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RESEARCH ARTICLE

The RNA Genome Sequencing Demonstrates Increased Production of Metabolic Genes in Late Ages in Highly Differentiated Tissues

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ABSTRACT
In this paper, we continue statistical analysis of RNA-Seq results of the whole genome of *Mus musculus* during their lifetime. We propose that the implementation of the developmental program by cells and their transition to the active performance of functions is the main mechanism of aging. The data obtained confirm the basis of our ideas that the triggering of aging processes is embedded in the very "design" of multicellulars. Previously, we noted a gradual decrease in RNA production in the part of the genome responsible for cellular infrastructure. At the same time, we noted a rise in the level of production of genes of this part in late ages. We hypothesized that this is associated with the increased demand of cells for energy production to maintain the weakening functions of the organism. We identified a block of 24 most productive genes responsible for metabolic activity and energy production in the cell. As shown by data analysis, it was these genes that appeared to be responsible for the rise in the overall activity of infrastructural genes in the late period. We also hypothesized that the rise in demand for cellular energy structures in the aging organism is most pronounced in highly differentiated tissues. For this purpose, we distinguished two groups of tissues, according to the level of their mitotic index. The results show that the rise in the production of the infrastructural part of the genome found in late ages is due to RNA synthesis of metabolic genes and is expressed only in the group of tissues with low mitotic index. We plan to further investigate the age-related dynamics of the proteome, comparing our results with other databases to identify similar patterns of RNA production dynamics in them.

Keywords: aging, RNA-Seq data analysis, metabolic activity, mitotic activity.

Introduction

One of the main questions in modern biology remains the problem of explaining the work of aging mechanisms and their connection with the processes of development and formation of multicellular organisms. Most scientists dealing with this problem agree in the opinion that aging is either the result of a purposeful evolutionary program inherent in the genome[1 - 6], or a consequence of spontaneous disturbances in the organism leading to maladaptation[7, 8]. At the same time both approaches to the causes of aging are not yet presented by the description of a specific mechanism explaining the emergence and acceleration of aging after reaching fertility[9, 10]. We explain aging by the gradual redistribution of limited resources between two main tasks of the organism: self-sufficiency based on the work of a group of genes responsible for the infrastructural support of cells, or housekeeping genes (HG), and functional differentiation provided by the integrative group of genes (IntG) [11,12]. For us, the fundamental approach is that we do not consider the role of individual genes in aging mechanisms, but rather their ontogenetically determined and different functional parts in their role in the organism. We are based on the assertion that an insufficient level of repair is the main cause of aging. In the case of a multicellular organism, its aging is also caused by a decrease in resources required by its cells for their repair and tissue regeneration and starts at the moment when its recovery begins to be incomplete[13]. Understanding exactly how this deficit arises is our primary concern. It is at this point that the whole cascade of aging processes is triggered and a situation arises in which aging becomes a side effect of the organism's development program. The connection between ontogenesis and aging is obvious to us, in which aging itself is a by-product of the ontogenesis program[14].

In the works devoted to age-related changes in the transcriptome, a smooth decrease in its production during life was found. At the same time, multidirectional changes in the level of RNA production were found for separate groups of genes[15, 16,17]. Summarizing the currently available data on age-associated decrease in gene expression, it can be stated that it corresponds to a progressive decrease in cellular functions both in separate tissues and in the whole organism. Obviously, the study of the causes and mechanisms that determine the aging transcriptome is necessary to understand the underlying mechanisms of aging[18, 19,20]. Thus, although the available data have not yet shown striking results, it is obvious that this line of research should be developed.

Analyzing the results obtained in the previous paper[21] we noticed a tendency to some increase in RNA production of HG genes after 21 months of age. This result somewhat contradicts our aging model and we assumed that an explanation can be found for this fact, which is the purpose of this paper.

To achieve our goal, we need to further analyze the RNA production database of the Mus musculus genome used by us in order to answer the following questions: In which tissues, in terms of mitotic activity, this elevation of HG gene production is more pronounced; Which of the genes of the HG part of the genome may be responsible for the rise in activity in late ages.

Materials and Methods

To analyze the results of RNA sequencing of *Mus musculus* genome production, we used the same material as in our previous article[21]. The RNA-Seq data on the *Mus musculus* mouse genome transcriptome is available in the GEO repository, under the accession number GSE132040 and can be found at the following link: www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE132040. The analyzed database contains the the RNA-Seq data from 17 tissue types: *Brain, BAT, Bone, GAT, Heart, Kidney, Lung, Marrow, MAT, Pancreas, SCAT, Skin, Small, Spleen, WBC, Limb, Liver*. Samples for each tissue type were taken from 5 or 6 male and female *Mus musculus* individuals, (3M+3F or 3M+2F). For the age dynamics a new group of experimental mice was used each time they reached the age of 1, 3, 6, 9, 12, 15, 18, 21, 24 and 27 months.

Selection into the HG gene part was performed according to the Housekeeping and Reference Transcript Atlas[22] (HRT Atlas v1.0, (www.housekeeping.unicamp.br). When the HG list was compiled, all variants of genes from those already listed, were additionally included. Total number of the Housekeeping genes was **5,101**. All remaining genes (**30,529**) were assigned to the IntG part. Genes with the same name in both groups were removed from the total list of the whole genome. The stochastic of the original data due to the use of genetically heterogeneous Mus musculus and the small (5 or 6) number of samples to represent the value at each age point impose certain limitations on the use of statistical methods for analyzing the results. These limitations excluded the use of polynomial analysis of variation in the resultant curve or cluster analysis.

The list of genes responsible for energy metabolism (**24** genes: *MT-ND1, MT-ND2, GM12895, M10722, MT-ND5, MT-ND4, ARS2, GM11168, MT-CO2, M10719, GM10800, MT-CO1, M10718, MT-CYTB, GM10721, M10715, MT-CO3, GM10801, RPL12, MRPL37, MRPL4, MRPL28, MRPL17, MRPL 42.*). These genes were isolated from the HG gene list using the NIH website showing their function and can be found at the following link: https://www.ncbi.nlm.nih.gov/guide/howto/find-func-gene/.

Results

The main amount of production falls on a relatively small group of genes with a high level of RNA synthesis in both HG and lntG parts of genes. Thus, the number of genes that produce more than 80% of all RNA production is only **4,850**, or 13.6% of the total number of genes. At the same time, the genes of the HG part **1,857** genes of total (**4,850**) have slightly higher productivity per gene. So to analyze the results of RNA sequencing within the HG part, we used **1,857** genes. The result we obtained in our previous work analyzing RNA sequencing data and presented in our previous paper is presented in (**Figure 1**). Differences in the level of RNA synthesis between the HG and lntG parts of the genome were statistically highly significant at all age points presented (p-value < 0.0001**).** The results also demonstrate a statistically significant difference (p-value = 0.0045) in the dynamics of the decrease in the activity of the HG part of genes compared to lntG in the period from the onset of puberty to the age of 18 months. At the same time, after reaching the age of 21 months, there is some rise in total RNA production in the HG part genes. No significant changes in the value of RNA production in the lntG part were observed. We believe that in the course of development and after puberty a large number of structures associated with the performance of organism functions accumulate in the cells of the organism. At the same time, their need for energy supply also increases. We assume that the increasing number of cells in the resting state during the age period creates an increasing number of specialized structures necessary for the functions of the organism. In turn, this should cause the increase in the production of HG genes responsible for metabolic processes and energy production in cells. To verify the validity of this assumption, we identified **24** maximally productive genes responsible for metabolic processes from **1,857** HG genes. Their total RNA production amounted to 53% of the total production of all **1,857** HG genes. The activity of our selected metabolic genes was high compared to other HG genes (p-value < 0.0001). The graph in **Figure 1** shows the averaged HG values for all tissues before and after subtracting the activity of metabolic genes from them.

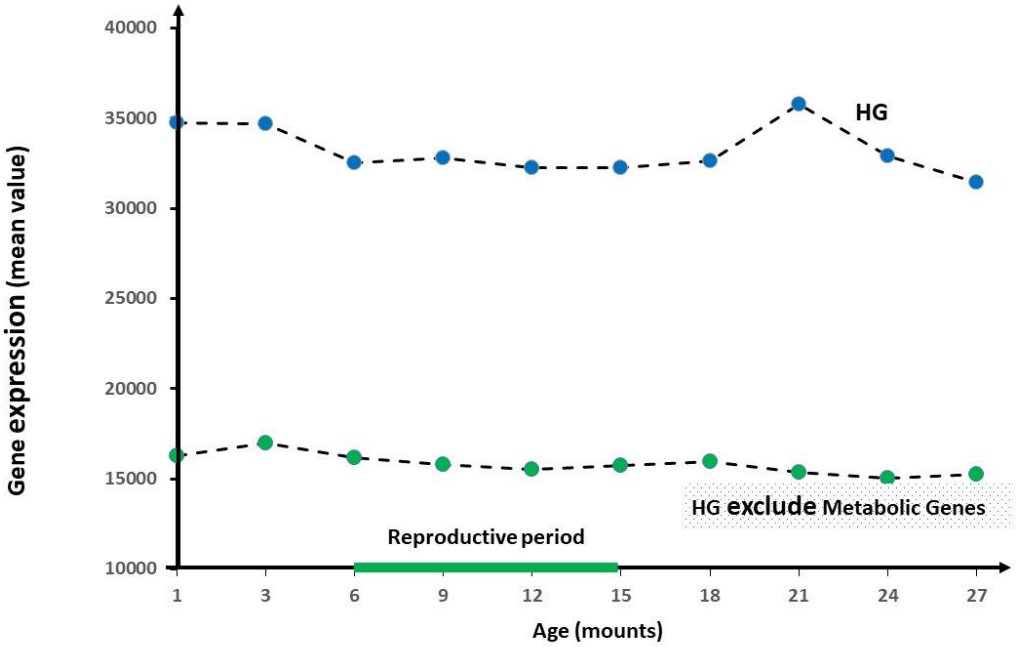

Figure 1. *Dynamics of RNA production value of the HG group (blue) and HG group exclude metabolic genes (green) in the period from 1 month to 27 months.*

As can be seen from **Figure 1,** the level of total RNA production of HG genes (highlighted in blue on the graph) increases significantly after the age of 18 up to 24 months. When excluding the activity of **24** metabolic genes selected by us from the total RNA production, the obtained curve (highlighted in green on the graph) took the form of an almost straight descending line. This result clearly proves that the increase in the activity of PG gene production at the age of 21 to 24 months is determined precisely by the activity of metabolic genes. Taking into account that tissues of the organism have different mitotic activity, we decided to select from all tissues present in the database two groups of tissues, as close in number as possible, related by their mitotic activity. The first group (**Group I**) with *low* MI values included tissues: *Bone, Kidney, Limb, Spleen, Liver*.

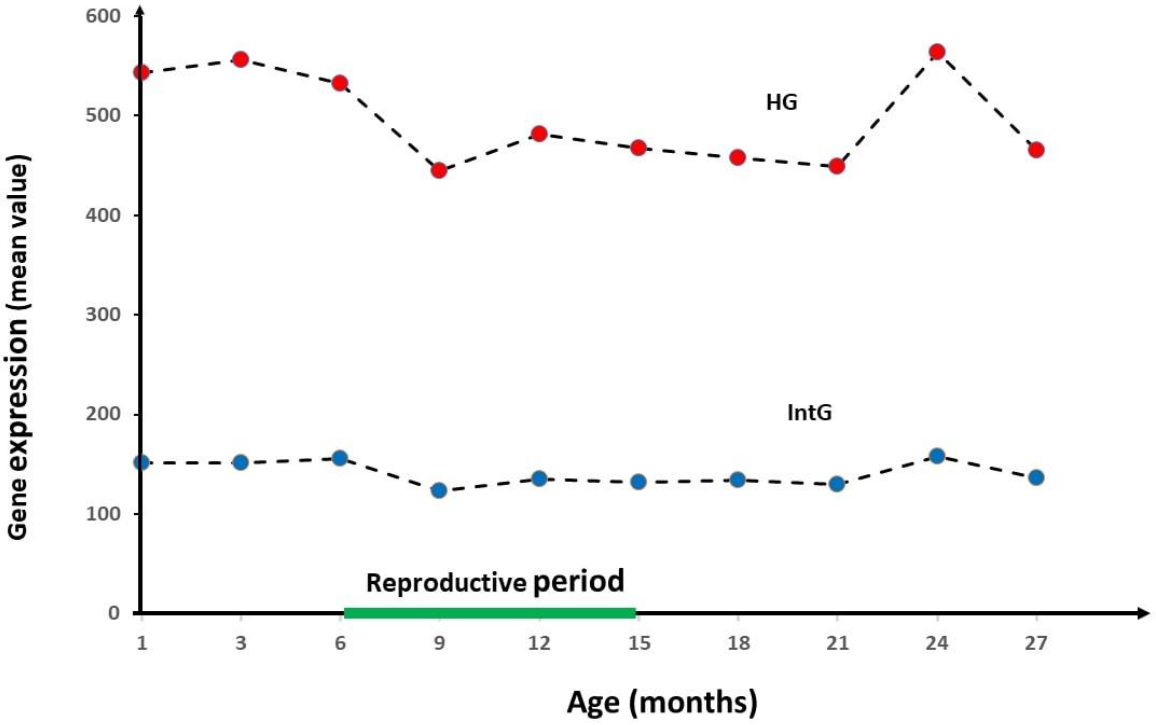

Figure 2. *Dynamics of RNA production value of all genes divided into HG and IntG groups in the period from 1 month to 27 months,* ***Group I*** *with low MI values included tissues: Bone, Kidney, Limb, Spleen, Liver.*

The graph presented in **Figure 2** demonstrates a pronounced rise in HG activity (marked in red color on the graph) after 21 months of age. It becomes evident that this rise is provided by the activity of metabolic genes that make the main contribution to the growth of total HG values shown in **Figure 1**. This result is associated with the overwhelming number of cells in the resting state and loaded with the fulfillment of body functions.

The dynamics of total HG and IntG values for another group of tissues is shown in **Figure 3.** The designations of the values of these genome parts are similar to the previous group. The second group of tissues (**Group II**) with high MI included: *Bone marrow, Skin, Small, WBC*.

As shown in Figure 3 in tissues with high MI (**Group II**) we observe only a small rise in HG gene production values at 21 months of age with greater overall variability in the data, which is significantly different from the dynamics of RNA production in **Group I.**

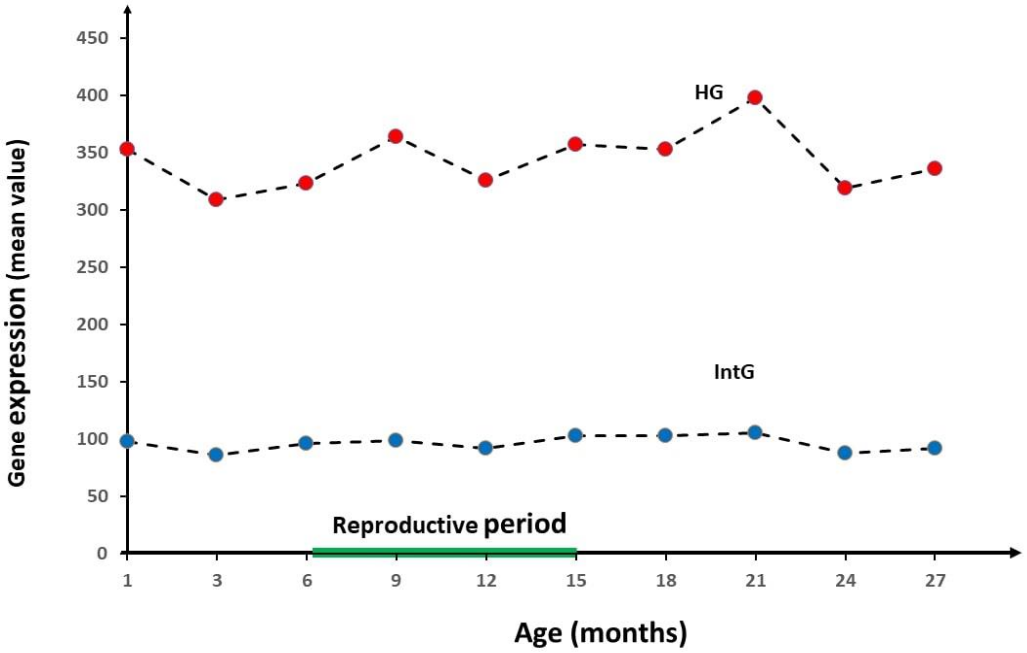

Figure 3. *Dynamics of RNA production value of all genes divided into HG and IntG groups in the period from 1 month to 27 months* ***Group II*** *with high MI values included tissues: Marrow, Skin, Small, WBC.*

Discussion

In this paper, we continue to analyze the changes in RNA production in *Mus musculus* by investigating its dynamics within the functional part of HG genes. We were motivated to do so by some specific characteristics of the results obtained earlier. Thus, in the period from 21 to 24 months, which has all the signs of aging in *Mus musculus*, we observed an increase in HG levels, which obviously required an explanation. In addition, it was shown that the level of metabolism, measured in a large group of people of different ages, remains unchanged and relatively constant between 20 and 60 years of age[23], after which it changes significantly. By comparing the age of humans and *Mus musculus*, we obtain an approximate correspondence in which the age of mice at 18 months corresponds to the age of humans at 60 years. This is exactly the age after which we observed a rise in the level of RNA production in Mus musculus. Taking into account the available data on changes in mitochondrial and metabolic gene activity in the organism of old age[24], we decided to consider the dynamics of RNA production of HG genes, some of which are responsible for these processes. The results obtained (**Figure 1**) prove the accuracy of our assumption about the reasons for the rise in the level of HG gene production in late ages. The graph shows that by eliminating the activity of **24** metabolic genes, we obtain a graph of smooth decrease in the values of total RNA production of infrastructural genes in all tissues. Analyzing the received result, we should pay attention to the fact that being engaged in the study of aging, most researchers focus on the age-related changes occurring in the organism in late ages. During this period, the various indicators under study reach their maximum differences from their normal values. Research focused on changes occurring in the second half of ontogenesis is doomed to deal only with the consequences of aging processes, missing their causes. It is also important to note that the animals involved in the experiments are a kind of long livers with certain genetic features that allow them to undergo a kind of natural selection and reach the age limits for the species. Thus, the experimental groups undergo their own natural selection, which essentially changes the general picture of the results obtained. It can be assumed that the increase in RNA production of HG metabolic genes is one of the conditions for survival in late ages and is associated with the need to compensate for the increasing energy consumption by the already existing specialized cell structures, responsible for the performance of organism functions[25]. The increase in the number of free radicals and associated damage caused by oxygen stress with age is also explained here. It is obvious that these processes occurring during late aging are primarily associated with changes occurring in the mitochondrial apparatus of cells[26, 27]. It should be emphasized that the increase in the activity of metabolic genes in late ages is associated not with an increase in the number of organism functional structures, but with the need to

preserve their energy expenditures in conditions of their decreasing efficiency. In addition, the decrease and dysregulation of RNA translation processes significantly increases in the process of aging[28, 29], contributes greatly to the increase of cellular protein demand.

In dividing the tissues of the organism on the basis of mitotic index (MI) we were guided by the way the organism uses its tissues. In one case, the cells themselves are needed as a consumable material (tissues with high MI), in the other case the constant work of cellular structures is necessary (tissues with low MI). In works on aging, a lot of attention is paid to cells and tissues with high mitotic index. These properties allow such cells to retain a high regenerative potential and make them a target for experiments aimed at rejuvenation[30, 31, and 32]. From our point of view, only influencing the epigenetic mechanisms of cells with high MI is clearly insufficient to achieve true rejuvenation of the organism. On the other hand, the bulk of the organism consists of organs and tissues composed of post mitotic cells and cells with low MI. There are many works devoted to aging processes in such cells, the influence of old cells on surrounding tissues and the reaction of the immune system to them. The contribution to the aging processes and the negative effects of old cells on the organism, have been shown in a number of articles[33 –37]. As demonstrated by our results in the group of tissues with low MI (**Figure 2**), where the total number of tissue cells increasing with age and passed into the resting state is significantly greater. This leads to a significant increase in the level of HG gene production in late ages. In tissues with low MI (**Figure 3**), no such pronounced rise was observed.

The confirmation of the fact that such a rise is realized at the expenses of metabolic genes is presented on the obtained graph (Figure 1). Thus, we can assert that the rise in HG gene production in late ages occurs mainly in tissues with low MI due to RNA synthesis of metabolic genes. The results we have presented from the analysis of our two selected tissue groups confirm our assumptions of a significant difference between the two main tissue groups. The analysis of age-related changes in these groups draw attention to a common strategy present at both cellular and species levels. Here we are talking about a choice between reproduction and survival strategies. While at the species level the result of this choice is reproductive activity, at the cellular level it is the transition from active division to a quiescent state. For cells, this transition is mainly associated with the use of regulation based on the activity of the mTOR pathway and the associated metabolic activity of the cell[38, 39].

In the course of evolution, the ability of cells to remain dormant under conditions of nutrient substrate deficiency was an obvious advantage, contributing to their survival. During mitotic rest, the cell's needs are minimized, and the activity of biosynthetic and metabolic processes is reduced, giving it the opportunity not only to save resources, but also to turn on repair processes if necessary. Another situation arises when a cell becomes a part of a multicellular organism, participating in a developmental program to form an organism. To maintain the continuity of the cell's organism functions, as well as to maintain the structural parts of the organism, it is necessary to keep the cell in the phase of mitotic pause for a long and sometimes permanent period of time. It is in this case that a contradiction between the needs of the organism as a whole and the needs of the cell itself arises. We believe that there is an optimal time between cell cycles, which is determined by the rate of attenuation of the impulse stimulating biosynthesis of cellular structures, including their reparative activity and represented by HG genes. Going beyond the optimal duration naturally leads to the situation when the total level of gene production within HG is redistributed from genes responsible for the process of biosynthesis and repair to the activity of metabolic genes.

Conclusion

Earlier in our previous work, we observed a rise in the level of HG gene production at the age of 21 to 24 months, when aging processes are already significant. To explain this phenomenon, we hypothesized that such an increase in the activity of the HG part of the genome in late ages is associated with an increased demand of cells for energy production to maintain the weakening functions of the organism. We identified a block of **24** most productive genes responsible for metabolic activity and energy production in the cell from the HG part of the genome. As data analysis showed, these genes were responsible for the rise in overall HG gene activity. Excluding their activity, the fall in the level of total HG production continued (**Figure 1**). We also hypothesized that the upsurge in demand for cellular energy structures in the aging organism is most pronounced in highly differentiated tissues. To this end, we distinguished two groups of tissues, according to the level of their MI. The results presented in the graphs allow us to conclude that such a rise in production in HG genes is especially pronounced in HG genes in the group of tissues with low MI in Group I as compared to Group II, which is shown in **Figure 2** and **Figure 3**. In conclusion, these results allow us to continue our

work on age-related proteome dynamics. We plan to compare these results with other databases to identify similar patterns of RNA production dynamics. This will allow us to come to a deeper understanding of the workings of aging mechanisms.

Conflict of Interest

Authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

L. Salnikov has proposed the theoretical model of aging based on functional genome partition and the role of ratio of the genome two functional parts activity in ontogenesis and aging. He also proposed a way to test the hypothesis using RNA-Seq data meta-analysis. **S. Goldberg** statistically processed the data and **E. Pinsky** organized the work and participated in the data analysis. All authors have made a contribution to prepare the article for publication, participated in the structuring of manuscript and helped with editing. All the authors reviewed, revised and approved the final version of the manuscript.

Authors Approvals

All authors have seen and approved the manuscript. The manuscript hasn't been accepted or published elsewhere.

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References

1. Larocca D, Lee J, West M.D, Labat I, Sternberg H. (2021) No Time to Age: Uncoupling Aging from Chronological Time. Genes (Basel). Apr 21;12(**5**):611. doi: 10.3390/genes12050611.
2. De Magalhães J. P, Church GM. (2005) Genomes optimize reproduction: aging as a consequence of the developmental program. Physiology (Bethesda). Aug;**20**:252-9. doi: 10.1152/physiol.00010.2005.
3. Bilinski T, Bylak A, Kukuła K, Zadrag-Tecza R. (2021) Senescence as a trade-off between successful land colonisation and longevity: critical review and analysis of a hypothesis. PeerJ. Nov **2**;9:e12286. doi: 10.7717/peerj.12286.
4. Gems D, Kern C.C., Nour J., Ezcurra M. (2021) Reproductive Suicide: Similar Mechanisms of Aging in C. *elegans* and Pacific Salmon. *Front Cell Dev Biol.*;**9**:688788. Published 2021 Aug 27. doi:10.3389/fcell.2021.688788.
5. Kirkwood, T.B.L., Holliday, R. (1979). The evolution of ageing and longevity. Proc. R. Soc. London Ser. B Biol. Sci. 205, 531–546.
6. Williams, G. C. (1957) Pleiotropy, natural selection and the evolution of senescence, *Evolution*, **11**, 398-411, doi: 10.2307/2406060.
7. Soto-Palma C, Niedernhofer LJ, Faulk CD, Dong X. (2022) Epigenetics, DNA damage, and aging. J Clin Invest. Aug 15;132(16):e158446. doi: 10.1172/JCI158446.
8. Kinzina E.D., Podolskiy D.I., Dmitriev S.E,, Gladyshev V.N. (2019) Patterns of Aging Biomarkers, Mortality, and Damaging Mutations Illuminate the Beginning of Aging and Causes of Early-Life Mortality. Cell Rep. Dec 24;29(**13**):4276-4284.e3. doi: 10.1016/j.celrep.2019.**11**.091.
9. Walker R.F. (2022) A Mechanistic Theory of Development-Aging Continuity in Humans and Other Mammals. Cells. Mar **7**;11(**5**):917. doi: 10.3390/cells11050917.
10. West M.D., Sternberg H., Labat I., Janus J., Chapman K.B., Malik N.N., de Grey A.D., Larocca D. (2019) Toward a unified theory of aging and regeneration. Regen Med. Sep;14(**9**):867-886. doi: 10.2217/rme-2019-0062. Epub 2019 Aug 28.
11. Salnikov, L., Baramiya, M. G. (2020) The Ratio of the Genome Two Functional Parts Activity as the Prime Cause of Aging. *Frontiers in Aging*, ***1***. https://doi.org/10.3389/fragi.2020.608076
12. Salnikov L., Baramiya M. G. (2021) From Autonomy to Integration, From Integration to Dynamically Balanced Integrated Co-existence: Non-aging as the Third Stage of Development. *Frontiers in Aging,* **2**. doi/org/10.3389/fragi.2021.655315
13. Naviaux, R.K. (2019) Incomplete Healing as a Cause of Aging: The Role of Mitochondria and the Cell Danger Response. *Biology*, **8**, 27. https://doi.org/10.3390/biology8020027
14. Salnikov L. Aging is a Side Effect of the Ontogenesis Program of Multicellular Organisms. Biochemistry (Mosc). 2022 Dec;87(12):1498-1503. doi: 10.1134/S0006297922120070. PMID: 36717443.
15. Santra M, Dill K.A, de Graff A.M.R. (2019) Proteostasis collapse is a driver of cell aging and death. Proc Natl Acad Sci U S A. Oct 29;116(**44**):22173-22178. doi: 10.1073/pnas.1906592116. Epub 2019 Oct 16.
16. Meyer, D. H., & Schumacher, B. (2021). BiT age: A transcriptome-based aging clock near the theoretical limit of accuracy. *Aging Cell*, *20*(**3**). https://doi.org/10.1111/acel.13320
17. Stoeger, T., Grant, R.A., McQuattie-Pimentel, A.C. *et al.* (2022) Aging is associated with a systemic length-associated transcriptome imbalance. *Nat Aging*. https://doi.org/10.1038/s43587-022-00317-6
18. Stegeman R, Weake VM. (2017) Transcriptional Signatures of Aging. J Mol Biol. Aug 4;429(**16**):2427-2437. doi: 10.1016/j.jmb.2017.06.019. Epub 2017 Jul 3.
19. Lu J.Y., Simon M., Zhao Y., Ablaeva J., Corson N, *et al.* (2022) Comparative transcriptomics reveals circadian and pluripotency networks as two pillars of longevity regulation. Cell Metab. Jun 7;34(**6**):836-856.e5. doi: 10.1016/j.cmet.2022.04.011. Epub 2022 May 16.
20. Ferreira M., Francisco S., Soares A.R., Nobre A., Pinheiro M., *et al.* (2021) Integration of segmented regression analysis with weighted gene correlation network analysis identifies genes whose expression is remodeled throughout physiological aging in mouse tissues. Aging (Albany NY). Jul 29;13(**14**):18150-18190. doi: 10.18632/aging.203379. Epub 2021 Jul 29.
21. Salnikov L, Goldberg S, Rijhwani H, Shi Y, Pinsky E. The RNA-Seq data analysis shows how the ontogenesis defines aging. Front Aging. 2023 Mar 14;4:1143334. doi: 10.3389/fragi.2023.1143334. PMID: 36999000; PMCID: PMC10046809.
22. Hounkpe B.W., Chenou F., de Lima F., De Paula E.V. (2021) HRT Atlas v1.0 database:

redefining human and mouse housekeeping genes and candidate reference transcripts by mining massive RNA-seq datasets. Nucleic Acids Res. Jan 8;**49**(D1):D947-D955. doi: 10.1093/nar/gkaa609.

23. Rhoads T. W., Anderson R. M. (2021). Taking the long view on metabolism. *Science*. 373(6556):738-739. doi: 10.1126/science.abl4537.
24. Amorim, J.A., Coppotelli, G., Rolo, A.P. *et al*. Mitochondrial and metabolic dysfunction in ageing and age-related diseases. *Nat Rev Endocrinol* 18, 243–258 (2022). https://doi.org/10.1038/s41574-021-00626-7
25. Lev Salnikov. Relation of Known Hallmarks of Aging to the Ontogenesis Program. OAJ Gerontol & Geriatric Med. 2023; **7**(2): 555706. Doi: 10.19080/OAJGGM.2023.07.555706
26. Mas-Bargues C. Mitochondria pleiotropism in stem cell senescence: Mechanisms and therapeutic approaches. Free Radic Biol Med. 2023 Nov 1;208:657-671. doi: 10.1016/j.freeradbiomed.2023.09.019. Epub 2023 Sep 20. PMID: 37739140.
27. Naia, L., Shimozawa, M., Bereczki, E. *et al*. Mitochondrial hypermetabolism precedes impaired autophagy and synaptic disorganization in *App* knock-in Alzheimer mouse models. *Mol Psychiatry* (2023). https://doi.org/10.1038/s41380-023-02289-4
28. Anisimova A.S., Meerson M.B, Gerashchenko M.V, Kulakovskiy IV, Dmitriev S.E, Gladyshev VN. Multifaceted deregulation of gene expression and protein synthesis with age. Proc Natl Acad Sci U S A. 2020 Jul 7;117(27):15581-15590. doi: 10.1073/pnas.2001788117. Epub 2020 Jun 23. PMID: 32576685; PMCID: PMC7354943.
29. Debès, C., Papadakis, A., Grönke, S.*et al*. Ageing-associated changes in transcriptional elongation influence longevity. *Nature* **616**, 814–821 (2023). https://doi.org/10.1038/s41586-023-05922-y
30. Zhang, W., Qu, J., Liu, G. H., & Belmonte, J. C. I. (2020). The ageing epigenome and its rejuvenation. *Nature Reviews Molecular Cell Biology*. *Nature Research*. doi.org/10.1038/s41580-019-0204-5
31. Olova, N., Simpson, D. J., Marioni, R. E., & Chandra, T. (2019). Partial reprogramming induces a steady decline in epigenetic age before loss of somatic identity. *Aging Cell*, ***18***(1). doi.org/10.1111/acel.12877.
32. Lapasset L, Milhavet O, Prieur A, Besnard E, Babled A, Aït-Hamou N, Leschik J, Pellestor F, Ramirez JM, De Vos J, Lehmann S, Lemaitre JM. (2011). Rejuvenating senescent and centenarian human cells by reprogramming through the pluripotent state. *Genes Dev*. **1**;*25*(21):2248-53. Doi: 10.1101/gad.173922.111.
33. Voutetakis K, Chatziioannou A, Gonos ES, Trougakos IP. (2015). Comparative Meta-Analysis of Transcriptomics Data during Cellular Senescence and In Vivo Tissue Ageing. *Oxid Med Cell Longev*. 2015:732914. Doi: 10.1155/2015/732914.
34. Van Deursen JM. (2014). The role of senescent cells in ageing. *Nature*. **22**;509(7501):439-46. Doi: 10.1038/nature13193.
35. Childs BG, Gluscevic M, Baker DJ, Laberge RM, Marquess D, Dananberg J, van Deursen JM. (2017). Senescent cells: an emerging target for diseases of ageing. *Nat Rev Drug Discov*. **10**:718-735. Doi: 10.1038/nrd.2017.116.
36. Mylonas A and O'Loghlen A. (2022). Cellular Senescence and Ageing: Mechanisms and Interventions. *Front Aging*. 3:866718. doi: 10.3389/fragi.2022.866718.
37. Huang, W., Hickson, L.J., Eirin, A. *et al*. Cellular senescence: the good, the bad and the unknown. *Nat Rev Nephrol* **18**, 611–627 (2022). https://doi.org/10.1038/s41581-022-00601-z
38. Panwar, V., Singh, A., Bhatt, M. *et al*. Multifaceted role of mTOR (mammalian target of rapamycin) signaling pathway in human health and disease. *Sig Transduct Target Ther* **8**,375 (2023). https://doi.org/10.1038/s41392-023-01608-z
39. Reinhardt HC and Schumacher B. (2012). The p53 network: cellular and systemic DNA damage responses in aging and cancer. *Trends Genet*. **3**:128-36. Doi: 10.1016/j.tig.2011.12.002.