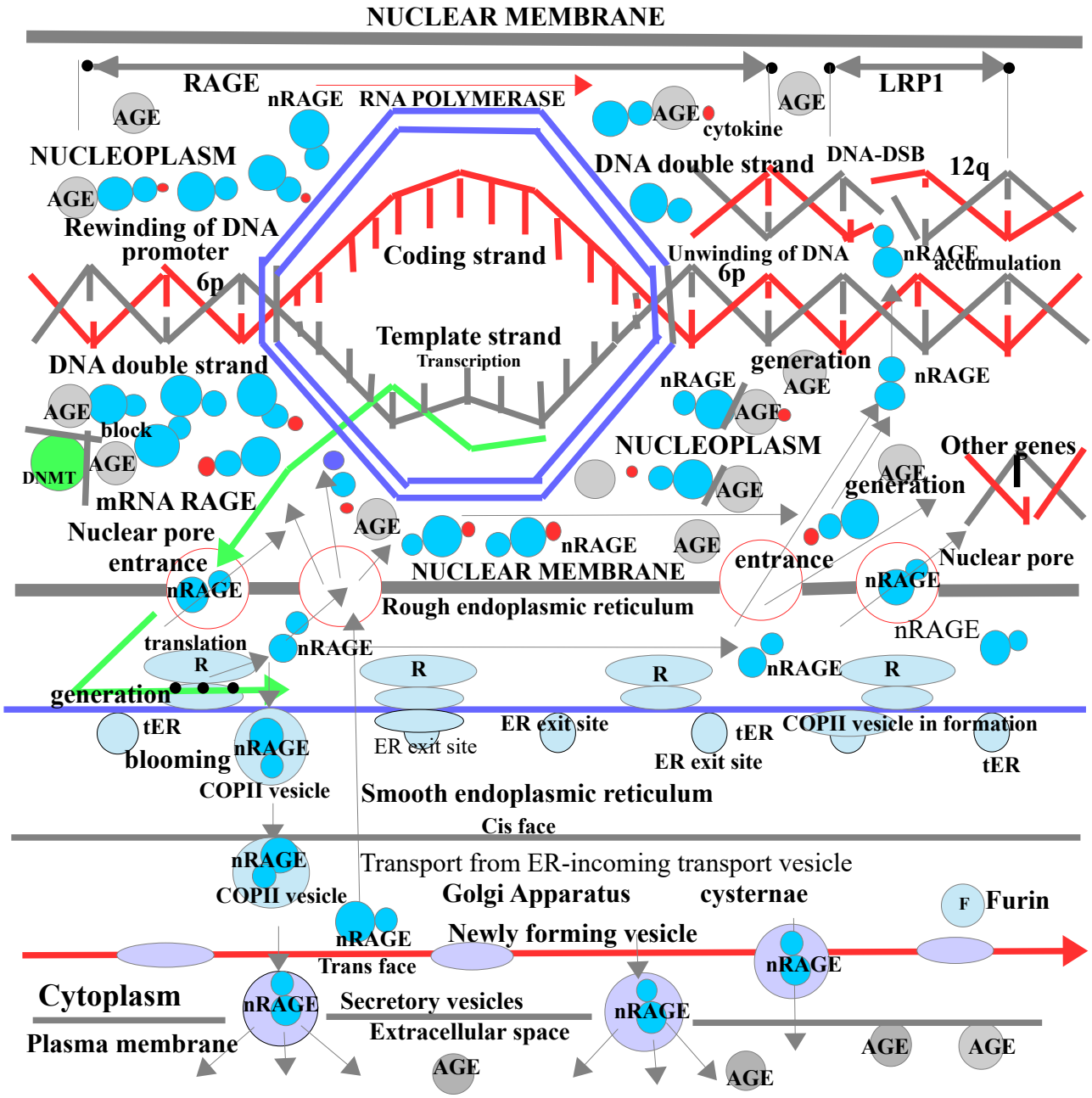

Fig. 6. **Schematic presentation of RAGE transcription.**

On the upper part of the figure, between two nuclear membranes (thick black lines), there is the nucleoplasm. Two thin black lines, bordered with black points and arrows, present the RAGE gene region (longer line) and the LRP1 gene (shorter line). The bigger white region, bordered with double blue lines, represents the RNA polymerase, which moves along the double strand DNA in the red arrow direction. Two especially important points are the rewinding of DNA and the unwinding of DNA. The DNA molecule is presented by the helical structure composed of short black (template strand) and red (coding strand) lines. The green broken arrow (mRNA RAGE), which begins under the transcription (template strand), passes through the nuclear pore in the nuclear membrane, enters the rough endoplasmic reticulum, and on the ribosomes is translated into nRAGE (nuclear RAGE). The small and big blue connected circles present the nRAGEs. On the inner nuclear membrane are visible four nuclear pores. Through these nuclear pores nRAGE returns back to the nucleus towards the RAGE promoter. By other, farther, pores, nRAGE returns back towards the LRP1 promoter region. The first mentioned nRAGE is included in the formation of the anti-methylation AGE/RAGE/cytokines network around the RAGE gene promoter, and the second mentioned nRAGE is included in the DNA-DSB repair in the LRP1 promoter. One part of the nRAGE, by COPII vesicles, passes across the smooth endoplasmic reticulum into the Golgi apparatus, and then by secretory vesicles enters the cytoplasm and extracellular space. nRAGEs, nuclear receptor for advanced glycation endproducts; LRP1, low-density lipoprotein receptor-related protein 1; COPII vesicles, A membrane structure responsible for transporting proteins from the endoplasmic reticulum to the Golgi apparatus; p = symbol for the short arm of the chromosome; q = symbol for the long arm of the chromosome; DNA-DSB, DNA-double strand break.

of the RP1 receptor muted expression, also in old age. Today, this is still in the experimental phase (experiments on animals), which calls for additional, narrowly focused studies on this issue. The detailed analysis of both, the attempts to slow down the aging process in humans by the FPS-ZM1 RAGE receptor blocker, and by LRP1 gene therapy, was presented in *Ref.4* and *Ref.24* [38].

Some additional considerations

Sp1 (Specificity Protein 1) is a zinc finger transcription factor that regulates gene expression by binding to GC-rich DNA sequences. It is involved in many fundamental cellular processes, including cell growth, differentiation, apoptosis, and immune responses. Sp1's activity can be modulated by post-translational modifications and it is linked to various diseases, particularly cancer.

Sp3 is a transcription factor that is a member of the Sp1-related family and can both activate and repress gene transcription. Its function is context-dependent, meaning its activity as an activator or repressor depends on the specific gene promoter and cellular environment. Sp3 plays a crucial role in various cellular processes by binding to GC and GT-box DNA elements.

Sp1 and Sp2 are members of the Sp protein family, which are transcription factors that regulate gene expression in cells. While Sp1 is ubiquitously expressed and essential for early development, Sp2 is also widely expressed but its function is distinct and can be context-dependent, with a mechanism that can involve recruiting other proteins to gene promoters independent of its own DNA-binding ability.

The transcriptor factors Sp1 and Sp3 induce the transcription of a number of genes, among them especially the transcription of LRP1, RAGE and DNMTs. Moreover, by binding with GC-box and GT-box in their own promoters, Sp1 and Sp3 transcription factors induce the transcription of their proper genes.

If the rate of the RAGE gene promoter region methylation decreases, at the same time RAGE expression increases. Enhanced RAGE expression induces the activation of PKC and NADPH oxidase, and these events are shown in this paper.

Enhanced oxidative stress induces the increase of NF-κB cascade and the generation of TNF-α. This is clearly presented in this paper. Increased NF-κB (NF-κB cascade) leads to elevated levels of cytokines, which support the phenomenon of AGE/RAGE/cytokines protective network, especially around LRP1 and RAGE promoters (genes).

Mechanism of ROS involvement

Various events involving ROS and AGEs have been reported *in vivo*. Introducing aldehydes into these events may provide a more detailed and clearer explanation. The carbonylation reaction between aldehydes and proteins can be broadly considered an oxidation reaction in terms of electron transfer. When aldehydes are oxidized to produce carboxylic acids, this is a reduction reaction mediated by the aldehyde. However, *in vivo*, glycative stress (excess aldehydes) is defended against by ALDH, GAPDH, and glyoxalase, in contrast to antioxidant enzymes (SOD, catalase, peroxidase), indicating that oxidation and glycation should be considered as completely independent phenomena. Regarding oxidative stress-related events occurring in the body, rather than simply describing them as involving ROS, it is important to clarify their mechanisms by focusing on the involvement of fatty acid-derived aldehydes generated by ROS. We would like to emphasize that research in this area needs to become more active in the future.

Conclusion

Aging is a natural, complex, multifaceted, inevitable and irreversible process. Its cause is still unknown. Aging essentially consists of two mutually closely related components: physiological or normal aging, and accelerated or pathological aging related to diseases. Physiological aging, according to a series of indications, occurs due to a certain program in the genome. Several prominent proteins play a crucial role in this process: LRP1, RAGE, three DNMTs and two Sp. Due to the different methylation conditions of their promoters, LRP1 is methylated faster and more strongly, while RAGE is methylated more slowly and weakly. This causes a faster weakening of LRP1 expression, as well as an entire complex of physiological and biochemical events related to this receptor during life, and especially in the periods of aging and old age. Conversely, RAGE expression increases during aging, and thus a series of pro-atherogenic events accelerates, all of which results in the acceleration of programmed aging.

Conflict of interest declaration

None in particular.

Competitive funding

None in particular.

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