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HIGHLY SPECULATIVE HYPOTHESES

The Redusome Hypothesis of Aging and the Control of Biological Time during Individual Development

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Abstract—The redusome hypothesis of aging and the control of biological time in individual development is proposed. Redusomes are hypothetical perichromosomal particles arising in differentiation events during morphogenesis of an organism. The linear molecule of DNA covered with proteins in the redusome is assumed to be a copy of a segment of chromosomal DNA. Redusomes are located mainly in subtelomeric regions of chromosomes. The redusome does not leave the body of a chromosome even in the course of cellular divisions, being kept in its chromosomal nest. Like telomeric DNA, redusome linear DNA is shortened step by step. Thus, tiny redusomes progressively decrease in size; it is from here their name originates. Together with loss of the length of DNA in a redusome, the number of different genes contained in it also decreases. Shortening of the redusomal DNA molecules (and, coupled to it, changes of the sets of genes in redusomes) is responsible for age-dependent shifts in the level of expression of different chromosomal genes. Owing to this, redusome DNA shortening serves as a key means of measuring biological time in individual development. The main part of DNA of most redusomes is postulated to be occupied by noncoding genes. Low-molecular-weight RNAs (micro RNAs and fountain RNAs, or fRNAs) are assumed to be transcribed from them. These RNAs are involved in regulation of various chromatin repackings that are specific to certain differentiations, while others modulate the levels of expression of chromosomal genes. Hypothetical fountain RNAs can quantitatively regulate the expression levels of chromosomal genes, forming specific complexes with fions. Fions are suggested to be specific sites of a chromosomal DNA which are complementary to different fRNAs. Fions reside in the vicinity of usual chromosomal genes. A complex of the fRNA–fion, specifically interacting with a closed gate of the corresponding ion channel of the internal nuclear membrane, initiates the opening of the gate for a very short time, thus organizing activity of an ion fountain which appears to be automatically aimed at the chromosomal gene nearest to the fion involved. The ion fountain creates, depending on specificity of matching fRNA, fion, and ion channel, a distinctive ionic environment near certain structural genes. Ion fountains exert their action on the configuration of corresponding segments of chromatin and on the transcriptional efficiency of chromosomal genes in a topographically specific manner. Hence, the fountain system of the nucleus is able to regulate the quantitative traits both of cells and organism; it can control dominance of alleles and plays a role in individual development. Significant and escalating truncation of the redusome DNA causes cell aging due to an arising and increasing deficit of fRNAs and, for this reason, the lack of required ions near certain structural genes. Progressive shortening of DNA of redusomes is proposed to result in cellular aging because of a constantly growing shortage of low-molecular-weight RNAs transcribed from redusomal genes. Two types of redusomes are postulated: chronosomes and printosomes. Linear molecules of DNA in these two types of redusomes are called chronomeres and printomeres, respectively. Chronosomes are responsible for measurement of biological time in nondividing cells of the CNS. Printosomes remember positions of cells in the course of interpretation of the positional information in morphogenesis. In accordance with the position of a cell in a morphogenetic field, printomeres do change cellular properties and remember the change made (this is a so-called printomere mechanism of interpretation of positional information). Besides, printomeres participate in maintaining the achieved state of cellular differentiation. Normally, the chronomere is shortened only on the maximum of infradian hormonal rhythm (T-rhythm) which initiates the act of a superhigh velocity of its transcription that is finished with truncation of the end of a chronomere (an effect called scrupting). The printomere can be shortened due to the effect of DNA end underreplication and owing to scrupting. The effect of the end underreplication of DNA in doubling cells occurs simultaneously both in printomeres and telomeres. Shortening of telomeres is just a bystander process of aging of cells, whereas the true cause of biological aging is only the shortening of redusome DNA. Processing of certain redusomes in terminally differentiating cells is a cause of a proliferation arrest. Linkage of genes in a eukaryotic chromosome is determined by the distances between genes and redusomes.

Key words: telomere, transcription, aging, biological time, ions, biological rhythms, differentiation, linkage of genes

The control of biological time in a multicellular organism is one of its major physiological functions that is also coupled with the process of organismal aging. This area, despite long-lasting theoretical and experimental efforts, in many respects remains terra incognita, and the proposed hypothesis is an attempt to promote progress in this direction.

It is supposed that as well as in the case of any other function, for the function of biological time control in an

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organism there should be some quite certain structure. In fact, there are no functions outside structures as there is no content outside the form. Before starting a statement of the hypothesis and consideration of offered processes, it is expedient to give a perforce rather long list of the hypothetical figurants involved in them, with their brief characteristics.

The redusome is a tiny nuclear particle constantly located on the chromosome body and decreasing in length in the course of time owing to the shortening of its linear DNA molecule covered with proteins.

The redusomal DNA (it can also be called a redumere) arises as a lateral amplification of a segment of chromosomal DNA; respectively, this segment is the protoredumere. Thus, the redumere is situated outside the chromosomal DNA, but physically near it, being kept in a chromosomal nest near its chromosomal original, that is, side by side with a protoredumere; retention of this DNA in the nest is supported by protein bridges and interguanine bonds between the redusomal DNA and chromosomal DNA.

The printosome is a type of redusome. Printosomes are present both in various dividing cells (for example, fibroblasts) and in nondividing cells not occupied with biological time control (for example, in cardiocytes). The printosome is absent from stem cells.

A printomere is a linear DNA molecule of a printosome. The printomere arises as a perichromosomal copy of the chromosomal original, or protoprintomere, laterally lying outside the chromosomal DNA (but near and along it). Being a perichromosomal amplification, the printomere molecule, in distinction from its chromosomal template, has free ends of DNA. The printomere is shortened, like telomeric DNA, basically due to the effect of DNA end underreplication. It contains various genes of micro RNAs and fRNAs. Decrease in the number of these genes in the printomere, due to its shortening, leads in cells to the change in levels of expression of the chromosomal genes.

The protoprintomere is a genetically inherited chromosomal original on which its perichromosomal copy, i.e., printomere, is formed.

The chronosome is a kind of redusome. It is involved in the control of biological time and operates only in nondividing neuroendocrine cells of the brain.

The chronomere is a linear DNA of a chronosome. Like the printomere, the chronomere has free ends, being a copy of a segment of a chromosomal DNA; the chronomere is also located side by side to its chromosomal template segment. The chronomere contains various genes of fRNAs and micro RNAs as well. The chronomere is shortening during the life of an organism owing to the activity of so called T-rhythms and scrupting, but not due to the process of DNA end underreplication that is impossible in nondividing neurons.

The protochronomere is a genetically inherited chromosomal original for synthesis of its somatic copy, or chronomere.

The acromere is a sequence of end segments of a linear redusomal DNA molecule represented by G-rich repeats. Acromeres are at the ends of all redumeres, i.e., on the termini of all printomeres and chronomeres. The acromere, as well as telomere, is protected from nucleases on the absolute DNA terminus. Acromeres of different printomeres and chronomeres are usually considerably less in their lengths as compared to telomeres. Acromeres, at least in some mammalian species, differ in their G-rich sequences from telomeric repeats and as a result are not recognized by telomerase.

The acrosome is a complex of proteins covering the very end of the acromeric double helix. Acrosomes are located at the termini of all redusomes. The acrosome participates in processing and immobilization of the very terminus of an acromeric DNA within the body of a chromosome, i.e., in the corresponding compartment of the chromosomal nest.

T-rhythm is a hormonal biological rhythm with a rather long period; in humans it is one of the infradian rhythms. On each peak of the T-rhythm, a single act of shortening of chronomeres is put into effect owing to the process of scrupting.

Scrupting is the shortening of a linear DNA molecule that is transcribed at an especially high rate. Truncation of such molecules is due to the occurrence of nonrepaired terminal breach. This end gap arises under conditions of mechanical strain that is created in the supertwisted DNA molecule in the course of maximally high rate of the transcriptional machinery operation, when the machinery is approaching most closely the very terminus of redusomal DNA which is physically immobilized on the chromosomal body.

The fountain system of the nucleus is a system of ion channels that are opening (in a short-term manner within the intranuclear membrane of the perinuclear cistern) and creating ion fountains near the structural genes. The gate of an ion channel is opening when contacting with the fion–fRNA complex. Fountains influence the productivity of genes, modulating their local ionic environment.

Fions are sites of chromosomal DNA in vicinities of structural genes; nonrandom localization of fions in genomes provides the topographically specific influence of ion fountains upon the configuration of corresponding chromatin segments and the efficiency of structural genes.

fRNAs are low-molecular-weight nuclear RNAs transcribing in redusomes from fRNA genes and directing the activity of nuclear ion fountains; they are coded by various fRNA genes of printomeres and chronomeres.

Micro RNAs are low-molecular-weight RNAs transcribing in redusomes from micro RNA genes; they are involved in creation of chromatin configurations that are specific for certain cytodifferentiations and regulation of RNA metabolism.

The chromosomal nest is a cavity in the chromosome created by three-dimensional setting of chromatin. It serves as a bed for the redusome. Each nest is always strictly specific only to the certain redusome.

Biological time is a duration of consecutive events during individual development. It is measured and controlled in higher animals by the organism itself at the expense of the change in genetic composition of the shortening chronomeres. Biological time flows in steps, rather than evenly and uniformly. Biological time proceeds irrespectively of astronomical time.

Having presented this list of characters, one can pass to more detailed consideration of their properties and activity.

THE REDUSOME—A NEW PERICHROMOSOMAL ORGANELLE OF THE CELL

For the basic structure considered here, the term "redusome" is offered (the term originates from "reducere" (to diminish, in Latin) and "some" (a body, in Greek)). The size of this structure decreases in the course of time because of shortening of its linear DNA, or redumere; it is here where the name of a new nuclear particle comes from. In one cell there can be one or a series of different redusomes. Each redusome is kept, even in mitoses, on a body of that chromosome on which it has been created in the course of differentiation, though cellular differentiations are not at all obliged to be associated with creation of new redusomes. Redusomes have not been found yet not only in view of the absence of the purposeful quest and their tiny sizes (the length of their DNA molecules is on average probably 10 kb). The main reason is a complete resemblance of redusomal DNA sequence with the sequence of respective chromosomal original, i.e., with the protoredumere sequence. The local amplification residing laterally, side by side on the chromosomal DNA (but outside its strands) and, hence, having its own free ends of DNA, is DNA of the redusome. As this DNA does not leave the body of chromosome, it should be more precisely considered as perichromosomal, rather than extrachromosomal DNA. Thus, this DNA fraction can escape attention during usual sequencing. Transcripts of redusomal genes coding for fRNAs and micro RNAs are processed and then function in ion regulation of the eukaryotic genome, as well as in regulation of chromatin configurations and RNA metabolism.

The redusome DNA has its own *ori* and promotors of transcription. There are also analogs of telomeres in it (in order to avoid confusion with telomeres, end structures of redusomal DNA may be called acromeres). It is important to emphasize that the redusome has no centromere and consequently its fate in dividing cells is entirely connected to the destiny of the chromosome, in whose body the redusome is embedded. The redusome does not abandon its chromosome even during DNA replication, being kept in the nest near the chromosomal original at the expense of protein bridges and G–G bonds between some G-rich sites of redusomal DNA and the DNA of the chromosomal nest. During replication, the redusome is kept in the nest, by turns, with the help of one of two of its arms; the arm of the redusome corresponds to a distance between the centrally located *ori* and the very end of the acromere.

Similarly to telomeres, linear molecules of redusomal DNA are stepwise shortening with time. But there are some differences. Owing to the DNA end underreplication, the truncation of redusome DNA proceeds only in dividing cells. In postmitotic cells, telomeric DNA can not be shortened at all, whereas the redusomal DNA can be shortened under peculiar conditions due to scrupting (the essence of scrupting is examined in other sections). The tiny redusomes, justifying their name, do decrease in size and lose their genes. Chromosomal originals, from which the redusomal DNA of somatic cells has been copied, are in tightly compacted form and not transcribed in normal cells (though transcription of protoredumeres is possible in cancer cells) and, immediately after creation of redusomes, protoredumeres again undergo compaction.

The main part of redusome DNA molecules is represented by different combinations of noncoding genes. Respectively, the majority of RNAs transcribing from different redusomes (i.e., RNAs marking the chromatin, fountain RNAs, etc.) do not code for proteins, and this circumstance simplifies performance of their missions. The presence in some redusomes of copies of protein coding genes, even if they are silent in the chromosomal DNA itself, would not in any way change, however, the basic properties of redusomes. The ability of some low-molecular-weight RNAs of a nucleus to carry out the epigenetic marking of chromatin and regulate the RNA metabolism has already been established experimentally. Respective low-molecular-weight RNAs are revealed in different eukaryotes, from worms to humans. Evidence rapidly accumulates concerning various micro RNAs transcribed from chromosomal genes and involved in a variety of species in diverse manifestations of the so-called RNA interference, as well as in chromatin reorganizations, including epigenetic marking of chromatin in infusoria, and also gene dose compensation in multicellulars, editing of nucleic acids, etc. [1-7]. Those of micro RNAs that are transcribed from redusomes, probably, form only a small fraction in comparison with chromosomal micro RNAs. Redusomal micro RNAs differ from the similar in size other nuclear RNAs, in that they, together with fRNAs, are at the top of the regulatory cascades in morphogenetic cytodifferentiations and determinations. They provide the establishing and maintenance of the specific chromatin configuration patterns and genetic activity switchings.

As a whole, in DNA of redusomes of different specificities, two groups of noncoding genes could be represented in different combinations. Some of the low-molecular-weight RNAs transcribed from redusomes are responsible for regulation of chromatin configurations, while others are able to modulate quantitative traits of cells. Among redusomal RNAs, fountain RNAs (fRNA) can be distinguished by their proposed ability to quantitatively change the levels of expression of chromosomal genes via regulation of the local ionic environment of the structural genes that modify the productivity of the genes. Since fountain RNAs have still not been revealed, it is expedient to consider their properties in some details. Low-molecular-weight fRNAs of different specificity form specific complexes with the complementary sites of chromosomal DNA (i.e., fions) located in vicinities of various protein-coding genes. Each fRNA–fion complex, interacting with the still closed gate of a certain ion channel of the inner nuclear membrane of the perinuclear cistern, provides the opening of a gate for the very short time. During this period, the protein–nucleic acid complex (the so-called fountosome consisting of fion plus fRNA plus accessory proteins plus protein gate of the channel) performs conformational reorganization, allowing a certain dose of ions to slip from the perinuclear cistern inside the nucleus.

The nature of incoming ion is controlled by structure of fountosome, i.e., it depends on specificity of fRNA, fion, and that ion channel which they have opened. The portion of ions thrown into the nucleus is designated as an ion fountain. The principal function of perinuclear cistern, which embraces karyoplasm and chromosomes by two membranes with a space between them, is assumed to be a depot of various ions. From the same perinuclear cistern, different ions are coming into different chromosomal genes due to specificity of fRNA-dependent ion channels. An ion channel produces a short volley in the direction of structural gene that has come near intranuclear membrane with the retinue of its fions. The channel activates for a very short time only in case of tight spatial approach of specific fRNA, fion, and gate of the channel of inner nuclear membrane. A short-term act of local injection of a portion of certain ions into karyoplasm from the perinuclear depot occurs only during conformational reorganization of the fountosome. Then the ion channel closes again, fountosome dismantles, and its fRNA inactivates presumably by means of endonucleolytic activity of fountosome itself. Reiteration of this cycle, i.e., a new act of formation of an ion fountain, is possible only with the novel fRNA molecule that will bind to the same fion. The data concerning the influence of various ions, which could act as ion fountains in a nucleus, on the mRNA processing and level of gene expression are discussed in [8].

According to the fountain model of eukaryotic genome regulation, the sense of the existence of the nucleus in eukaryotes is to modulate the quantitative traits of cells and the organism as a whole, whereas prokaryotes are devoid of this possibility. Evolutionary translocations of fions along the genome, as well as changes of sets of genes within chronomeres and printomeres in evolution, are among the most important causes creating the huge biological variety of eukaryotic organisms on our planet. Each fountain delivers only one ion (for example, Ca^{2+}, Zn^{2+}, Mg^{2+}, K^{+}, or Cl^{-}), but dissimilar combinations of fions in vicinities of certain genes can provide them with such ion environment that drastically distinguishes around different target genes. In its turn, this circumstance exerts local effects on transcription and processing of transcripts, DNA methylation, phosphorylation and acetylation of nuclear proteins, etc. In particular, change in a local level of zinc ions is able to modulate activity of proteins having zinc fingers, especially transcriptional factors, and by that to locally adjust the transcription process. The changes in calcium ion levels nearby genes could control, for example, the duration of existence of complexes of mRNA with proteins, thus changing the productivity of genes, and so on. Beside their effects on quantitative traits, ions could also influence some qualitative characteristics of a cell, more particularly acting via alternative splicing, a process that, apropos, can be somewhat different in aging cells. Those fions, that are separated from their gene by spacers, can be tightened to the gene, by means of folding or unfolding the spacers (and, on the contrary, fions can be kept away from the gene in the course of developmental regulation). This allows the creation of enormous polymorphism in productivity of cells, considerably expanding the capacity of eukaryotic genomes. One can even assert that the principal sense of eukaryotism generally consists in the possession of the ion fountain system, creating huge additional opportunities in achieving of the variety and adaptations in plants and animals, dominance and recessivity of many alleles as well as some epigenetic effects in development [8, 9]. It may be very advantageous for a cell to have the possibility to force one and the same structural gene to provide diverse levels of productivity at the different steps of individual development, and just the fountain system, being controlled by differing sets of redusomes in different cells, could provide such possibility.

Two varieties of redusomes—chronosomes and printosomes—are involved in the processes associated with the control of biological time and aging, but their activity is rather different. The common property of these structures is the aptitude of their DNA, respectively the chronomeric and printomeric DNA, to trim with time, though even their shortening is implemented in quite different ways.

The term "chronomere" ("khronos"—time, in Greek) reflects the role of this structure in measuring biological time, while term "printomere" is used to underscore the idea that cells receive and memorize a peculiar

print about their positional information in morphogenesis (see next section for more details). The principal biological function of chronomeric DNA, or chronomere for brief, consists in the biological time control during individual development, and also (after achieving the maturity state) in the control of duration of the subsequent process of aging that manifest itself in age-related weakening of functions of many organismal systems. It is important to emphasize at once that chronomeres do not impose the process of aging by special genes. Decreasing in length (cause of this shortening is examined below in another section), chronomeres forcedly enfeeble the ionic servicing of chromosomal genes, imitating in this way the chronic weakening of cells, which is an equivalent of cellular aging. Hence, chronomeres can be considered as a physically decreasing carrier of the program that is aimed at the maintenance of viability of the organism, and just this decreasing compels an organism to age as its chronomeric content of genes diminishes. If to designate the length of chronomeres as L, and the rate of their exhaustion as V, then the program of life span should be the ratio L/V, rather than values L or V taken separately. The program of the length of the aging respectively is the ratio L_a/V, where L_a is the length of chronomeres that has been preserved in the central nervous system (CNS) by the onset of aging of corresponding systems, controllable by these chronomeres, and V is the rate of their shortening, which, as it is appropriate to note, can to a certain extent be slowed down in the very advanced age. To carry out their mission, chronomeres should be located in such types of cells that are able to coordinate activity of the whole organism, but do not divide, thereby avoiding those alterations in length of chronomeric DNA that are related to DNA end underreplication and are not connected with the biological time measurement. Neuroendocrinal and neurotrophic cells of the CNS are apparently the best candidacy for this role.

Another variant of redusomes, viz printosomes containing printomeres, or printomeric DNA, serves for many dividing cells. Printomeres have no direct relationship to the measurement of biological time in the organism, though they show *in vitro* the capability to control cell aging. This is probably due to the shortening of the end-underreplicated printomeres, the dividing cells, such as, for instance, fibroblasts, do age at the background of excessive loss of printomeric genes. As well as in the case of chronomeres, the loss of printomeric genes leads to weakening cellular productivity and, in a stepwise mode, to aging. Many types of dividing cells, probably, do not have the opportunity to completely spend *in vivo* the length of their printomeres, even for the whole life of the organism. Therefore, printomeres do not bring crucial contribution in organismal aging which is mainly directed centrally, i.e., through chronomeres of the CNS. But, then, why are the printomeres present in cells at all? The following section answers this question.

PRINTOMERES REMEMBER POSITIONS OF CELLS IN THE COURSE OF INTERPRETATION OF THE POSITIONAL INFORMATION IN MORPHOGENESIS AND, ACCORDING TO THE POSITION, THEY CHANGE FOREVER THE CELLULAR PROPERTIES. SHORTENING OF PRINTOMERES AND AGING OF DIVIDING CELLS

It is known that determination of the whole usually occurs in embryonic development earlier than determination of parts. This statement is related to the universal phenomenon of embryonic regulations which were registered for embryos of numerous species and stages of individual development. The essence of this phenomenon is in that the development of the whole embryo realizes successfully regardless of shortage, surplus, or reshuffle of cells in morphogenetic field (embryonic territory along which the gradient of morphogen concentrations is created) that is exposed to an inducer, or morphogen. It is most essential that the formation of parts of an embryo occurs under these various conditions all the same in the geometrically homologous positions, that is independently of the number of cells and their initial locations. The fact is that the destiny of parts is connected, in an invariant mode, with coordination of these parts relatively to the whole [10]. Such result is achievable (through a system of induction interactions) only due to the establishment of the gradient of inducer concentrations in a morphogenetic field. Cells are able to read and interpret their positional information, i.e., information about their individual positions relative to the source of morphogen (which is situated at one pole of the morphogenetic field). It is supposed here that cells perform this task by creating those printomeres which correspond to a position occupied by a certain cell in the morphogenetic field. A notion concerning printomeres, discussed below, would apparently allow the unification of the so-called genocentric and morphocentric guidelines in embryology that have until now coexisted a bit separately [11, 12].

The concrete way of reading the positional information is one of the crucial, but still unresolved, problems of developmental biology [13]. The mission of printomeres is a resolution of the problem of interpreting and memorizing the positional information during morphogenetic differentiations. In a morphogenetic field, progenitor cells, as is known, should define their own positions on the axis of a concentration gradient of a chemical inducer. Cells recognize somehow, if they are far from or close to the source of morphogen in the morphogenetic field. Initially being quite identical, the cells differing only by location in a field must remember this difference and, moreover, should transfer this memory to descendants. Just this ability lies at the basis of distinction between so-called regulatory morphogenesis (printomeres should exist) and mosaic, or autonomous, morphogenesis (each

cell is differentiated irrespectively of its position relatively to surrounding cells and, hence, printomeres are not required). Regulatory morphogenesis is typical for humans, while mosaic morphogenesis is characteristic, e.g., for *Caenorhabditis elegans* that does not have, by the way, typical heterochromatin where the protoprintomeres could be hidden. In regulatory morphogenesis, those cells that are equipped to make the decision (so-called competent cells) may apply the binary strategy, or choice from two opportunities. Such cells have two protoprintomeres of different specificities prepared for unpacking. The first protoprintomere is unpacking only in that case when the high concentration of inducer is around the cell; territorially that occurs only in close proximity to the source of inducer. On the contrary, the second protoprintomere unpacks very easily, even at low concentration of morphogen, i.e., at the disposition of the cell far apart from the source of inducer. As a result, near to a source of inducer, both protoprintomeres will be unpacked, and both these perichromosomal copies, that is printomeres, will emerge. These printomeres are then replicated and inherited in a train of somatic cellular generations. And vice versa, far from the source of inducer, i.e., at its low concentration around the cell, only the least resistant (to unpacking agent) protoprintomere will appear to be unpacked to produce corresponding printomere. Consequently, the cells located in the morphogenetic field far from inducer source should receive only one variant of printomere. That is why the cells opposed in a position in a morphogenetic field do acquire and can further sustain the diverse specificities controlled by the proper set of acquired printomeres. Exactly this forms the basis for creating of heterogeneous structures from initially identical cells in morphogenesis.

If lipids are used to seal heterochromatin with its protoprintomeres, then the role of the unpacking agents could be performed possibly by free radicals [14] that would initiate lipid peroxidation of corresponding seals of heterochromatin. DNA and proteins are relatively more resistant to destruction by free radicals in comparison with lipids, which are especially vulnerable to destructive chain reactions acting in the course of lipid peroxidation. The intranuclear concentration of free radicals is presumably increased in cells that settle in the neighborhood of the source of inducer. Free radicals could play a role of a can-opener or only the role of the signal that, working through calcium and other secondary messengers, orders enzymes to break the lipid seal on heterochromatin "jug" and to let the protoprintomeric "genie" replicate in order to create printomere. The stated assertion about the reason for printomeres existence is the hypothesis of "printomeric interpretation of the positional information". The main thing in it is, of course, the role of printomeres in interpretation of a position of cells, rather than a concrete nature of the agent leading to unpacking of protoprintomeres. It is necessary to note that, in general, even in regulatory morphogenesis it is not necessary for absolutely all cells to know about their positions. The very important exclusion concerns stem cells, as well as many cells of vegetatively propagated plants, etc. The circumstance that printomeres, with their shortening, are absent from animal stem cells and corresponding cells of vegetatively propagated plants, does help these dividing cells to be potentially immortal (but, certainly, in case of an adequate compensation by telomerase or transposons, etc. of the telomere attrition occurring due to the effect of DNA end underreplication).

Thus, both printomeres and chronomeres perform important missions in ontogeny, though at diverse levels of its regulation. Chronomeres originate in evolution from printomeres not only because a function of definition of the cell positions in morphogenesis should arise earlier than a function of time control. In the course of the brain morphogenesis, cells should interpret their positional information and hence neuroendocrine cells also need printomeres. Afterwards, in the course of phylogenesis, the functions of printomeres could become more complicated and, beside interpretation of positional information, they began to specialize also on temporal functions. The simplest way is to combine both these functions in one structure if the prospective chronomere operates firstly as a printomere, and then (by now in postmitotic neurons) it would process up to the actual chronomere of standard species length. After being processed, the chronomere commences its principal function—it starts to measure biological time.

The number of copies of printomeres in one printosome can vary from one copy up to a whole group of identical printomeres, whereas the number of chronomeres in postmitotic neurons does not possibly significantly vary. It is important to emphasize, however, that even the presence of different number of chronomeric copies in a cell should not radically change their temporal function, as the chronomere shortening takes place only at the end of each linear chronomeric molecule that is irrespective of number of chronomeric copies lying side by side in a chronosome. In order to replicate together with a cell, all its printomeres are obliged to have their own *ori*, whereas the activity of the *ori* should be switched off to prevent an independent replication of printomeres in terminally differentiated cells. As to chronomeres, they should also have their own *ori* if, in the course of brain morphogenesis, they should firstly execute a printomeric function of reading positional information and only later they begin, at last, to perform their main, namely temporal, function. However, even if the chronomere is arising in the postmitotic neuron for the first time, it must nevertheless have the copy of the segment of chromosomal DNA (protochronomere). Since the copying of this segment is impossible without *ori*, both protochronomere and a newborn chronomere in a neuron should have, like protoprintomeres and printomeres, their own *ori*.

Strict observance of a certain number of printomeric copies in a printosome is not, by any means, anything critical for functioning of printomeres, i.e., for reading positional information, as well as for organizing specific expression patterns of chromosomal genes at certain cytodifferentiations. Both these functions are carried out with participation of the fRNAs modulating activity of the nuclear fountain system and the micro RNAs involved in remodeling of chromatin and other processes of chromosomal gene regulation. On the whole, the number of printomeres in a printosome can not be strictly identical in various cells of the same cytodifferentiation, and, as it will be shown in the final sections, has far-reaching biological consequences.

Printomeres of dividing cells are subject to DNA end underreplication that is the same process that has already been predicted and confirmed for telomeric DNA. The printomere has *ori* in a medial part, while acromeres are located at its ends. Acts of printomere replication during each cellular doubling should lead to the consecutive loss of those redusomal genes that are located closer than others to the neighboring acromere at a corresponding arm of a printomere. Immediately after exhaustion of the acromere, the nearest subacromeric gene undergoes nuclease destruction. After this, the nearest acromere-like spacer, having received the free DNA terminus, is transformed into the novel acromere of this truncated printomere. Some types of printomeres can possess a considerable number of repeating specific genes whose gradual lessening in number does not at once affect the state of cellular differentiation. Nevertheless, this process guides a growing decline of proliferative ability of cells in process of cellular doublings. If printomeric acromeres are very long, a significant stock of genes is not required. In view of the inevitability of acromere shortening in dividing cells, those of them, which normally are expected to perform a large number of divisions, should possess printomeres with long acromeres. The length of acromeres is sacrificing simultaneously with telomeres to the process of DNA end underreplication. In all cases, the shortening of printomeres leads to the stepwise slackening cellular functions at the expense of changes in a ratio of the concentration of various cellular factors, and eventually to proliferative arrest. On the whole, it is the shortening of printomeres that appears a key thing that can play a role of that inner mitotic counter whose potential existence was for the first time revealed in Hayflick's studies [15-17].

Each arm of a redusomal DNA is a distance between *ori*, located in the middle part of linear DNA molecule, and the absolute end of a corresponding acromere. With other things being equal, it is favorable for a cell to have acromeres of dissimilar initial lengths on two different printomeric arms. In this case, disappearance of subacromeric genes on both arms will happen orderly, one gene from the first arm, then another gene from the second arm. Such a variant is more profitable with respect to economy of DNA in comparison with simultaneous loss of genes on both flanks. If a printomere has, for example, two genes in each arm, and if they are lost consecutively (each loss, one can assume, does occur after 10 mitoses), then such shortening printomere has capacity for supporting 40 divisions. After a loss of each of these four genes, homeostatic resources of a cell will reduce, and progressing cellular aging will eventually lead to replicative arrest. If genes located and were lost symmetrically, the clone would receive this result earlier.

In a printomere of a young cell, there are more genes than in an aged cell, including the fRNAs genes involved in the maintenance of productivity of structural chromosomal genes that are necessary for efficacious activity of a cell. Such argumentation could be used to explain the facts according to which cells of a young donor migrate from an explant with a faster rate [18], they propagate more rapidly [19], and larger size colonies grow from one cell [20], than in case of cells from aged donors.

What initial length should a printomere have, for example, in young human fibroblasts? If the telomere of a fibroblast is shortening approximately at 100 bp for each of 50 doublings during a proliferative life of a clone, it decreases by 5 kb [21-23]. The same can be attributed to printomere as to linear DNA molecule since it is also shortened owing to the terminal underreplication in the course of cell doublings and synchronously with telomeres whose shortening can be accepted for the approximate reference point. Therefore, 50 doublings could be executed by the fibroblast if the entire length of the acromeric DNA of one arm (an acromere itself and all acromere-like spacers that in a series are converted into acromeres) equals 5 kb. All this is considered without taking into account a role of the second arm of the printomere and the length of all its genes. The lengths of redusomal genes encoding, correspondingly, fRNAs and micro RNAs are, probably, rather moderate, for example, about 50 bp. fRNAs should be small, for instance, in order to successfully interact with DNA of small fions. The number of genes in a single printomere can also be few. For many cases, it will be enough to have, possibly, 5-6 genes in one printomere having long acromeres. If one takes into account the length of both arms, as well as the space for *ori* and transcriptional promoter, then a general suppositional length of printomeric DNA can be estimated as 12-15 kb. An assumption has earlier been declared that the shortening of printomeres (instead of telomeres) could be the initiator of the aging of dividing cells [24]. However, the notion of chronomeres (this is the main hypothetical figurant of the whole-organismal aging) and an idea on existence of the so-called scrupting were still absent. The next section is devoted to the consideration of how chronomeres operate, first helping to create a mature organism, and then, with the same methodicalness, dragging it into the abyss of aging.

PROPERTIES OF CHRONOMERES. BIOLOGICAL TIME IS MEASURED IN INDIVIDUAL DEVELOPMENT BY A CHRONOMERIC RULER. WHAT IS SCRUPTING AND WHY IS IT NECESSARY FOR THE CHRONOMERE?

As distinct from printomeres, the function of chronomeres is to provide the directed current of biological time during eternal recurrence of individual development of organisms. The principal distinction between chronomeres and printomeres is a difference not only in functioning, but also in the way of shortening their DNA. How can chronomeric DNA measure a flow of biological time by its presumptive shortening if neuronic cells do not divide at all over the most part of the organism life and, hence, the process of end underreplication of chronomeric linear DNA cannot take place? Before considering a feasible answer in some details, it is necessary to focus upon some additional properties of chronomeres.

A chronomere is a linear molecule of DNA formed on a protochronomere as its lateral amplification. Chronomeres as well as printomeres, together with proteins covering them, are located in chromosomal nests, or three-dimensionally stacked chromosomal chromatin, forming similarity of a bed for the postulated organelle. The chronomere does not compose a uniform molecule with chromosomal DNA. Like printomeres, chronomeres are, so to say, a perichromosomal DNA, as against an extrachromosomal DNA abandoning chromosomes. It is postulated that measurement of the biological time current with the help of chronomeres is managed by means of the shortening of chronomeric DNA throughout special style of its transcription. The shortening of chronomeres is caused by the process of so-called "scrupting" proposed here and examined below. Scrupting causes formation of the nonrepairing terminal breaches in linear chronomeric DNA. This results, in a stepwise manner, in a consecutive shortening of the chronomere. Disappearance from the chronomere of its next genes encoding various fRNAs and micro RNAs (and, possibly, even copies of some protein-coding genes if such genes are also built in some chronomeres) serves as a temporal signal for the neuroendocrinal cell where the chronomere is located. This signal testifies that the next interval of biological time has expired. Different types of neuroendocrinal cells have chronomeres of different specificity, and, as a whole, in the CNS there is an entire network of a variety of chronomeres assisting with their functioning to order in time all processes of individual development. Not all types of chronomeres should exist in neural cells of an early embryo. It is acceptable that there is a peculiar relay-race of appearance and subsequent disappearance of certain chronomeres. If chronomeres localized in different brain centers do take up the mission of time measurement not all together, but by turns (i.e., in a relay-race mode, that is firstly in one neural ensemble, then in another, and so on) then, if that is the case, the starting length of chronomeres could be greatly reduced, and that would allow the organism to economize the size of the DNA of a nuclear genome. Everything that is required for this purpose is the selective response of respective cellular ensembles to the signal that impels chronomeres in a corresponding neuronal ensemble to shorten. A minus of such tactics is the necessity of existence of the nonrandom succession organized on the basis of these cellular ensembles. Loss of an acromere, i.e., the chronomeric buffer, results in an immediate loss of the nearest subacromeric gene, that is now unprotected by the acromere, and hence it should be destroyed right away by nucleases. With the loss of an acromere, the next acromere-like intergenic spacer must be recruited, being subjected to processing converting this spacer into a novel acromere, thus protecting the remaining genes in the chronomere (as well as in the printomere). So, the chronomere is a peculiar dynamic structure. The length of the chronomere correlates with the number of genes contained in it. As the number of residuary genes decreases in chronomeres, it allows the cells owning chronomeres to estimate the value of biological time that has already elapsed.

While the acromere, even though it is decreasing in length, has not disappeared yet, the biological time is being kept unchanged, it is "frozen". Therefore, at least at the level of an individual neuroendocrinal cell, biological time does not flow smoothly. Measuring time in a cellular biochronometer is carried out by spurts since chronomeric genes disappear in a stepwise mode. If cells of corresponding neural ensembles operate strictly synchronously, then the levels of different hormones and other factors controllable by the CNS should also be altered stepwise, rather than smoothly, during the life of an organism. If, however, the cells within a neural ensemble do not coordinate themselves too strictly in this respect, then such cell groups hand out, at the levels of different releasing factors and hormones, some averaged characteristics of biological time. In this case biological time at the level of the whole organism does flow more smoothly.

The starting length of acromeres, undoubtedly, is an object of evolution, as well as genetic composition of chronomere. Because of this, in order to correctly exercise the function of biological time measuring, the length of chronomeric acromeres should not be a target of telomerase activity under any circumstances. For this reason, in neuroendocrinal and neurotrophic cells of the CNS where chronomeres are in action, at least one of the two conditions should be obeyed: a) the sequence, recognizable by telomerase in acromeres, should be absent from chronomeres, or b) the telomerase activity itself should be strictly prohibited in corresponding neurons. As distinct from the human or murine CNS, the telomerase activity in the brain of adult rainbow trout is not

switched off [25]. Hence, the acromeric sequence in their chronomeres should unconditionally not contain the sequence T_2AG_3 recognizable by telomerase.

If any species, having reached a state of maturity, passes to use the other chronomeres which would be extended by telomerase (or if some species refuses to use chronomeres at the adult stage of ontogeny) then such organisms could not potentially become old at all. It is usually considered that nondividing cells cannot avoid the process of aging. But what does an opportunity of existence of nondividing human neurons that effectively works during decades of years testify to, meanwhile the fruit fly neurons do age and die during only several months? Is it explainable via the weakness of the fly repair system? Perhaps the true reason consists in some other circumstance, namely in that the most differentiated eukaryotic cells (including the nondividing neurons) can maintain their young state only while they have a sufficient reserve of redusomal genes. Owing to these genes a differentiated cell can keep ability for an efficient repair of casual damages. In addition to the above mentioned opportunity to protect the organism from aging (substitution of chronomeres with acromeres of T_2AG_3 type, that can be lengthened by telomerase, with novel chronomeres having acromeres of non-T_2AG_3 type), there is another possibility to cease the time measuring and, hence, to stop aging. Instead of expressing the novel chronomeres, some species could have, from the very beginning of their development, chronomeres with acromere-like spacers of two types. Distant chronomeric genes are protected in them with acromeres of non-T_2AG_3 type (they do not lengthen even in presence of brain telomerase and, being normally shorten, are used during individual development to measure a time), whereas acromere-like spacers located in the middle part of the same chronomeres are of T_2AG_3 type. When organismal maturity is achieved, these medial acromere-like spacers may be converted into acromeres which now can be elongated by telomerase. This will stop the current of biological time and prevent further changes of biological age of the individual.

The organization of the correct schedule of events of individual development which should be precisely allocated in time rather than only in space of a growing organism is, thus, delegated to chronomeres, including their length and genetic content. Disappearance of some genes from chronomeres which, for example, have supported the syntheses of inhibitors of certain developmental processes, should elicit the onset of deployment of such processes, etc. On the whole, as postulated here, the set of chronomeres of the CNS, as well as the so-called system of T-rhythms and fountain system of a nucleus, constitute conjointly the mechanism which works as a timer of individual development.

Maintenance of stable functioning of chronomere should be supported by protection from unscheduled nuclease attacks and, undoubtedly, it should not leave its chromosomal nest. Fastening both chronomeres and printomeres in their chromosomal nests is executed at the expense of interduplex G–G interactions and with participations of proteins bridges. However, only a part of redusomal DNA is involved in such interactions, mainly it is its G-rich acromere-like intergenic spacers and acromeres. Interduplex G–G interactions take place between the specified sequences of redusome DNA and the homologous sequences of chromatin of the chromosomal nest. Both in chronosome and printosome, the ends of linear DNA molecules should be protected from nucleases. Loss of acromere as a buffer DNA immediately results in the loss of the unprotected subacromeric gene, and the next acromere-like intergenic spacer begins to process in chronomeres just as in printomeres. The absolute termini of acromeric DNA of these organelles are fixed by their contacts with DNA and chromatin of chromosomal nest that reminds of interactions of the absolute end of the telomeric DNA. It is known that telomere DNA forms a loop and the telomeric end becomes locked within its own chromosome. In case of redusome, however, the termini of redusomal DNA become locked on the body of chromosome, rather than on the body of redusome itself, and owing to this, at least two purposes are achieved. Besides protection from exonuclease, the second, not less important for chronomeres, purpose is pursued by this locking of acromeric terminus in the chromosomal nest. This goal is a creation of the precondition for the subsequent display of the main ability of scrupting, namely mechanically induced destruction of the DNA terminus. This event can be realized only if the end of acromere has preliminarily been immobilized (variants of scrupting are considered below).

Shortening of chronomeric DNA is carried out at the expense of a process of so-called scrupting. The term "scrupting" is formed by elimination of unnecessary letters from the expression "transcription-coupled rupture of terminus". The scrupting is implied to be of the excessively high-rate transcription of the redusomal DNA resulting in a mechanical break of the connection between the terminus of acromeric DNA and the site of its anchoring within chromatin of the chromosomal nest. Physically, the cause of this event is a creation of critically high mechanical strain in the redusomal linear DNA molecule that is formed near the immobilized terminus of transcribing acromere. Redusome genes encoding untranslatable small RNAs do not have terminators at their ends, thus permitting continuous transcription of redusomal DNA beyond the end of last subacromeric gene. Hence, acromeric DNA can be transcribed together with redusomal genes, although it is a noninformational buffer DNA. Detaching the end of acromeric DNA from the anchoring site (i.e., break of noncovalent connection) as well as a break of covalent bond in acromere DNA, is possible only when the RNA polymerase comes

at an extremely high rate in close vicinity to the immobilized absolute terminus of redusomal DNA, creating in it the highest tension of the DNA molecule. Acts of scrupting recur on a regular basis, but this recurring is rare. Normally, these acts are designed only for the shortening of chronomeres rather than printomeres. Nevertheless, printomeres, especially under stress conditions of cell culturing *in vitro*, also can become subject to scrupting. How often do acts of scrupting normally recur and how are the intervals between them regulated? These questions are considered below in a section dealing with the T-rhythm.

Unlike printomeres, chronomeres reside in nondividing cells of a brain and consequently cannot be shortened at the expense of DNA end underreplication that happens only in dividing and, hence, replicating cells [26-28]. For that reason, nature had to invent, for the sake of functioning of presumptive chronomeres, a system that could work even in the absence of replication. Transcription that is in action in every cell could, to all appearances, especially match this goal, would it actually be able to lead to shortening DNA in the course of scrupting. If that is possible, nature hardly could pass by. The mechanism of scrupting could function for the welfare of the embryonic and postnatal development, including the achievement by the organism of the maturity state. Further, the scrupting continues to serve for cells, but now scrupting results already in aging because of a continued expenditure of the remaining genetic resource of chronomeres. With loss of these last genetic "emergency ration", the activity of an organism fades.

Scrupting produces terminal unrepaired breach at the very end of acromeric DNA and just that results in the shortening of chronomeric DNA. A chronomere created by copying of a protochronomere has at the very end of each its acromere the 3′-terminal overhang, like telomeric overhang. So, initially the chronomeric acromere, already on the start of its existence, has a terminal irrepairable breach, and henceforth this state of affairs is being maintained, yet the end breach is stepwise displaced to the center of the shortening chronomere. During acts of scrupting, transcription of acromeric DNA stops near the shorter 5′-terminus of transcribing strand of the chronomeric DNA. Conditions of DNA repair at the end of a linear DNA molecule have fundamental features distinguishing this process from DNA repair at the medial part of a molecule. If, for example, a one-stranded break appears near the absolute end of DNA, it should inevitably result in the process of so-called DNA end underrepair [29, 30]. The process of DNA end underrepair is partly reminiscent of the process of DNA end underreplication. It can take place even in nondividing cells. The inherent inability of DNA polymerase to repair the short terminal breaches in redusomal DNA is related by two principal circumstances.

If the 5′-overhang (that is the 5′-terminus protruding) locates in a breach, while a shorter 3′-end is in the second strand at the distance of one or several nucleotides from the longer 5′-terminus, then the end of the shorter strand cannot be used as a primer. The reason for that is a protruding state of the repair machinary outside a template; such "protrusion" is incompatible with the normal work of the enzyme that, apparently, is not able under these conditions to bring its catalytic center to the terminal nucleotide(s) of the template, not having lost the normal full contact with the template. Therefore, a repair of the short end breach that has 5′-overhang appears to be impossible. This leads to DNA shortening owing to the subsequent destruction of 5′-overhang at this irreparable short breach. It is an essence of the first variant, but one more is also possible. If the end breach has 3′-overhang, then the DNA polymerase of repair synthesis cannot restore terminal gap simply due to the incapability of all polymerases to extend the 5′-terminus of DNA. In both considered cases, the linear DNA molecule appears to be shortened by the length of the unrepaired terminal breach. This is an essence of the phenomenon of a DNA end underrepair. Owing to it, the consequence of scrupting, i.e., just the occurrence of small terminal breach at the end of redusomal DNA, appears to be fundamentally significant for the fate of chronomeres and their ability to measure biological time. As to the telomerase, it could interfere with the length control of chronomeres, increasing their lengths and, hence, impeding the time measurement. However, such hazard is possible if telomerase is present in neural cells and only if their chronomeric acromeres have telomere-like T_2AG_3 repeats recognizable by telomerase.

Having accepted that occurrence of terminal breaches actually could result in shortening of chronomeres, it is necessary to answer in more detail the key question: what is the primary cause of occurrence of such breaches, in other words, what are the details of the scrupting? Common acts of transcription proceed constantly and if each of them were able to remove even a single nucleotide from DNA, then any acromere and the whole chronomeres would be destroyed very quickly. Therefore, if the proposing mechanism of scrupting, i.e., the shortening of linear DNA molecule in the course of its transcription, is really used in biological time measuring, the scrupting should take place only under truly unique conditions. Such unique and relatively seldom recurring situations are the postulated acts of the so-called "superhigh rate" transcription of chronomeres. High speed of movement of polymerase machinary along DNA molecule is required here in order to initiate the critically high torsional strain in it. This strain could be able either to provoke the break in acromere or execute the tearing off of the terminus of the acromeric DNA from its site of anchoring in the chromosomal nest. In the latter and most probable case, that is after disconnection

of noncovalent connexion of the acromeric terminus and chromosomal nest, the supplementary enzymatic processing of acromeric terminus could be carried out. This processing will enzymatically and strictly regulatorily have truncated the acromere terminus by the small and standard number of nucleotides before this terminus is again hidden in the nest and anchored there at the chromosomal DNA.

In the course of progress of RNA polymerase along the non-elastic DNA molecule, the torsional pulse-like waves should migrate [31]. However, the fixedness of the end of acromeric DNA in a chromosomal nest puts an insurmountable hindrance for further spreading of the waves. In case of extremely high-speed motion of the transcriptional complex, the collision of waves with the obstacle can indeed generate tension in redusomal DNA. The tension reaches its maximum near the immobilized end of the acromere, namely between the last nucleotides of the acromeric DNA anchored at the chromosomal nest and the transcriptional machine swiftly coming nearer to the fixed DNA terminus. In this narrow zone of the highest local torsion of DNA, the topoisomerases, to all appearance, have insufficient free space to carry out the activity normally accessible to them, i.e., to drop an excessive strain of the locally supertwisted acromeric DNA. If termini of DNA have no possibility to rotate, the supertwisting arises within any transcribing DNA molecule, including acromeric DNA whose terminus is immobilized at the chromosomal body. This creates a precondition for scrupting, paving the way for discharging the acromeric terminus from the captivity for subsequent processing and shortening.

The just now shortened chronomeric acromere should necessarily be protected from the recurring unscheduled acts of its shortening. How to avoid this situation, i.e., how to exclude the possibility according to which any next molecule of RNA polymerase would immediately lead to one more loss of several nucleotides from the acromere terminus, and so on. If the suggested scenario is true, something preventing such a turn of events should exist. One can predict that there is a mechanism providing realization of only a single act of scrupting during one peak of the special hormonal biorhythm, or the T-rhythm (see about it in the following section). The maximal hormonal and other activity related to T-rhythm peak lasts not very long—probably nothing more than around 10 min. This peak may recur again only the next day, one or two weeks later or, for example, even once per month—in different animal species it occurs, obviously, in a different way. However, even ten minutes are actually enough to totally ruin the whole acromere, would the acts of scrupting follow one by one continuously. Nevertheless, the required rare and solitary acts of detachment of acromere from chromosomal nest, with the background of superintensive transcription of chronomeres, are achievable and, moreover, very easily realizable. The matter is as follows. The single detachment of acromeric end from the site of its immobilization will relax DNA immediately. An uncontrollable unwound chronomere DNA will not be a very convenient template for a highly intense transcription; at the same time, RNA polymerases will not now be able to recreate a superstrain state in the acromere terminus, since it is, at this time, loosely dangling, having been liberated from captivity within the chromosomal nest. This state, in its turn, prevents further shortening of the chronomere terminus since it cannot now be forcedly broken. At this stage the terminus must, of course, be protected by accessory proteins to avoid its unscheduled hydrolysis by illegitimate nucleases. Thereby an opportunity for immediate recurring acts of scrupting is abolished. The shortened chronomere restores protecting its absolute acromeric DNA termini by hiding them again into the chromosomal nest with participation of chromatin proteins and the chromosomal DNA itself. A full restoration of a three-dimensional design of the acromere concealed in the chromosomal body allows reinstating a normal torsional state of chronomere DNA which is optimal for future effective transcription of chronomere. However, this process of re-establishing the completely functional acromere construction within chromosomal nest is long, actually it requires probably several hours. A quick guard of the free acromere end by protective proteins is only the beginning of the long procedure dealing with reconstruction of the necessary three-dimensional nucleoproteinic structure, or the acrosome, built anew in a chromosomal nest; this is a very complex and time-consuming procedure. Apparently, the time is mainly spent for the correct intrusion of the acromeric terminus into the chromosomal DNA double helix. After the scrupting has been completed, the arm of chronomere with its acromere should be settled again in the same nest which size, however, has remained totally invariable, since a nest is the cavity that is spatially arranged by chromosomal chromatin folding rather than by configuration of the redusome itself. The unchanged chromosomal bed appears to be rather big for the redusome that has been truncated in the course of the recent act of scrupting. Because of this, both interacting structures, that is, a redusome and its nest, are needed in the mutual spatial adjustment that requires motions of redusomal and chromosomal chromatin, and all that can, probably, demand up to several hours. Only after such time interval, one can await that chronomere will again be potentially ready to be subjected to the next act of scrupting. Before that its acromeric DNA cannot be "scrupted". However, for the superhigh-rate transcription that is necessary to scrupt an immobilized DNA terminus to occur, as already mentioned, the maximum of hormonal activity is required that happens only at the T-rhythm peak. The T-rhythm is probably adjusted in evolution in such a way that its duration is relatively short (for instance, 10 min). If so, up to the moment when the total

completing of scrupting (including the acromere shortening and its final processing) is successfully accomplished, the peak of the T-rhythm has already been a very long time ago safely over. That is the reason why one and the same peak of the T-rhythm cannot induce two or more consecutive acts of scrupting in a chronomere within a short time interval (e.g., within several seconds or minutes). A new act of scrupting is possible only with the arrival of the next "decuman wave" of the T-rhythm whose peaks are recurring only, for example, in a couple of weeks or in a month, etc. depending on a specific type of the T-rhythm, typical for certain species of animals.

It is appropriate to mention that the essence of reasoning concerning acromere shortening through scrupting does not vary significantly depending on its different variants. One of them is simply a mechanical break of the acromeric DNA which is not able to withstand the pressure of the of transcription machinary impelled to a superhigh-rate movement along a template by factors at the peak of T-rhythm. The second variant of scrupting could be realized a little otherwise. A single-stranded break near the end of acromeric DNA appears due to some endonuclease of the transcriptosome that activates only in that rare case when the transcriptional complex finds itself at the critically supertwisted DNA segment. This variant, however, is hazardous to any cell since it is coupled with fabrication of a significant number of nicks even in chromosomal DNA, would transcriptional machine produce them, having met with a mechanical obstacle during a very intensive transcription; though it is known that cells have a mechanism of a transcription-coupled DNA repair. One more, the third, so to say, mechano-enzymatic mechanism of scrupting could exist. Its essence consists in the following. In the course of a superhigh-rate transcription, transcriptosome, having created the extremely high torsional strain at the acromeric terminus, could pull out, literally with a root, the absolute end of the acromeric DNA that has been anchored within the chromosomal body. Immediately after this mechanical uprooting has been performed, the enzymatic processing of the liberated end of acromere begins, but it lasts a very long time (probably several hours). The purpose of this processing is to shorten enzymatically the acromeric terminus up to the strictly standard size and, after that, to prepare and execute the process of intrusion and anchoring of the very end of acromeric terminus (which is now short-cut) into the chromosomal DNA of the nest. In this third variant, the size of shortening is under control of the protein complex, or acrosome, which is immediately forming at the butt of acromeric DNA double helix just uprooted from its chromosomal nest. This third variant should be enzymatically similar to the events occurring during transformation of an acromere-like spacer to acromere when the initially intergenic spacer appears to be on the free end of a decreasing chronomere. Such event regularly happens right after the chronomere, having already lost its acromere, loses the nearest subacromeric gene, too. Processing comes to an end, as mentioned, by immobilization of the redusomal end within the chromosomal nest. As to the acrosome structure, it is most probably evolutionally related to those protector protein structures that exist at the very end of telomeres [23], though acromeric terminus is built in a non-self DNA molecule (that is into chromosomal rather than redusomal DNA), whereas the telomeric end, making a loop, inserts, as known, into its own chromosomal DNA. The latter two variants of redusomal DNA shortening do have some advantages over the first variant with its purely mechanical breakage of DNA, because they permit to increase the accuracy of the length which the acromere will be shortened by. Finally, if to choose between them, the latter variant, that is the mechano-enzymatic variant, seems to be the safest for the genome, as it does not elicit off-scheduled damages within the chromosomal DNA; besides, it is supervised by acrosome proteins constituting a specific ensemble at the butt of the acromere. Proteins of the acrosome can identify the acromeric sequence represented by certain repeats, like a telomere, but not obligatorily by definite telomeric hexanucleotides. The presence of certain repeats in an acromere would facilitate recognition of proteins of acromeric terminus and assemblage of multiprotein acrosome at it. In addition, the presence of definite repeats in an acromeric DNA would, probably, help to accurately orientate acrosome proteins when they choose the precise point of the single enzymatic incision at the acromeric DNA. In this most feasible variant of scrupting, the acromere length will be spent especially economically and in a very controllable mode. Under enzymatic processing of the acromeric end, just liberated from an immobilizing captivity, the enzymatic complex of the acrosome, being guided by the acromeric butt and repeats (e.g., hexanucleotides by analogy to telomere), could remove, for example, only one terminal repeat, and finish on it the shortening of acromere. Thus, in this case, six nucleotides would be deleted from the DNA strand for one act of scrupting. The nucleotide sequence of an acromeric repeat as well as the removing length of DNA can be, however, distinctive for different species.

Genes of chronomeres and printomeres (in contrast to usual structural genes of chromosomes) have no terminating sequence. As a consequence, RNA polymerase, leaving the confines of the uppermost (i.e., subacromeric) gene, continues transcription, now transcribing a non-informative acromeric DNA. In a usual (not a superhigh-rate mode) of transcription, RNA polymerase, at the occurrence of the ineradicable DNA supertwisting near the acromere end, simply disconnects from the template, without rendering any harm to the DNA molecule. Because of this, the acromeric DNA, even near its terminus fixed in the nest, keeps native structure and is not

exposed to a shortening in intervals between peaks of the T-rhythm. But as soon as there comes the next "decuman wave" of T-rhythm, the act of scrupting reiterates owing to the sharp acceleration of movement of RNA polymerases. By the way, the ability of individual RNA polymerases to run along the template with different speeds has been shown by direct observations [32-35].

It is necessary to notice that the presence of transcription terminators in chronomeric genes would deprive a chronomere of its ability to keep measuring time. The long transcripts arising in the absence of terminating sites at the end of redusomal genes should process up to the low-molecular-weight RNAs, starting then the marking, repacking of chromatin, and the regulation of the chromosomal transcripts processing (in case of micro RNAs) as well as the quantitative regulation by ions of the levels of chromosomal gene expression (in case of fRNAs). Off-scheduled acts of a superhigh-rate transcription, e.g., under intensive stresses, can accelerate the beginning of aging. On the other hand, an artificial creation, for example, in an adult organism, of transcriptional termination at the border of subacromeric genes would be equivalent, by its consequences, to immortalization, since in this case chronomeres would cease to be shortened. So, rhythmically repeating acts of scrupting of chronomeres could be used in order to ensure ticking of the biochronometer in the CNS whose operation is vital for the individual development. Having discussed the process of scrupting, which is initiated by peaks of T-rhythms, it is necessary to consider in more detail the questions concerning these biological rhythms.

VARIETY OF BIOLOGICAL RHYTHMS—THE T-RHYTHM AND ITS ROLE IN BIOLOGICAL TIME MEASURING

Time, as is known, is a form of consecutive change in the phenomena and states of matter characterizing a continuance of their existence. Biological time is duration of consecutively superseding events of an individual development, measurable and controllable in higher animals on the basis of changes of a genetic composition of shortening chronomeres. The totality of chronomeres makes the basis for a peculiar biochronometer located in the CNS and operating through rhythmically repeating acts of the shortening of chronomere length. Biological time does not flow smoothly, but stepwise. The course of biological time is independent of astronomical time.

Was it possible to pay off for solving the key problem of biological time control by a certain reasonable nucleotide price, i.e., not too large losses of chronomere length? It is important to take into account that the price can be reduced by means of increasing intervals between acts of scrupting, would they reiterate with some low frequency, in once-a-day mode or even more seldom, e.g., once in many days. For organizing required rare acts of a superhigh-rate transcription which would lead through scrupting to shortening of chronomeres, the peaks of an already mentioned T-rhythm involved in regulation of the individual development and its final phase—a biological aging—could be used. Long intervals between maximums of T-rhythm conform to the intervals when biological time does not flow; it is as though frozen. In the term "T-rhythm" the letter "T" reflects its participation in a temporal function, i.e., in the control of biological time. T-rhythms are probably certain infradian hormonal biorhythms. A complex system of biological rhythms, as is known, includes the ultradian rhythms (they have a period between two maximums less than a day), as well as circadian (daily), infradian (the period is often equal to some days or even to several weeks), seasonal, circannual (yearly) and other rhythms. Infradian biorhythms of the neuroendocrine system could be most suitable for the role of T-rhythms. T-rhythm can be based on one hormone, but a combination of several hormones and other factors is more feasible. Infradian T-rhythms are especially optimal for the postnatal period of higher animal development, whereas short-term T-rhythms are, probably, more propitious for some stages of a prenatal development when biological time should flow especially rapidly, as the events of individual development during this period are strongly pressed in time. For animals, whose species life span is only a couple of months, as for some insects, it is more favorable to use circadian T-rhythms while in an adult life of long-living animals, such as elephants, the infradian T-rhythm seems to be more appropriate for their chronomeric regulation of individual development. Thus, for each species, the corresponding parameters of T-rhythms could differ considerably.

The main target of T-rhythm signals is the brain. Hormones secreting at the peak of T-rhythm may exert stimulating influence upon neural cells bearing chronomeres. Intensive stimulation of transcription in target cells is supposed to be achieved not so much at the expense of the high concentration of hormones produced in an environment of cells but at the expense of the specific frequency of the hormonal bursts following one after another. The maximum of hormonal secretion of T-rhythm is organized in such a way that a pattern of bursts at the peak of T-rhythm is quite nonrandom, and very short intervals (minutes and seconds) separate individual bursts within a single peak of T-rhythm. The frequency of succession of these splashes bears important information, whose sense is to specifically impel the neuroendocrine target cells to begin a superhigh-rate transcription of chronomeres, and biochronometer hands in the brain make the next step. The sense of such selectivity is to prevent an idle shortening of chronomeres at peaks of any transcription stimulating factors. The levels of different stimulating factors can oscillate in organisms, as is known, both with a frequency of short-term ultradian

rhythms (their periods are from several minutes up to several hours) and, for example, even with frequency of seasonal biorhythms. Hence, T-rhythmic bursts should be very specific to exclude any unfavorable interference with any unauthorized factors. In reply to specific frequencies of T-rhythm hormonal bursts, the target cells generate, at the nonrandom frequency, oscillations of intracellular calcium. In their turn, these calcium oscillations launch, in a rather selective and precipitous, abrupt manner, the very intensive syntheses in target cells, providing realization of the superhigh-rate transcription of some genes, including chronomeric genes, which is necessary for scrupting. Among hormones involved in the organization of T-rhythms, one can envisage in particular, the growth hormone and/or insulin-like factor. Further in this section, some facts and considerations testifying, though indirectly, in favor of such order of events are expounded.

Why is not it possible to use, as a signal for target cells, simply the highest concentration of released hormonal factors? This pathway is unrealizable because it demands from receptors that they overcome an insuperable hindrance, namely, to recognize the suprathreshold hormonal concentrations that would initialize a hyper intense transcription. Such way is dead-end due to the existence of the phenomenon of desensitization via dereceptorization (that is, a removal of receptors from the cell surface in the presence of a redundant concentration of specific ligand outside the cell). Contrary to this, the use of frequency of hormonal bursts is deprived of such a difficulty because the very short as to their duration hormonal bursts within one peak do not result in dereceptorization, and burst frequency can be transformed into oscillations of secondary messengers of a cell, at least at the level of calcium oscillations.

What kind of hormones should be indispensable participants of T-rhythms? The growth hormone (GH) plays one of the key roles in energy metabolism, and the insulin-like growth factor (IGF-1) is one of the principal hormones involved in realization of effects of GH and proteins (GHBP, IGFBPs) coupling the activity of these two hormones [36]. In *C. elegans*, for example, mutations weakening signals of insulin/IGF-I system have been shown to be able to redouble the life span of the worm, and it appeared to be possible to easily observe, using Nomarsky optics, the alterations inside its aging tissues composed by transparent and nondividing cells [37]. In mutants, these changes have been developed in a slowed-down rate. Taking into account a special significance of the energy metabolism for the rate of aging, it seems reasonable to assume that T-rhythms, with their crucial role for performance of scrupting should, by all means, be coupled with a rhythmic hormonal activity of the GH/IGF-I axis. This rhythmicity should occur as rather seldom recurring peaks of infradian T-rhythm, for example, in an adult elephant (e.g., one peak per month) and, besides, in a specific pattern of rhythmic hormonal bursts that quickly follow one after another during this T-rhythm peak lasting only, e.g., 10 min. Each maximum of T-rhythm organized with participation of the GH/IGF-I axis and other coupled systems carries away, through scrupting, a small, but irreplaceable, part of chronomeres. Functioning of T-rhythms can be connected besides to the activity of the hypothalamus–pituitary–adrenal axis also with other endocrinal axes and systems, among them an important role being played by the epiphysis producing melatonin. This hormone is synthesized on the basis of a rhythmically carried out transcription [38]; it can influence the expression of genes regulating in liberation of calcium from cellular depots [39]. It is a hormonal pacemaker governing a number of physiological functions [40, 41]. As noted above, it is expedient to use the frequency of hormonal bursts as a signal for initializing the critically high-rate transcription, which results in chronomere shortening. This approach allows not only to bypass the problem of desensitization of target cells concerning ligands, but also to make the whole process entirely independent of individual deviations in absolute concentrations of various hormones, which are to a certain extent dissimilar in various individuals of the same species. Different systems of intracellular regulation, including the calcium system and some other secondary messengers, should, probably, respond specifically to the corresponding hormonal burst frequencies. Concerning the calcium system, it is known to be competent to change, in an oscillatory manner, concentration of free ions of calcium, redistributing them between various compartments. There are observations indicating that intracellular calcium oscillations are competent to modulate the process of transcription of nuclear genes. Normally, inositol-1,4,5-trisphosphate (InsP3) releases calcium from intracellular depots, and this results in generation of complicated waves and oscillations of free calcium in cytosol. It was shown that the induction in cytoplasm of these oscillations, which were induced with the help of an analog of InsP3, was able to raise the intensity of specific genes transcription in T-lymphocytes. Moreover, gene expression turns out to sharply increase if the analog of InsP3 has been added to cells with 1-min intervals, not less often and not more often. Furthermore, when the plateau of InsP3 concentration was kept unchanged, the mentioned effect of sharp stimulation of transcription did not take place [42]. It was also revealed that oscillations of cytosolic $[Ca^{2+}]_i$ allow to lower a calcium threshold during activation of transcriptional factors, thus enabling the cell to effectively respond even to very low levels of stimulating agents [43]. In addition to this, it was found that a quite certain frequency of oscillations is important, rather than merely any periodicity as such. Fast oscillations of $[Ca^{2+}]_i$ were able to stimulate all the three investigated transcriptional factors: NF-AT, Oct/OAP, and NF-κB, whereas rare oscillations stimulated only the NF-κB. The biological sense of such a char-

acter of this activation could be connected to the ability of cells to respond via calcium oscillations to periodic hormonal emissions and, by means of the hormonal orders, to change consistently the levels of gene expression depending on the situation. There are various examples of calcium oscillatory signaling that transmits the information from the outer cellular membrane activated by interaction of its receptors with various ligands, including hormones, to genes [44]. Synthesis of mRNA of one of the gonadotropin subunits was selectively stimulated *in vitro* in pituitary cells after they have been subjected to rinsing by the activator of calcium channels every 15 min, rather than one time per hour [45]. It is observed that liberation of calcium from cytoplasmic stores accompanied by a certain oscillatory pattern was coupled with the action of nuclear calcium upon the CREB-dependent transcription in neurons [46]. Efficiency and specificity of activation of genes in T-lymphocytes were influenced by the amplitude, duration, and pattern of calcium oscillations, i.e., their kinetic characteristics [47]. Induction of low-frequency calcium oscillations evoked in epithelial cells the activation of transcriptional factor NF-κB [48]. It is proved that it is the frequency of cytosolic calcium spikes (but not their amplitude) that is responsible for the specific increasing of the transcriptional activity of factor NF-κB in nuclei of endothelial cells of the aorta [49].

The specified calcium oscillations themselves are hardly capable to choose in chromosomes precisely those genes which they should stimulate. How then is the revealed specific stimulation regarding certain genes reached? The solution of this mysterious question proposed here is as follows. Cytosol calcium oscillations are indirectly reflected, through interaction with perinuclear factors, either: 1) in the total concentration of free calcium within the whole perinuclear cistern, or 2) on the availability of free calcium (and, possibly, of other ions that are also displaced from the bound state) just for the intracisternal face of each ion channel of the perinuclear cistern. The proper ions, being directed by topographically specific activity of fRNAs bound to complementary fions of chromosomal DNA, come, through these channels of the fountain system, to the definite chromosomal genes. It is supposed that calcium oscillations concerning the perinuclear cistern could exhibit themselves, under these conditions, through one of the two following ways or in their combination. First, a local increase in concentration of the intracisternal $[Ca^{2+}]_i$ in a close proximity to the entry of the intracisternal face of a channel can increase the portion of particular ions entering via the channel when the channel gate will be fRNA-dependently opened from the karyoplasm side of the inner nuclear membrane. Second, oscillations of intracisternal calcium could probably influence the conformational activity of channel proteins in the inner nuclear membrane. It is even possible that, among the ion channels of an inner nuclear membrane, there is a specialization in the ability to be "preactivated" under the influence of certain patterns of calcium oscillations occurring inside the perinuclear cistern. This implies that in the intranuclear membrane, there is a series of subfamilies of ion channels which, probably, respond only to the double-sided stimulation: 1) stimulation by a certain frequency of calcium oscillation at the intracisternal face of a channel, and 2) simultaneous stimulation of the channel gate by the fRNA-dependent nascent fountosome formed at the karyoplasmic face of the same channel. Thus, here postulated is the double-sided activation of the ion channel that opens for a short time only under the action of two keys at once, like some safes. Free calcium could play a role of the regulatory key not only for calcium channels, but also for other ion channels, liberating (inside the perinuclear cistern) ions of the other nature, including zinc ions, from their bound up with proteins state. However, a topographic specificity regarding chromosomal genes, manifesting itself in selective influence of different ion channels of the inner nuclear membrane in quite certain chromosomal regions, is reached in all cases just at the expense of the mechanism of the fountain ion system [8, 9].

So, it is supposed that the definite frequencies characteristic of the hormonal bursts operating in T-rhythms, acting through the intracellular calcium oscillatory system, create preconditions for organizing a necessary super intensive transcription of certain nuclear genes. In the context of the redusomal mechanism of biological time measuring, those biological rhythms which allow the performance of acts of scrupting with observance of an optimal balance between the economy of chronomeric DNA, and the necessary rate of biological time current are of especial interest. There are a number of infradian biorhythms whose length of period is principally favorable as a basis for corresponding T-rhythms. Different rhythms with long periods have been found in humans and animals. In prematurely born children, the circaseptan (about a week's duration) and circasemiseptan (of a half week's duration) rhythms of arterial pressure manifest themselves even earlier than the circadian (daily) rhythm. It was asserted that the weekly rhythm has been set by the moment of a birth, and that is not a sequent of influences of a social week cycle [50]. Circaseptan rhythm is typical for a human electrocardiogram [51]. Studies of growth periodicity of healthy children and rodents have also revealed an obvious infradianity of corresponding rhythms. Measurements of augment in length of a shin have specified sharp spurts of growth each 30-55 days; these spurts are a consequence of interfering of the more short-term mini-spurts following one after another with different shorter periods among which intervals in 7-9 days prevailed [52]. Similar measuring of growth in rats has also allowed establishing nonlinearity of the augments of shin length. In rodents, the mini-spurts followed one after another with intervals about 4-5 days; in females, the

periods of a growth rhythm appeared to be a little bit shorter than in males [53]. The rhythm of changes of the rate of growth in length in salmon fish *O. kisutch* has a period about 14-15 days [54]. In dogs of grey collie breed, cyclic hematopoiesis was found with a period of about 14 days; this cyclicity was steadily supported despite such provoking interventions as bleeding, hypertransfusion, and hypoxia [55]. Cyclic erythropoiesis with a periodicity in 16-19 days has been found in some strains of mice, and it was possible to eliminate it by splenectomy [56, 57]. Cyclic fluctuations of diverse factors (such as hematopoietic oscillations, changes in ferritin concentrations, and body temperature), that recurred with the regular intervals in 7-8 days, were ascertained for a patient of a hematological clinic [58]; in another patient, synchronous cyclic neutropenia and thrombocytopenia have been shown to repeat every six weeks [59]. The analysis of cases of the spontaneous pneumothorax in men has revealed a rhythm with a 14-day-long period, and the maximal number of cases was grouped around a new moon [60]. The periodicity, with the period of a half of a lunar month and with the minimum at a full moon, of acts of aggressive behavior was observed in the Czech population. Periodicity of cases of sudden cardiovascular casualties [61] was a fortnight also. The maximum of sharp infarcts of myocardium among Chinese patients corresponded to the first day of a lunar month, and the minimum was after two weeks [62]. Probably a certain endogenous rhythm, like a daily rhythm, is synchronized in these cases with external influence. But whereas the circadian rhythms correlate with daily light exposure, the endogenous lunar rhythm coordinates its frequency, perhaps, with the gravitational field of the Moon. The influence of the Moon was ascertained even for bees, whose weight and steroid contents do fluctuate consistently with the synodical lunar rhythm (period equal to 29.5 days). Circaseptan and circadiseptan (fortnight) harmonics for levels of some lipids and carbohydrates were identified in hemolymph of these animals [63]. For many land mammals the duration of their pregnancy is a multiple of ~30 days, which is close to the length of a lunar month [64]. The duration of menstrual cycle of women is also close to the length of a lunar month, and almost one third of all menses falls on a new moon [65]. For healthy normal people, the existence of an endogenous lunar rhythm is ascertained concerning the increase in amount of consumed food and the decrease in taking of alcohol (both peaks fall on a full moon) [66].

In adult humans, T-rhythm has, possibly, a time interval between two peaks (that is a length of one period) approximately one month (a lunar rhythm) or about two weeks (circasemilunar rhythm), operating, certainly, both in men and women. Like other infradian biorhythms, T-rhythm is, probably, a result of an interference of different hormonal and other biological rhythms of an organism. Not only hormones circulating in blood are involved in this complicated system of rhythmic interactions, but in all probability, also the locally operating in the CNS neuropeptides and neuromediators. Specificity and selectivity of signals of the T-rhythm, which periodically evokes the superhigh-rate transcription of chronomeres in neuronal targets, are, apparently, achieved owing to the observance of two principal conditions. First, it is a specific frequency of the action of ligands on the target neuroendocrine and neurotrophic cells of a brain possessing chronomeres. Second, it is a nonrandomness of functional interposition of target cells and cells generating signals of T-rhythm, or T-signals. In a complicated system of lasting many day interactions, which come to the end with a short episode of T-signal generation, the different centers of the brain, peripheral endocrine glands, and other systems of an organism could participate sequentially and in different combinations. Moreover, even the target cells do, undoubtedly, participate at certain stages of the infradian rhythm in preparing the final episode of emission of T-signals at a peak of T-rhythm, which will be eventually aimed at these cells themselves to initialize scrupting of their chronomeres.

The longer the periods of the T-rhythm are, the less size of a fraction of the genome is required which should be predestined in a chromosomal DNA to the protochronomeres. But is it impossible to economize a DNA length somehow else, in particular, at the expense of the design of chronomeres? This possibility is examined in the following section.

IS IT POSSIBLE TO ECONOMIZE THE LENGTH OF DNA REQUIRED FOR CHRONOMERES AT THE EXPENSE OF FEATURES OF A CHRONOMERIC STRUCTURE?

The size of redusomal genes is rather small since the low-molecular-weight fRNAs hardly need more than 50 nucleotides for organizing a fountosome, while the micro RNAs marking the genome for its repackings and participating in other forms of genetic activity could be still less. Without taking into account the genes contribution, the size of one arm of a human chronomere could make ~7 kb. This estimation is drawn from the following. Let one act of scrupting take off only one hexanucleotide repeat from a chronomere and let scrupting recur in the adult human life only once a month. For the hundred of years, i.e., for the time interval approximately matching a human life span, 7.2 kb of each chronomere will be spent (6·12·100). If the second arm of chronomere is transcribed irrespectively of the first arm and has the same length then a general length of chronomere (without taking into account its short genes) should be doubled. Contribution of ten short (i.e., 50-100 bp) genes would not radically increase a chronomeric arm and the general length of a

chronomere with two equal arms will be nothing but about 15 kb. However, even this value can be almost halved, if a chronomere were asymmetric. If to put the transcription promoter rather close to one of the two acromeres, but to direct a rapid advancement of RNA polymerases along DNA only to the opposite end of a chronomere, then a critically high torsion strain may be created close to the immobilized 5′-terminus of a single transcribed strand, i.e., at the very end of, so to say, "5′-acromere", which is designated according to the 5′-end of the transcribed strand where a transcription process is finalizing. The scrupting will then happen only at the end of this 5′-acromere. The acromere at the opposite side of a chronomere, that is the "3′-acromere", is not transcribed, and a would-be torsional tension at the area of the transcription promoter that locates between this 3′-acromere and the nearest subacromeric gene can be easily relaxed by topoisomerases owing to the necessary space within an untranscribed 3′-acromere. Contrary to this, the same topoisomerases are unable to relax the escalating torsional strain nearby the absolute 5′-end, having there no space for their maneuvers under conditions of a superhigh-rate motion of RNA polymerases quickly approaching the transcribing and immobilized 5′-terminus of a 5′-acromere.

This is the rationale of a conceivably wide occurrence of the asymmetric one-armed chronomeres. This most effective variant of a chronomere would require on average, under the above mentioned conditions, nothing but the same 7-8 kb. The length of an asymmetric chronomere, thus, appears to be like a length of the presumedly two-armed printomere, which, for example, may be at the disposal of human fibroblasts and which in accordance with the above mentioned estimation could have ~12-15 kb in young cells.

WHY SHOULD MEASURING INSTRUMENTS OF TIME—CHRONOMERES—RESIDE IN CELLS OF THE CNS?

The central nervous system coordinates functioning of the whole animal organism. For the reason that the monitoring of biological time which is very important for the orderly performance of individual development (including the duration control of consecutive developmental events, the monitoring of continuance of state of maturity, as well as the tracking both of onset of aging and an endurance of aging as a part of organismal development), time control should be carried out, most rationally, just with participation of cells of this crucial command system of an animal organism. Different facts show in total that the brain is an initial substratum of aging, and DNA of neural cells is primary substratum of this process. It was proved that a selective irradiation of an animal brain does evoke accelerated aging in absence of a radiation disease [67-70]. The irradiation of the whole fruit flies larvae did shorten the life span of the imago [71]. Drosophila metamorphosis is characterized, in particular, by that circumstance that only nondividing cells of nervous ganglia are able to keep the label of a radiosensitizer 5-bromo-2′-deoxyuridine (BrdU) from the larval stage up to the adult state. Being administered at the larval stage, BrdU deteriorates photosensivity of an imago. Taking into account, that radiosensitizer BrdU is able to cause the accelerated, though postponed in time, radiation aging of an adult organism (that was irradiated while being a larva) and considering that such an effect can be explained only by preservation of a specified label in DNA of the CNS, Akifiev and Potapenko have concluded that the initial substratum of an imago aging was solely the DNA of nervous ganglia. They have suggested that only some minor fraction of chromosomal DNA, which does not code for proteins, is responsible for aging [71].

Age-dependent changes of the epiphysis that is a part of the CNS bring a significant contribution to organismal aging [72]. Reasoning from endocrinological studies and clinical observations, Dilman and coauthors long ago drew the conclusion that the animal brain is a primary substratum of aging and, moreover, they have specified which of its compartments makes, in their opinion, the most important contribution to aging; for such a role the hypothalamus–pituitary complex has been proposed [73]. Still more important is that in these works the concrete physiological mechanism of participation of the hypothalamus in aging has been offered for the first time. Dilman assumed that the age-dependent changes in energy metabolism are most decisive for the aging of an organism and that they are caused by the gradual raising of a threshold of sensitivity of the hypothalamus–pituitary complex to the common inhibitory signals incoming from the peripheral endocrine glands. This compels the complex to work more and more constrainedly that gradually disables its homeostatic systems, triggering a number of the major pathologies of an old age [73]. As to the primary cause of the mentioned shift in the threshold of the hypothalamus sensitivity, it remained unknown. However, now it is possible to explain that cause by the shortening of chronomeres in cells of the CNS and, as a consequence, by alteration of the chronomere-dependent levels of expression of receptors and other factors in neuroendocrinal and neurotrophic cells of a brain, which later do lead to the advent of pathologies of advanced age.

Studies in different vertebrate and invertebrate species not only unanimously testify that the brain is a pivotal organ in organismal aging. In addition, they multifacetedly confirm an especially significant role of energy metabolism of the CNS itself in aging. Mutations of Snell and Ames dwarf mice provoke the underdevelopment of the hypophysis [74] secretion, in particular, a

growth hormone which is coupled with an energy metabolism in its operation. As it turned out, the mice with deficiency of a growth hormone live much longer than the common mice [75]. Investigation of mutations in the nervous system of worms also yields fruits. Mutations of the genes encoding proteins of the insulin signal pathway, which operates in neurons, influence the life span of the adult animal [76]. The most demonstrative changes in the work of a neuroendocrinal system of *C. elegans*, which were caused by mutations in *daf-2* and *age-1* genes of the insulin signaling pathway, were immediately involved in energy metabolism regulation [77]. These mutations considerably increased the life span of the worms [78, 79]. A study by Wolkow and his coauthors [80] is especially remarkable in this context. Working with *daf-2* and *age-1* genes coding in *C. elegans* respectively the homologs of insulin receptor and phosphatidyl-inositol-3-kinase, researchers used the promoters specific to the particular types of cells. They have forced an expression of the *daf-2* or *age-1* wild type genes in the mutant worms that do live for an especially long time just due to mutations of these *daf-2* and *age-1* genes. The expression of *daf-2* or *age-1* wild type in muscles or cells of the intestine of mutants did not in any way change the life span. But when an expression of the same *daf-2* or *age-1* wild type genes, i.e., normal genes, has been exercised in neurons, the longevity of the mutant worms appeared to be sharply reduced. This expression returns the nematode life span to the wild type that reduces the duration of their life to the "wild" norm. These experiments directly and unequivocally specify a key role of the nervous system and its energy metabolism in the life span of animals [80]. It is hardly possible to interpret the observed effect as an unfavorable change in formation of free radicals since their production in wild type should as much as possible be compensated by a protective action of antioxidant systems [76]. What is the actual mechanism of the beneficial effect of mutant neurons on a *C. elegans* life span, i.e., why does a change in their energy metabolism remarkably delay the rate of aging? This question still remains unanswered [76].

Within the framework of the redusomal hypothesis, it appears justified to accept the existence of the dependence of regulation of the scrupting process on the features of the energy metabolism control. It is possible to admit that a decrease in frequency of the T-rhythm peaks and/or attenuation of the rate of transcription of a redusomal DNA could be coupled either simply with reduction in energy metabolism level or with a change of ways of its regulation. By the way, a calorie restricted diet is the major among the already known ways of effective life span extension in rodents. It was shown that such diet elevates in primates and rodents the sensitivity of cells to insulin and glucose tolerance as well. This emphasizes once again the importance of the energy metabolism modulation as a geroprotective intervention [81]. In connection with the discussed role in aging of the nervous system and its energy metabolism, it is important to mention that a calorie restricted diet promotes an increase in producing the neurotrophic BDNF factor and also the nerve growth factor as well as other substances capable of enhancing the viability of neurons [82-84]. One of the ways of coordination by brain of the energy status and food requirements of an organism, and additionally, of coordination of the functioning of different biorhythms, including the T-rhythm, could be participation of the circulating factors such as, e.g., leptin. It, for example, is produced at a periphery, but participates through a feedback system in regulation of the neuronal cell activity of the hypothalamus [85].

Thus, estimating the significance of the listed evidence from the viewpoint of the redusomal hypothesis, it is possible to assume that, under restricted undernourishment, the intensity of chronomere transcription and/or the frequency of T-rhythm bars (that is, the frequency of the acts of scrupting in brain cells) is reduced. In its turn, this favors the economy of chronomere length and, by that, increases the life span of the organism. In this context, it is possible, by the way, to reconsider the role of the energy metabolism in aging as follows. Under the redundant consumption of calories, just the accelerated exhaustion of chronomeres (due to the speeding up of the acts of scrupting), rather than the intensification by itself of the fabrication of active species of oxygen, or ROS, is the triggering factor of aging. Overeating requires the intensification of the functioning of different cellular systems, the antioxidant system including. Hence, overeating demands also enhancement of the transcription of various redusomes in order to support increased cellular activity, thus forcing redusome shortening. However, further, already at the background of pathologies primarily arising only owing to the excessive shortening of a redusomal DNA, the ROS start to be produced in excessive quantities. These excessive parasitic ROS exert a noxious action on cellular structures, getting more and more dangerous just with further stepwise redusome reduction.

IS SCRUPTING POSSIBLE ONLY FOR CHRONOMERES OR ALSO FOR PRINTOMERES? WHY DO NONDIVIDING CELLS SENESCE *in vitro*?

The question is pertinent, whether the printomeres could owing to scrupting shorten like chronomeres? In fact, all occurs in an organism as a single whole and in the presence of one and the same neuroendocrinal products. This question, however, should, in all appearance, be negatively answered, and here is the reason. The T-rhythm that is created in an organism only for the sake of

realization of the chronomere scrupting is a series of T-signals. They are periodic, with a rather high frequency, splashes of the levels of secreted hormones and/or neuromediators and neuropeptides, designed to target other neuroendocrinal and neurotrophic cells predominantly within the limits of the CNS itself. Moreover, the proper targets of T-signals are the topographically nonrandom neuroendocrinal cells. When the respective molecules responsible for the T-signals spread across the whole body, the sharp differences in local levels of the secreted endocrine factors as well as a frequency pattern of the original T-signal (whose transcription stimulating message is additionally specific only for a strictly definite group of neural cells) will already have been smoothed out. Hence, the nascent T-signals generating at a peak of T-rhythm will hardly be able to enforce printomeres to a superhigh-rate transcription and to an unscheduled scrupting-dependent shortening of printomeres in cells outside the brain, independently of dividing and nondividing state of those cells. If T-signals are very short-lived, they will not at all survive until approaching the distantly located peripheral cells and, hence, any off-scheduled T-signal influences will occur negligible. It is especially true if T-signals, finalizing by themselves (as the last unit of a chain) a long, lasting many days, physiological relay of an infradian T-rhythm, are in their nature nothing else but the flash of a specific pattern of the nervous impulses that are strictly aimed at the definite neural ensemble bearing the corresponding chronomeres.

From the assumption that scrupting hardly encroaches *in vivo* upon the length of printomeres of most non-neuronal cells, it does not, however, follow that the same is true also for an *in vitro* situation. In stress conditions of cellular culture [86], cells could force their printomeres to be transcribed in a much more strenuous mode. Having experience of cultural discomfort, cells via regulatory feedback systems, undoubtedly, could send the biochemical demands dealing, in particular, with requirements for the enhancing of fRNA levels to raise the productivity of chromosomal structural genes. Such off-scheduled commands would drastically intensify transcription of printomeres, leading to their scrupting. The sustained stress of high intensity is able, actually without any T-rhythms, to impel the cultivated cells to begin a very intensive transcription of some groups of their genes that is especially perilous with respect to printomere scrupting. The scrupting of printomeres is a hazardous process for any nondividing cells, including for instance, the *in vitro* resting aged fibroblasts or cardiocytes of the heart working in an old body. Stress-dependent shortening of printomeres in these cells should inevitably lead to an accelerated loss of cellular functionalities. Having lost a critical number of redusomal genes, a cell remains with two choices at its disposal—to die, being normal, or to transform, becoming malignant. Some cells choose the latter.

NUCLEASE SHORTENING OF REDUSOMAL DNA AS A CAUSE OF PROGEROID WERNER'S SYNDROME

In connection with problems of cellular aging, it is pertinent to mention about progeria, or a range of diseases of accelerated aging. One of the most widely studied syndromes in this field is the so-called Werner's syndrome. With this suffering, in some facets of its nature remaining so far elusive, fibroblasts, for example, do senesce *in vitro* in an accelerated mode, performing the diminished number of cell doublings comparing to the Hayflick limit of control fibroblasts from a healthy donor of the same age. Werner's syndrome is a rare autosomal recessive disease. It is already known that mutations responsible for this syndrome reside in a single gene coding for a helicase/exonuclease (WRN). The minimal exonuclease domain of a WRN protein possesses two species of nuclease activity. They are the 3′-5′-exonuclease activity attacking a single-stranded DNA and endonuclease activity operating at the 5′-end of a protruding strand of DNA at the border of a single-stranded and double-stranded DNA, and also within a single-stranded DNA [87]. In application to printomeres, this hazardous ability of a WRN protein means that its exonuclease and endonuclease activities can be directed on the shortening of ends of a printomeric DNA when they become accessible to this protein. Apparently, that happens at the moment of replication of the acromeric DNA of printomeres. Normally, in order to be replicated, the terminal part of an acromeric DNA should carefully and slowly (to avoid an imitation of events leading to scrupting) be drawn out from the anchoring site located within the chromatin of a chromosomal nest. At this very moment, the acromere terminus, being slowly extracted from this anchoring site of the chromosomal nest, appears to be vulnerable to possible attacks of the nuclease activity of a WRN protein. Therefore, printomeres in dividing and replicating fibroblasts from patients with a Werner's syndrome can be subjected to a shortening at the expense of two factors: 1) the off-scheduled nuclease trimming of the very end of an acromeric terminus, and 2) the, now well known, DNA end underreplication of the same terminus. Due to its postulated capacity to forcedly accelerate printomere shortening, a WRN protein is also able to hasten the onset of cellular aging.

Concerning chronomeres, the WRN protein is not less dangerous for them compared with printomeres, especially when neural cells still divide and replicate their DNA. In the presence of a WRN protein, the replicating chronomeres should shorten faster due to the same reason as printomeres. As a result, the duration of a forthcoming life span of the owner of these chronomeres is significantly reduced, and the signs of aging will manifest themselves anomalously early. In addition, one more variant is possible, namely, the WRN protein could attack the acromeres

of chronomeres during their processing that proceeds immediately after each act of the scrupting of each chronomeric acromere. In this case, redusomes will untimely be shrunk even in the adult brain whose neurons have already ceased to divide long before.

TELOMERES ARE ONLY THE BYSTANDER, RATHER THAN THE PARTICIPANT OF IMMORTALIZATION OF CELLS. ACROMERES AND THE IMMORTALIZATION OF CELLS BY TELOMERASE

Depending on the nucleotide sequence of an acromere, telomerase can either recognize and extend it, and by this immortalize a cell, or not recognize an acromere at all. It is possible to suggest here that, in case of successful immortalization of human dividing cells by a telomerase [88-90], nothing else but the artificial extension of acromeres happening concurrently with the elongation of telomeres took place in reality. Owing to this, cellular printomeres with their fRNA genes, micro RNA genes, and acromeres occurred to be protected from the shortening and loss of genes. Though telomeres were also extended, it was merely extension of the bystander of immortalization rather than the active participant of maintaining a young state of cells.

Telomerase potentially could be used to protect also the acromeres in chronomeres of an adult organism. However, in a developing organism, such act of the so-called "acromerization", or the protective elongation of acromeres in a redusomal DNA by a telomerase activity, could lead to the arrest of development. This procedure, that is, acromerization, being performed in neurons of the developing brain of an embryo, would be able to stop the chronomere-dependent process of measuring biological time in the CNS. Possibly, it is just the proposed opportunity of acromerization that is a genuine reason why the telomerase activity is absent from adult brain, as, for instance, in mice and humans. It is especially important to emphasize that it is not found even in a brain of the transgenic mice whose genome does contain a vector encoding the catalytic subunit of a murine telomerase, or mTERT, under a strong promoter [91]. The reason for the last circumstance is still unknown. Probably, some embryos that express mTERT in their brain cannot survive due to the chronomere elongation, i.e., distortion of a temporal component of development; if this is the case, only those transgenic mice, whose brain, because of a mutation or for other reasons, has been able to reliably suppress the telomerase activity, do survive. There are higher animal species that, like a rainbow trout, normally have telomerase even in brain, but, nevertheless, they all the same become aged. In this connection one can assume that this enzyme, most likely, does not recognize (and does not lengthen) their chronomeres. The forcedly expressed telomerase activity in an adult brain can bring a geroprotective success only in the case if the chronomeric acromeres of a given animal species are indeed recognized by telomerase. If the acromeres of a given animal species hold non-T_2AG_3 repeats, then one can use such geroprotective vector that codes for the acromere-specific RNA (or "acromerase" RNA, for short), supplying the brain with the acromerase RNA to substitute by it the common RNA cofactor of telomerase. The concurrent expression of acromere-specific RNA and telomerase catalytic subunit would be an effective means to compensate the inability of a mammalian common telomerase to recognize the non-T_2AG_3 repeats in corresponding chronomeric acromeres in brain. The telomerase, which has been transformed into an acromerase (actually a novel, artificial enzyme) in presence of the acromere-specific RNA, will be compelled to lengthen any acromere, whichever chronomeres are in brain. To fabricate a necessary artificial acromerase, one can use such acromere-specific RNAs that are complementary to the particular acromeric DNA repeats (and that are also able to bind to a telomerase catalytic unit, playing the role of its specific cofactor). Telomerase, exploiting different acromere-specific RNAs as variable cofactors, would be competent to protect from attrition any redusomes in any animal species.

By the way, one more geroprotective means could be invented, namely, such neurotropic vectors that by themselves encode the needed fRNAs and micro RNA, thus recompensing the defective capacity of the too shortened chronomeres to govern a genome. Another geroprotective alternative is a compulsory re-synthesis of chronomeres in neuroendocrine cells of a mature organism.

Dividing cells such as, for example, human fibroblasts, being immortalized by a telomerase, cease to count mitoses. Does this occur due to the breakage of some telomeric counter of mitoses? Now, one can answer negatively. The genuine reason is in that circumstance that the redusomal mitotic counter is switched off owing to excessive elongation of redusomal acromeres by a telomerase. It is interesting to note that the state of affairs with murine fibroblasts is, however, quite different. In order to clarify the question regarding the possibility to immortalize the cells of mice, and a murine organism as a whole, in mouse oocytes there has been introduced the vector that codes the catalytic subunit of a mouse telomerase, that is an above mentioned mTERT [91]. It was not possible to find telomerase activity in the murine brain, so the second goal, i.e., immortalization of the whole organism, was abandoned. However, transgenic embryonic fibroblasts did contain a high telomerase activity, and their telomeres were considerably elongated compared to control. Nevertheless, and this is the most fascinating, the Hayflick limit of these fibroblasts remained unchanged. This means that the mTERT subunit, elongating murine telomeres, cannot immortalize mouse embryonic fibro-

blasts [91], though the similar subunit of a human origin, or hTERT, successfully immortalizes several types of human cells [88, 89]. This result can be explained in the framework of the redusome conception through the failure of telomerase to find the T_2AG_3 repeats in printomeric acromeres of rodent fibroblasts, because there are no such repeats there. At the same time, telomerase finds these repeats and elongates them in all mammalian telomeres and, for example, in printomeres of human fibroblasts, but not in mouse fibroblasts.

ROLE OF PRINTOMERES IN THE TWO PROLIFERATIVE BARRIERS

It is known that normal human somatic cells have two proliferative barriers, and in case of getting over them, aging cells can lose normalcy and become malignant. The first of these barriers is often designated by the term senescence that denotes such a degree of cellular aging which is incompatible with further divisions, although a senesced cell can keep its viability *in vitro* for many months [22]. The second barrier is a so-called crisis, or the period of mass destruction of cells. It occurs after some rather prolonged time interval afterwards the onset of a senescence state. Transformation with viral oncogenes extends the duration of proliferative status of cells beyond the first barrier limits, allowing overcoming the replicative arrest, or blocking of the proliferative growth of senescent cells. Nevertheless, even transformed dividing cells become a victim of crisis all the same. However, Counter and coauthors have shown that the ectopic expression of a catalytic telomerase subunit, which has been carried out in due time (after the achievement by cells of senescence, but still before the onset of the phase of crisis), allows these "postsenescence" cells (they were, besides, transformed with virus oncogenes) not only to reach the second barrier but even to overcome it, successfully continuing to divide even at that time when a mass cellular destruction has already taken place in the control [92]. The offered explanation of these, yet not completely interpreted in the literature, events is as follows. The definite levels of numerous factors of the cell cycle, as well as quite certain relationships of these levels, are required for the cell cycle to normally operate. Levels of expression of corresponding chromosomal genes coding for these factors are under the strict regulatory control performed, in particular, via the intranuclear ion fountains controlled by printomeric fRNAs. Micro RNAs transcribed from the printomere micro RNAs genes also make a significant contribution to chromatin configuration. How does a cell "sense" that it has already exhausted its doubling limit? An answer to this question is a subject of the current debate in the gerontological literature. May a cell stop dividing simply when the concentration of necessary factors of the cell cycle falls below the critical threshold due to the fRNA level decline occurring as a result of printomere shortening? Such conclusion, however, may occur a bit smattering since it does not take into account a possible role of the qualitative variety of different redusomal genes. It seems pertinent to assume that some printosomes (though it is not obligatory that all types of them) contain special genes crucial for the unlimited continuation of cellular doublings. One can designate such hypothetical genes as the "nonstop" genes. A universal feature of the nonstop genes could be their ability to direct synthesis of such products (some proteins or some untranslatable RNAs) that will inhibit the syntheses of inhibitors of the cell cycle. In often dividing cells, the nonstop genes should be localized at the greatest distance from the termini of printosomes in order to avoid the untimely loss of these genes and, hence, to avoid the too early ceasing of proliferative activity. While the printosomal nonstop genes are still present in redusomes, cells can make divisions. As soon as these genes have been lost, a cell starts to accumulate the inhibitors of a cell cycle and eventually enters a state of senescence.

Cells transformed by viral oncogenes have at their disposal the virus oncoproteins, which presumably can counteract with respect to the suppressing activity of inhibitors of the cell cycle. Therefore, the virally transformed cells can continue their mitoses beyond the limit posed for control nontransformed cells by the beginning of the senescence state. However, printomeres, because of the effect of DNA end underreplication, continue to shorten even in the virally transformed dividing cells. They proceed in this activity within the whole way between senescence and crisis. And when, at last, most printomeric genes have been lost, virus oncoproteins will not already be able to preserve the situation any more, since, being able to compensate the action of some cell cycle inhibitors, viral oncoproteins are useless with respect to compensation of other vitally important cellular proteins. For this reason, even among cells having the proper viral oncoproteins, total destruction, i.e., a crisis, begins eventually.

If, however, senescing transformed cells such as human fibroblasts were enriched additionally with virus oncoproteins and also with a telomerase, then the protected, actually immortalized, printomeres would not lose their genes any more. That is why these cells can continue to double, not noticing the second proliferative barrier at which their normal cellular sisters stumble and disappear.

HOW CAN THE NOTION OF REDUSOMES HELP TO SOLVE THE CURRENT PRINCIPAL DIFFICULTIES OF THE BIOLOGY OF AGING?

At the beginning of this section, the basic notions of the redusomal model are summarized, and then its potentials regarding some unresolved problems of aging

biology are examined. According to the offered approach, after achievement by an organism of its physiological maturity, redusomes persist to lose their genes, and just this loss causes cellular aging. Loss of genes by printomeres of dividing cells is less significant to the whole organism than an excessive shortening of chronomeres in the brain. The reason of such state of affairs consists in that many of the *in vivo* dividing cells simply have no necessity to make numerous mitoses, and consequently they do not exhaust the entire reserve of their redusomal genes over the life span of an organism. The continuing aging of cells dividing *in vitro* and the ceasing of proliferation after they have already performed a definite number of doublings (Hayflick's limit for mitotic population of normal cells) are caused by the process of the ongoing shortening of cellular printomeres owing DNA end underreplication of these linear molecules. The cells, which have stopped proliferative activity, still keep a small stock of printomeric genes and, consequently, they can be forced to make an extra series of divisions, after which the cells can even completely lose their printosomes, and at last there comes a phenomenon called in cytogerontology a crisis. At crisis, as mentioned above, the cells, being totally deprived of the support of printomeric genes, are extensively dying. Some of them are transformed that can be coupled with an occasional expression of telomerase or, otherwise, with the re-synthesis of some printomeres, former or novel, and also with abnormal use of such genes for fabrication of the required fRNA and micro RNA molecules, which belong to protoprintomeres (i.e., they are chromosomal templates of printomeres) and are normally never used for such transcription.

In all cases biological aging is normally associated with the lessening of gene dose in redusomes. The DNA end underreplication happens in doubling cells synchronously both in redusomes and telomeres. The shortening of telomeres in *in vitro* and *in vivo* dividing cells is not a primary cause of cellular aging. Instead, telomere shortening is merely a molecular bystander of this process. Redusomes of dividing cells can generate "a signal of aging". This signal does not consist in occurrence of some mythical proteins of aging coded by mythical genes of aging, though. The redusomal signal of aging is simply a long-lasting decline (below the level of a mature norm) of concentration of different redusomal RNAs and the resultant decreasing of the fRNA-dependent ion supply of chromosomal genes, distortion of proper configuration of chromatin due to the shortage of needed redusomal micro RNAs, etc. An optimal productivity of chromosomal genes becomes thereof impaired in comparison with the state typical for young cells, and the quantitative traits of cells vary, i.e., the levels of some cellular factors are anomalously high due to the absence of necessary levels of their antagonists, whereas other factors are unfavorably low. Because of this, aging can be considered as nothing else but a "disease of quantitative traits" that concerns cells, and the whole organism as well.

However, shortening of chronomeres, as distinct from printomeres, is crucial for aging of higher animals. The length of printomeres is designed in evolution with some reserve that can be spent for any off-scheduled divisions necessary for deviations of sizes and for regeneration. A linear chronomeric DNA carries in itself that temporal program of development, which operates at the expense of a stepwise disappearance of different specific genes from this carrier. In addition to genes coding for untranslatable nuclear RNAs, some chronomeres may contain also copies of protein-coding genes that constantly keep silence in chromosomes, but may be activated as part of a chronomere to participate in the certain switchings from one stage of development to another. To the point, in subtelomeric regions of a chromosomal DNA, where redusomes can be predominantly located, some structural chromosomal genes are present. The loss from a chronomere of a corresponding gene (and the loss of a related inhibitor of the next developmental stage) could serve as a signal starting the following step in ontogeny.

Chronomeres reside in the cells of various centers of the CNS. Hence, disappearance of the next set of chronomeric genes in a particular neural ensemble should result in a respective change in the ratio of activities of different chromosomal genes in proper tissues and organs, which are under control of neuroendocrinal and neurotrophic systems with their chronomeres. Probably, in many cases the loss of redusomal genes can play for the developing organism a role of temporal signals to perform the next stages of individual development, including puberty and achievement of a state of maturity by its various systems. The achieved state of maturity is maintained for a certain time interval, but then the state of maturity is superseded, owing to the ongoing loss of redusomal genes, with a stepwise and heterochronous deployment of aging in various body systems. The gene composition of chronomeres and the length of their acromeric DNA at a stage of the already achieved maturity on the whole are not only a part of a program of the duration of mature state maintenance, but they are also a pivotal component of the program of organismal aging, which was selected in the course of evolution. It is necessary to note that some biodemographers [93, 94] deny the possibility of existence of a program of aging, not seeing the ways of its material realization.

The fundamental feature of the program of aging operating at the expense of interaction of T-rhythms and chronomeres is a condition according to which the material carrier of this program itself is dynamically altering with time. In the offered program of aging, there are no special genes of aging. Chronomeric genes functioning in it are constantly used for the greatest possible efficiency of maintaining the viability of cells, while a pivotal factor

of aging is solely a continuous diminishing of the number of these genes. The process of aging, being dependent on the reducing of redusomal DNA length, cannot be in principle circumvented in evolution, even if it is adverse for every individual of a species. The variety of effects, which chronomeres render on an organism, depends on several factors, including both the plurality of different chronomeric genes and a variety of groups of neuroendocrinal or neurotrophic cells of the CNS where the proper chronomeres are localized. Governing the neuronal cells, chronomeres via the products of these neural cells do direct the activity of subordinated to them peripheral cells of a body. All that, taken as a whole, explains why the shortening of chronomeres during aging results in the fading of practically all systems, organs, and tissues of a multicellular organism occurring not synchronously, though. That is why the ability for stepwise diminishing chronomeres to control the appearance of manifold developmental traits in a growing organism should later inevitably lead to manifestation of multifarious signs of its aging.

In the context of developed notions about redusomes, the question why the germ line cells are able to support themselves in an uncountable train of generations, if even these cells resort, for example, to the service of fRNAs depending on printomeres in order to modulate through the ion fountain system the activity of their genes. The answer to this question consists not so much in a potential possibility of a telomerase to protect acromeres in such cells as in the opportunity to create anew the necessary redusomes in the course of gametogenesis during every subsequent sexual generation. As a consequence of this, a germinative cellular line does not accumulate senile injuries which could not be attained, e.g., only by an intraorganismic selection of the highest quality cells. On the other hand, if some germinative cells should not interpret the positional information at all, and hence, they have no need to have printomeres at their disposal, then aging of these dividing cells, being connected first of all with the loss of printomeric genes, cannot threaten them in general.

A range of problems has been accumulated in the literature that is hard to explain, remaining on the positions of a telomeric aging model. Although it has indeed brought obvious progress in a molecular biology of aging, but, apparently, it is exhausting its last potential now. The unresolved questions and contradictions of biogerontology are summarized in many publications [23, 95-101]. Listed below are the main current difficulties together with those answers that can be given, reasoning from the redusomal concept.

Some of biogerontological problems are complicated (or even are begotten) by the fact that the *in vitro* studied processes of a cellular aging have the obvious indications of stochasticity. It is reflected in the peculiarities of behavior and in changes of the traits of dividing cells, whose many functional troubles could be ascribed to this or that form of disappearance of genes from printosomes.

The shortening of all printomeres happens in a cell irrespective of the number of printomeric copies in a printosome. Hence, for cellular activity it is hardly insignificant how many copies of printomeres of certain specificity are at a cell's disposal. Other conditions being equal, the greater the number of copies of the identical printomeric linear DNA molecules a printosome has, the more, e.g., fRNAs will be transcribed from this printosome and, therefore, the more effective, in certain limits, cell systems will function depending on the nuclear fountain system. As a result, properties of a cell are influenced by any factors capable altering the printomere "copyness", or the number of copies of identical printomeres inside a particular printosome. Structural features of chromosomal nests belong to such factors. Each nest is always highly specific, as concerns its DNA sequence and spatial chromatin configuration, which is fitted solely to a definite printosome composed of a set of identical printomeres. However, the affinity of a chromosomal nest concerning its own printosome depends not only on the DNA sequence and configuration of the chromatin surface, but also on the size of the nest. Hence, both the affinity and physical capacity of a nest (relative to the number of printomeres held in the nest) can be different in two homologous nests belonging to two homologous autosomes of a karyotype. At each cellular doubling, the process of replication of the printomeres and their fastening in homologous nests recurs. Origination of distinctions between different cells, as concerns the number of printomeres keeping in homologous autosomal nests, includes distinctions between even two sister cells. This should inevitably lead to the appearance of cells with increased and decreased potential to survive in adverse conditions of *in vitro* cell culturing. A casual loss of a whole printosome by a normal cell should result in an immediate loss of the proliferative activity of this cell, and moreover, in appearance of the signs of an aging cytophenotype. Printomeres, being, probably, needless in stem cells, are necessary for their descendants interpreting their positions in a morphogenesis; therefore, the fates of different cell types as concerns mortality can depend both on possession of printomeres and on their absence. Taking all this into account, one can estimate in a new way the corresponding blank, blank spots of cytogerontology, which, apparently, can cease to exist.

So, for example, stem cells show practically not signs of exhaustion of cell doubling limit, performing not less than a thousand divisions in the renewing tissues during a lifetime [95], which sometimes is considered in the literature as a challenge to an idea concerning the existence of a counter of mitoses. The true reason for such behavior of stem cells consists, however, in that the cell doublings limit is caused solely by possession of printomeres, which

should be absent from stem cells not interpreting their own positional information. If, in a clonal prehistory of some stem cells, they, nevertheless, had utilized some printomeres for special variants of morphogenesis, these printomeres had to be used only during a strictly fixed number of mitoses, whereupon such clone of stem cells had to come back into a "printomereless" mode (long before a critical exhaustion of printomeres); it would prevent aging of a respective stem clone, endowing it with immortality. The above-mentioned immortal germ line cells, being nothing else but stem cells, also benefit from immortality only owing to the absence of the printomere-shortening problem.

Some authors, considering certain discrepancies between the predictions of a telomere mitotic clock model and peculiarities of aging of cultured cells, generally come to a conclusion, probably quite erroneous, that cells do age *in vitro* simply due to the random deteriorations of cell structures in an uncomfortable environment. Staying in artificial culture conditions is by itself a culturing shock for the cells [86, 95, 97]. Consequences of a culture shock [86] are, probably, most dangerous just to redusomes. The culture shock could initialize the following different adverse effects especially critical for printosomes. First, the stress in culture could demand the drastic intensification of a printomere transcription and, as a result, a culture shock could induce in printosomes the acts of printomere scrupting which are not coupled with T-rhythms in any way. However, this stress would lead to the scrupting-related shortening of printomeres both in dividing and, what is especially important to emphasize, even in resting cells. Second, an occasional loss of individual printomeres from a printosome could take place in stressed cultured cells. Third, the shock (as well as stresses of other origin, including some intense stresses *in vivo*) could provoke even the loss of the whole printosome from its chromosomal nest with the subsequent destruction of the falling out printomere. In more aged dividing cells, whose redusomes have already been considerably diminished, spontaneous acts of the loss of these perichromosomal organelles from their chromosomal nests, in all probability, happen with higher frequency.

Difficulties in interpretation are connected in the literature also with the following circumstance. Under conditions of culturing according to Hayflick, when evaluating the number of passages performed at a high cellular density, each cell makes on the average one mitosis per one population doubling. However, for some unknown reason, already from the very first day of culturing at a low cellular density, the rate of duplication of mouse embryonic fibroblasts is distinctly declining [102]. The suggested solution of this fact will be that a delay of growth during cell aging *in vitro* is a result of the summation of the two processes—the shortening of printomeres in all cells and the loss of printosomes by some of the cells; to all appearance, the latter happens most often under non-optimal conditions for culturing, in particular, at a low cellular density.

One more variation on the same theme is a surprisingly wide distribution of proliferative potential in any cultured population of normal cells taken from humans or laboratory animals, and some cells senesce already during the first mitoses; and what is more, the frequency of occurrence of aged cells increases as the number of the mitoses already performed increases [95]. The effect may be explained by the existence of unequal lengths of printomeric DNA molecules in different cells, whose proliferative prehistories are different. It was shown that, e.g., the quantity of human fibroblasts and glial cells which have stopped divisions rises in a linearly increasing rate with each population doubling, beginning from the very moment of explantation [103-105]. The indicated phenomenon probably alludes to the increasing divergence between the reducing sizes of redusomes of dividing cells and the constant sizes of their relatively stable chromosomal nests; owing to this the area of contact of the redusome with its nest diminishes and, therefore, the strength of retention of the organelle in a nest declines. This process apparently goes as a process with a positive feedback. The progressively smaller the redusomes become, the more easily they drop out of their own nests. As a result, the frequency of losses of the redusomes by cells is growing, and the viability of cells remaining without these nuclear organelles descends at once.

In conformity with the stated above, heterogeneity of affinities of printosomes in relation to their nests could be recruited also for elucidating the phenomenon of the heterogeneity of cell aging [96]. Study of many individual cell clones has revealed a high interclonal heterogeneity in the size of colonies and in the lifespan of clones, notably when the first signs of replicative aging manifest themselves [103, 106]. The reason for the noticed heterogeneity can consist in the following. One nest of a heterozygous allele nest pair could behave itself as a dominant allele. This occurs when the nests' binds a printosome more tightly than a recessive homologous allelic nest holds its own printosome of the same specificity. If the dominant homologous chromosomal nests' residue at both homologous autosomes of a karyotype, then such cell has a high chance to become a progenitor of the most long-lived clone. Polymorphism of homologous nests, including their three-dimensional configurations, is a base for the possibility of existence of some especially effective nest designs allowing the firm and reliable holding of specific redusomes in the nests, and even when these organelles were to a great extent already decreased in size compared to the size of the nest. Just such clones with nests of high affinity should compose the main part of the cellular population approaching exhaustion of its Hayflick limit. It goes well with the evidence that the Hayflick limit of population doublings is applicable exactly to the most long-lived clone [107]. Hayflick and

other cytogerontologists have shown that individual clones of cells senesce at different rates. Being doubled more and more slowly, clones for a half of the time of their existence are able to accomplish on the average about 8 cell divisions [80, 88]. The clones effectively propagating for the most prolonged time interval are responsible for the establishment of a final population with the maximal Hayflick limit for a given cell population, e.g., 50 doublings [95, 103]. Within the framework of the offered conception, one can assert that a material basis of the Hayflick limit estimated at the level of an individual mitotic cell is exactly the length of DNA in its redusomes.

An opportunity for asymmetric distribution of the copies of replicated printomeres between two sisterly cells is of special interest. As mentioned above, the number of printomeres arising during replication and the features of nests expecting them can vary. As a result, even sister cells, i.e., two daughters of a single, just divided cell, having received and holding the dissimilar number of printomeres in their homologous (but not identical) nests, can get unalike cytophysiological status and nonidentical ability for further doublings. The established spatial structure of a nest is maintained over a series of cellular generations, most likely epigenetically. Hence, two clonal populations, which have arisen from two sister cells, can possess different replicative potentials. Reasoning from this account, it is possible to explain a bimodality of distribution of doubling potential among individual cells [108].

One more uncertainty existing at present in the literature is why the length of telomeres does not correlate very strictly with a species lifespan. In wild mice the average length of telomeres was slightly more than in humans [109], while in laboratory mice telomeres are still longer than in their wild relatives. And nevertheless, the mean species lifespan in mice is tens of times less than the mean species lifetime of humans. The offered explanation is based on the assumption that chronomeres of mice are shorter than in humans and/or that they shorten more quickly (for example, in view of the presumably shorter periods of a murine T-rhythm). As to printomeres, they should be unconditionally shorter in mice than in humans if to agree with postulated idea that a counter of cellular doublings is nothing else but the printomere mitotic clock. Within the framework of the redusomal hypothesis, only in such way one can interpret the well-known fact that the Hayflick limit for normal mouse fibroblasts is lower than that for human fibroblasts [91]. Correlation between the species lifespan of an organism and the Hayflick limit for its cells proliferating *in vitro* is not actually strict, and it has significant deviations from direct proportionality [110]. The most feasible explanation will be that though both the Hayflick limit and specific lifespan are really encoded in DNA of redusomes, there are no bases for natural selection to care about the strictly coordinated exhaustion of the reserves of chronomeres and printomeres. The "bottleneck" that limits the span of the species lifetime is the chronomeres, instead of printomeres; the length of printomeres is created in evolution with some redundancy in order to keep it in reserve for the growth and regeneration needs. This is actually the reason for lack of a reliable correlation between mentioned characteristics.

The length of printomeres in dividing cells such, for example, as fibroblasts depends not only on the number of doublings that have already been performed, but also on conditions in which they have been executed. For example, to the shortening of redusomal DNA occurring due to the common DNA end underreplication some other factors could be also added and, in particular, DNA shortening proceeding at the expense of oxidative breakages of DNA, like those observed for telomeric DNA [111]. One cannot exclude an opportunity of a nuclease shortening of acromeres in the case of their insufficiently effective capping in a chromosomal nest. Decline in overall performance of cells aged *in vivo* is actually promoted by the impairment of an intercellular milieu which is, certainly, worsening as the chronomeres in the CNS (and, respectively, the hormonal and neurotrophic supply of cells in a body) do deviate more and more from the optimum that was achieved in the past, namely when the maturity state of an organism came. The gradual deviation of young physiological indices from the already established physiological optimum proceeds in the course of aging; it was named by Dilman as the "law of deviation of homeostasis" [112]. The still unresolved problem of the heterogeneity of aging, or irregular manifestation of signs of aging at different parts of tissues and organs, could also be interpreted here as a consequence of the locally heterogeneous deficiencies of neurotrophic factors. The cause of that is nonuniform distribution of the nerve endings in target tissues. This circumstance, which is of little consequence in youth, becomes crucial on the background of growing shortage of neurotrophic factors in an aged organism. Just so one can interpret, for instance, the results of measuring duration of cell cycles in the intestinal tract functioning the whole life. The study on mice has revealed *in vivo* a considerable increase in heterogeneity concerning the duration of individual cell cycles in various groups of cells as aging of the intestinal tract is persevering [113]. It is possible to assume that the shorter chronomeres become in the CNS, the less effective neurotrophic supply of the body periphery will appear, being dependent on the chronomeric length. If local innervation of a definite tissue segment was formed during embryo morphogenesis in a topographically nonoptimal manner (for example, some tissue segment has received too little nerve endings), then this may become an important factor of the aging pathology on the background of deepening deficiency of the chronomere-related neurotrophic factors whose shortage was of no importance in youth, when

each of the endings still delivered a sufficient concentration of neurotrophic factors.

Both printomeres and telomeres, being replicated, are compelled to shorten concurrently, and by the same length on the average. There are data that are categorically incompatible with the telomeric approach, though they could receive a verisimilar explanation on the basis of the idea of the printomeric mitotic clock. The printomere counter is more adequate for elucidation of some facts than the telomeric counter just because printomeres, in contrast to telomeres, do contain and lose regulatory genes. The results of DePinho's team [91], which have been obtained on mouse fibroblasts, are in this respect especially demonstrative. Mouse embryonic fibroblasts, containing a high telomerase activity as a result of transgenic introduction of murine mTERT activity, had telomeres even longer than in control; however, on this background, the limit of cell doublings did not increase at all, i.e., mouse fibroblasts have not been immortalized by a murine telomerase. This result, being incomprehensible if reasoning from a telomere model of aging, can be explained through the supposition that mouse printomeres, unlike printomeres of human fibroblasts, in their acromeres do not have the T_2AG_3 repeats easily recognizable both by murine and human telomerases. Instead, the acromeric repeats of murine printomeres possess some other, though also G-rich, repeated sequences unrecognizable by mouse telomerase. The reason for various tactics regarding printomeres in humans and mice appears to be explicable if one takes into account that, in the overwhelming majority of cells in humans, telomerase is switched-off or its level is negligibly low. Hence, human cells can have just the T_2AG_3 repeats at the ends of printomeres, which are very effective for protection of DNA termini, as is proved by their broadest prevalence among telomeres of higher animals. On the contrary, in mice a telomerase is active in many cell types (though it is absent from the primary cultures of fibroblasts) [114]. In the evolution of mouse acromeres it was apparently easier to refuse from services of T_2AG_3 repeats in all (or in many types of cells), because the presence of this sequence distinguishable by mouse telomerase would permit the unnecessary immortalization of somatic murine cells through protection of their acromeres. Mice, by the way, should pay off something for the refusal of this very effective protective sequence and for the application of another, less efficient, non-T_2AG_3 sequence in acromeres of their printomeres. It is possible to assume that the "mouse payment" for all that consists in more frequent losses of printomeres from chromosomal nests and/or in less effectual capping of acromeres fraught with danger of more often destruction of individual printomeres in printosomes. On the whole, it decreases the homeostatic capabilities of mouse cells and raises the probability of cancer occurrence. If some types of human cells are for some reason compelled not to use the T_2AG_3 repeats in acromeres of their printomeres, then such cells could meet a threat of an increased probability of carcinogenesis because an excessive loss of redusomal genes should bring an imbalance into work of many cellular systems. Interaction of telomerase with telomeres resulting in immortalization of all tissues cannot be approved in evolution by natural selection, and here is the reason. Only aging causes relay of generations of various species of higher animals in the overwhelming majority of cases, even in wild nature. Though, as is well known, animals in wild nature do not usually survive until the onset of absolutely overt signs of aging, nevertheless the aging-related attenuation of speed, force, hearing, vision, etc. gradually appears to be in such conditions that an aged predator, as well as an aged victim of the predator or of parasite, would hopelessly lose in a struggle for life. A preferable death of organisms, which have even just begun to age, will automatically result in replacing one generation by another. This generation relay is an evolutionally favorable process maintaining status quo in the ecological niche, and in this sense August Weismann was absolutely correct when in his earlier statements he pointed out at the evolutionary benefit of individual aging of each member of a species for the same species as a whole.

Another problem repeatedly discussed in connection with telomeres is the exponential increase in cancer frequency with age, with the higher incidence in the elderly. According to a rather widespread point of view, aging is a protection against cancer [115]. The opposite point of view is also shared by a number of authors [95, 116, 117] and just it is, probably, true. Many cases of cancer are themselves a consequence of aging in the sense that the loss of redusomes accompanying aging attenuates the functional abilities of cells, whereas aging as such does not protect from cancer in any way. On a background of a growing imbalance of levels of intracellular factors, which is cropping up because of regular losses of the genetic reserve of redusomes, the efficiency of repair and other cellular systems should apparently descend. Inevitably, this results in an extremely slow, though progressive, accumulation of deteriorations, including mutations, in cell populations. Increase of genome instability in the course of redusome-dependent cell aging is, supposedly, the primary cause of age-dependent advancing in frequency of the incidence of malignancy. Cell aging is an ineffective way to struggle against cancer through delay of cellular divisions, apoptosis, etc., as it is often, but erroneously, assumed in the literature; instead, aging of cells is, most likely, the major factor provoking, even in the absence of carcinogens coming from outside, the origination and growth of tumors. The effect of the exponential rise in cancer frequency with age is sometimes explained as a plain consequence of accumulation of oxidative damage of cells [95]. As a basis for such viewpoint the fact is sometimes used that both the rate of aging and the incidence of cancer decrease under caloric restriction. The

notion of redusomes permits however to highlight the key points otherwise. The shortening of printomeres and the casual shedding of printosomes from chromosomal nests probably become more and more frequent as the sizes of printosomes are becoming too small for their excessively loose nests in senescing cells, and this, in turn, reduces step by step the homeostatic abilities of dividing cells. On a background of attrition of chronomeres in the CNS of an aging organism, this situation is aggravating by a rising impairment of the supply of tissues with hormones and neurotrophic factors. As a result of these initial redusome-dependent changes, the pathological deviations both in regulation and metabolism spring up in dividing cells, and this could lead to anomalously high formation of reactive oxygen species, that behave themselves here as a parasitic factor; hence, the role of ROS promoting intracellular disorders is secondary compared to the primary role of diminishing redusomes in the whole process of the onset of aging and of aggravating infirmities of very advanced age. If this conclusion is correct, the redusomal concept could send a challenge to the free radical theory of aging that has raised a claim for explaining the primary driving factor of aging. Rejecting the idea about free radical damage as an initial mechanism of aging, one can henceforth leave only a secondary role for the pernicious action of excessive production of ROS in various pathologies. It is pertinent to note, by the way, that medical statistics has already testified to the utter inefficiency of antioxidants in the prophylaxis of aging of humans living in the rather comfortable conditions of a contemporary civilization [93, 118]. Although the sensitivity of tissues to the oxidative damage of DNA and other structures can actually become a real threat for the old heart, brain, or skeletal muscles [119], and also probably for old brain itself, one should search for the first and genuine cause of all these events both in the age-dependent shifts in levels of the chronomere-dependent neurotrophic and neuroendocrinal supply of target tissues as well as in the changes of expression of chromosomal genes that are monitored by local redusomes resident in the considered targets, i.e., in heart, skeletal muscles, etc. Products of redusomes optimize genome functioning and also protect its integrity. The far advanced losses of redusomal genes lead to the incurable decline of cell viability and capabilities of their homeostasis. Increasing shortage of redusomal fRNAs leads, in particular, to a growing deficiency of ion servicing of chromosomal genes and provokes a growth of instability of chromosomes owing to the reduction in accuracy of nuclear systems demanding the strictly defined ionic environment for proper performance of replication, transcription, etc. In turn, these circumstances could make a significant contribution to the rise in cancer frequency in advanced age.

Delay of the further deepening of processes of aging in the very advanced ages that is reflected in lowering of a rate of mortality is considered as probably the most intriguing finding in current demographic studies of life span [120]. Similar delay of mortality rates in advanced ages was reported also for insects [121]. The cause of this enigmatic phenomenon could be the declining rate of transcription of chronomeres and, consequently, the more economical expenditure of a redusomal genetic reserve, which still remains at the disposal of the very old organism in its declining years.

REDUSOME LOCATION AS A CAUSE OF STRICTLY DEFINITE LINKAGE OF GENES IN CHROMOSOMES

Why are genes linked in an absolutely nonrandom fashion in each individual chromosome? The reason for this phenomenon still remains largely mysterious. Of course, as is well known, this linkage complicates the possibility of interspecies hybridization in wild nature, promoting the reproductive isolation of species. However, isolation is effectively achievable also along other pathways, such as different peculiarities of a behavior, distinctions in the anatomy, etc. To this day it is not clear in the literature what dictates the great differences in sizes of various chromosomes and in their number in karyotypes. Are these distinctions reached solely by accident, or not? In connection with a role of the postulated fountain system that is controllable by redusomes it is possible to consider the sense of existence of the nonrandom gene linkage from an entirely new viewpoint. The pivotal cause of preservation of the definite patterns of structural gene linkage, which is specific for every chromosome, could be interpreted via necessity to maintain the strictly appointed by natural selection intrachromosomal distances between each redusome and the structural chromosomal genes (and their accessory sequences), which should receive adequate RNA-messages in adequate concentrations from the related redusome. Redusomes lying in their own chromosomal nests are apparently located predominantly in subtelomeric regions of chromosomes, though some evolutionally younger redusomes could meet also in paracentromeric regions.

The subtelomerically located redusome can admittedly serve by means of its transcripts for the whole length of a chromosomal arm. Not all chromosomes are obliged to bear redusomes for each cytodifferentiation. However, those of chromosomes, which, nevertheless, do have redusomes, should be generally serviced, for example, by fRNAs transcribed from their own redusomes; owing to this service, the chromosomal genes are receiving a topographically specific and appropriate ion surrounding. In a subtelomeric region of any given autosome, usually only one redusome is at work during a particular differentiation, while in the next differentiation another active redusome is created for servicing chromosomal genes. This will be done using the neighboring sequence of the same

subtelomeric region as a chromosomal template (or even subtelomeric DNA of another chromosome). In one nucleus, several different redusomes may operate simultaneously, but they will probably tenant the space in different chromosomes. In all these cases, the most important item, however, is the distances between a redusome and its chromosomal targets. These distances should be optimal for upholding (in close surrounding nearby the adequate chromosomal genes) the most favorable concentrations of specific RNAs, which are transcribed from the given redusome. Different and nonrandom concentrations of redusomal RNAs near each gene are necessary to guarantee, in particular, the optimal ion servicing of a corresponding set of structural genes located one after another along the chromosome arm. For the reliable functioning of the genome, it is necessary to maintain not only the optimal distances from each gene up to a proper redusome. Not of less importance is the ability to keep up the optimal ratio between all these distances in every arm of each chromosome. The individual fRNAs, for example, could serve sometimes also for some genes on other chromosomes positioned not far from a given redusome in the same nucleus. Nevertheless the physical linkage of the redusome and all targets of its redusomal RNAs within its own chromosome as the single whole is an indispensable condition for the most advantageous servicing of all genes of the chromosome by means of redusomal RNAs, including organization of the fRNA-dependent ion fountains and chromatin remodeling. The farther from the subtelomeric region with its redusome the chromosomal gene is located, the lower the concentration of redusomal RNAs attaining its region is. Therefore, the more the structural gene is remote from a telomere, the more, for example, the fions, as centers of fRNA binding, this structural gene should have in its retinue in order to remain in equal conditions with other chromosomal genes that are placed in the same chromosome (but closer to the redusome that resides in a subtelomeric region). The stated condition regarding the "redusome–genes" distances does matter both for the fountain RNAs and the micro RNAs having their own nuclear targets.

In chromosomes, as is known chromatin is organized in the so-called chromomeres and interchromomeric regions [122, 123]. Chromomeres are the segments of chromosomes capable of local dynamic compaction and decompaction that are specific for definite stages of ontogeny [124]. Patterns of chromomeres and interchromomeric regions are widely used in cytogenetic identification of chromosomes. The true sense of the existence of genes within chromomeres (which are like the beads of a chromosomal necklace) still remains not quite clear. Is the matter solely in that it was easier to inactivate genes by compaction when they were in chromomeres? What is the sense of interchromomeric spacers? As it was noted for the first time by cytogeneticist Lima-de-Faria, the sizes of chromomeres have an obviously expressed tendency to increase in the direction towards a centromere (the so-called chromomere size gradient). This probably testifies to a predominantly subtelomeric, instead of paracentromeric, disposition of the majority of redusomes. It was cytogenetically demonstrated that chromomeres with genes inside them do keep in the kindred biological species their mutual disposition along the chromosome length both relative to each other and in relation to telomeres and centromeres; strikingly, this regularity was true even despite variability of lengths of corresponding chromosomal arms [125-129]. In this connection one can deduce that dependence of the cell activity on a ratio of expression levels of its chromosomal genes should be coupled with the local concentrations of RNAs transcribed from the redusomal genes. Since the quantity of these transcripts near each target chromosomal gene depends on chromosomal distances between particular genes and the corresponding redusome lying in a subtelomeric region, the relationships between aforementioned distances should be vigilantly upheld by natural selection in chromosomes of the organisms belonging to kindred taxons possessing comparable quantitative traits.

In accordance with the chromosome field theory proposed by Lima-de-Faria and the facts confirming it, each DNA segment has the optimal territory sited between a centromere and telomere, tending to occupy the same territory after any evolutionary chromosomal reorganization [128]. More than a half of all retroviral oncogenes are situated close to telomeres. All oncogenes avoid the shortest and the longest arms in chromosomes [126, 127], apparently, shunning in this way the formation, in close vicinity to specific targets in chromosomal DNA, of the surplus or lack of the concentration of the RNA molecules transcribed from the redusome. It is most likely that the key genes of a cell cycle, like many other genes, could search and find in evolution their own optimal chromosomal distances in relation to corresponding redusomes. So, though the redusome could send signals for its chromosomal arm from either of the two arm ends, the observations by Lima-de-Faria [125] concerning a regular increase in the chromomere size in a direction to the centromere (the slope of the chromomere size gradient was a function of the length of the chromosome arm) can be interpreted as follows; the subtelomeric region does grant certain advantages to redusomes, both in regulatory and evolutionary attitude.

Thus, chromomeres are probably the larger, the greater the number of targets for redusomal micro RNAs and fRNAs they contain. The farther from the redusome any structural chromosomal gene is located, the greater number, for example, of fions it should have in its retinue to be able to catch by them a sufficient number of the redusome-related fRNA molecules. That is why structural genes located far from the redusome, i.e., far from telomere and close to centromere, should be situated in especially large chromomeres. The presence of special

nuclear organelles—redusomes—that reside in the special chromosomal nests puts the topography of structural genes (which are in need of fountain system servicing that may be efficiently executed only in the presence of optimal levels of redusomal RNAs) in a strict dependence on the topography of redusomes. That is the primary cause of the linear association of genes linked to each other in a quite definite order in chromosomes. Such is the quintessence of the suggested solution to one of the most aged riddles of genetics—the mysticism of nonrandom linkage of genes in eukaryotes.

IS THERE ANY CYTOGENETIC EVIDENCE THAT, WOULD IT BE INDIRECT, COULD INDICATE THE POSSIBLE EXISTENCE OF REDUSOMES?

Uruguayan cytogeneticists, analyzing the results of T-banding, have found unusual tiny cavities in chromosomes at the place of a removed chromatin fraction. Intrachromosomal cavities were found in paracentromeric, i.e., on both flanks of a centromere, and also in subtelomeric regions of some chromosomes [130, 131]. The cavities were completely free of chromatin, and in homologous chromosomes they were situated in identical places. Not only the position, but also even their form was strictly preserved on homologous chromosomes. As earlier pointed out, when considering a role of printomeres in differentiation, such nonrandom tiny cavities could correspond to the former chromosomal nests, whose printomeres had dropped out in the course of cytogenetic treatment [24]. The redistributions of the chromosomal segments encoding the protoredumeres (protoredumeres are the chromosomal templates coding for redusomal DNA in the process of the redusome creation during morphogenesis) undoubtedly could occur during chromosomal evolution. This evolutionary redistribution of the aforementioned segments between various chromosomes could be carried out at the expense of the well known processes of fusion of the ends of chromosomes that follows by the recurring breakage–reunion acts, as part of chromosomal rearrangements, which take place predominantly within the telomere and centromere regions. For this reason, redusomes should have a propensity to appear closer to the subtelomeric and sometimes, though not for a long time, near paracentromeric regions of chromosomes.

Studies of transcription-coupled excision repair had revealed that this process has rather unusual allocation in genomes. While the common sites of DNA repair are evenly dispersed along chromosomes, the clusters of sites of this type of DNA repair were identified among early replicating bands, which are enriched with genes and which (what is the most essential thing in the considered context) were found in the telomeric regions of some chromosomes. As to preferred localization of the sites of transcription-coupled excision repair, the involvement of just the T-bands has been revealed, and moreover the corresponding sites appeared to be GC-rich [132]. As noted above, G-rich sites are most suitable for organizing the interguanine bridges between redusomes and their own chromosomal nests, as well as for using these sites in acromeres and intergene acromere-like spacers of redusomal DNA. Therefore, properties of G-rich sites in T-bands make them quite adequate candidates for the role of those sites where redusomes and their nests could be localized in a chromosome. These sites of the transcription-coupled excision repair appeared to be located just there, where redusomes should predominantly be. It might be considered as a hint at the passing there of a superhigh-rate transcription of redusomal genes. Reliability of the work of the redusomal genes will be the highest only in that case if their transcription is coupled to such DNA repair system that is able to protect these genes from destruction, which had occurred long before the redusomal DNA termini were subjected to scrupting. Here is the reason why these examples can be envisaged as at least not contradicting what is necessary to expect from the viewpoint of the redusomal hypothesis.

What is biological aging? This question can be answered in different ways [133-135]. “Aging is a disease of quantitative traits”—such a formulation was offered in [136]. It is possible to add—a disease which is universal and chronic. A progressive loss of genes from redusomes eventually leads to escalating imbalance in activities of the redusome-dependent factors, thus violating the functioning of the nuclear fountain system and shifting towards the unfavorable side the quantitative traits of cells. The qualitative changes occurring in the course of aging, both at the level of cells and of the organism as a whole are also a consequence of decreasing the number (or even a complete loss) of regulatory redusomal genes. Aging can be envisaged as a universal and, above all, scheduled, evolutionally programmed redusomal disease. Aging is one side of a redusomal medal. Another side is maintenance of individual development of organisms by means of organization of the vitally important process—the monitoring of biological time passing. On the whole, the account of a redusome role allows removing many of the current contradictions in the biology of aging. Among them are debates about whether a program of aging occurs at all [93]. The program of aging of an organism, undoubtedly, not only exists, but also is a part of the wider strategy of life aimed at protecting the species gene pool [137]. One of the two components of the program of aging is a redusomal DNA of brain. Working in the developmental program making living creatures and continuing to decrease in size, the redusomal DNA measures, by its own decreasing length, the value of a remaining fraction of the life span of an individual. Redusomal DNA performs this task conjointly with the second component of

the aging program. This second component is the T-rhythm, which governs the rate of a shortening of chronomeres; parameters of the T-rhythm depend on endocrine and other systems of the whole organism. Taking into account that the time of existence of an organism, or so to say, a time in the way from the start until finish, is defined by the length of chronomeres (L) and by velocity of chronomere shortening (V), which is dependent on the T-rhythm parameters, it is possible to suppose that all three programs—the program of biological time passing, the program of the life span, and the program of duration of animal aging—are basically described by the expression $T = k \cdot L/V$, where the corresponding lengths of chronomeres and rate of their exhaustion (the exhaustion is determined by fluctuations of T-rhythm) are specified. Stresses and other factors that influence the performance of T-rhythms and the rates of chronomere exhaustion, and thereby the value of factor k, turn a life span of individuals into a variable. It varies in not too large limits, though.

Thus, aging of an organism, as well as deployment in time of its developmental events, are running on the basis of a universal mechanism of the chronomere shortening. Truncation of the ends of DNA in redusomes of the brain is a result of the scheduled acts of molecular vandalism committed by transcriptional machinary and its accomplice—a special variant of biological rhythms. These acts are used to provide in the central nervous system the ticking of the biochronometer of life. If some species do not use a biochronometer in their development (as for example, a vegetatively propagated potato), such species are not subjected to aging. All species of higher animals do develop, applying monitoring of biological time, and consequently they cannot avoid the onset of aging, if they have already established the state of maturity and continue to measure the biological time flow. The initial substratum of aging of higher animals is redusomes. Inevitability of natural aging, which comes with 100% probability, is caused by the fact that the shortening of redusomes lying in its basis is an indispensable part of the developmental mechanism and therefore, of life itself. Only an artificial compensation for redusomal losses, being implemented in different ways after maturation of an organism, can offer a reliable alternative to this scenario.

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