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HYPOTHESIS

Notes on a "Printomere" Mechanism of Cellular Memory and Ion Regulation of Chromatin Configurations

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Abstract—According to the proposed hypothesis, the memory of a cell about the achieved state of cytodifferentiation is based on the existence of a postulated genetic structure termed here as a "printomere". A printomere is a relatively small linear DNA fragment which is laterally located on the chromosomal body and armed at its termini with peculiar analogs of chromosomal telomeres, which in this case are designated as "acromeres". The printomere locates along its chromosomal original—protoprintomere—and is bound to this chromosomal segment via proteins. The printomere codes for so-called fountain RNAs (fRNAs). Molecules of fRNAs as a part of ribonucleoproteins, or fRNPs, specifically bind to the complementary for them DNA sites, or "fions", that are dispersed nearby many structural genes. fRNP–fion complexes help to open, for a very short time, closed ion channels in the inner nuclear membrane, and this occurs strictly nearby corresponding genes. Dosed and local entry of the specific ions from the perinuclear cistern of the nucleus modifies the local pattern of the chromatin decompaction and modulates the expression level of the corresponding genes. The implied role of the fRNAs was considered in the so-called "fountain theory" (A. M. Olovnikov (1997) *Int. J. Dev. Biol.*, 41: 923-931; A. M. Olovnikov (1999) *J. Anti-Aging Medicine*, 2: 57-71; A. M. Olovnikov (1999) *Advances in Gerontology* (St. Petersburg), 3: 54-64). Transcripts (fRNAs) coded by printomeres participate in the creation and maintenance of the specific patterns of decompaction and compaction of chromatin, which are characteristic for corresponding cytodifferentiations. Printomeres of various differentiations differ in their nucleotide sequences. The printomere and its chromosomal original, the protoprintomere, located co-linearly, side by side with it, have their own *ori*. Their length may vary from several thousands of base pairs to tens of thousands of b.p. Printomere bound by its arms to the chromosomal DNA with chromatin proteins is able to pass over the replicative forks during printomere replication and replication of the chromosome. That is why any printomere can be stably retained on the chromosomal body in the course of numerous cell divisions. Owing to printomeres, cellular memory about the proper structure of chromatin decompactions is created, kept, and can be carried through the succession of doublings of differentiated cells.

Key words: cellular memory, differentiation, fountain RNA, printomere

It is known that cells are able to create and memorize their state of cellular differentiation, i.e., differential activity of genes is achieved by a cell—the founder of a cellular clone. Nevertheless, a satisfactory model explaining at the molecular level the way non-identical groups of genes are activated in initially identical cells is still absent; and what is most important, it is unknown how the cellular memory of the necessary pattern of chromosome decompaction reproduced in every subsequent cell cycle is organized. The current paradigm assumes that the mechanism of cellular memory is determined by a strictly specific set of chromatin proteins. But is this sufficient?

Determinations and differentiations of somatic cells are in many cases reversible as it was demonstrated, in particular, by the feasibility of artificial construction of creatures by transferring the nucleus from a differentiated somatic cell into an enucleated egg (e.g., creation of a ewe, carrot, tobacco, etc.), that is by cloning of plants and animals. Similar results show that the genome of somatic cells is in principle not changed during development of an individual. Equivalency of the genome of differentiated cells within the organism is typical for the overwhelming majority of species, and alterations of cytophenotypes are gained through specific repackings of the chromatin.

Thus, the known phenomenology concerning cell determination and differentiation is based on the ability of cells to somehow memorize and then maintain the cytophenotype which has been made in a cell under the influence of a specific inducing factor.

Under conditions of equivalency of the genome of differentiated cells, distinctions could have, as it is currently assumed, epigenetic nature, which means that the

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cellular memory, keeping unchanged nucleotide sequence of the chromosomal DNA, could be based on local changes in methylation of DNA sites and on binding of different sets of nuclear proteins with DNA [1].

In the shadow of the great and necessary work on deciphering the structure of nuclear proteins, chromatin packing, etc., an old and seemingly unsolved problem has so far remained. This problem is the so-called regulation of regulators. Its essence is in the following. Proteins capable of recognizing certain DNA sites should be synthesized under the control of other genes, for recognition and activation of which new proteins are required, and these new proteins should be synthesized under the control of a third group of genes, and so on, thus creating a vicious circle. Remaining in the framework of the current paradigm, it is difficult to exit from this circle; however, cells circumvent this difficulty with ease. How exactly? I suppose that this goal is achieved with the help of so-called "printomeres" and ion regulation of the genome.

ABOUT THE POSSIBILITY OF ION REGULATION OF CHROMATIN REPACKINGS

The question how exactly the cellular memory maintaining the ordered chromatin structure is organized still remains open. The main player in the novel scenario of the cell memory organization proposed here is the "printomere", a linear double helix DNA molecule, which is bound to the surface of a chromosome. As seen from the following, the activity of printomeres is based on the earlier proposed "fountain mechanism"; that is why consideration of the proposed model of cellular memory begins with discussion of the essential principle of the fountain mechanism; whereupon the examination of the role of the printomere utilizing the fountain mechanism for establishing the cellular memory will be given.

According to the fountain theory [2-4], the configuration of chromatin and its transcriptional activity is changed depending on strictly dosed entry of ions that are coming in a controlled manner from the perinuclear lumen via the inner nuclear membrane into the vicinity of certain segments of chromosomes. Ions can enter via ion-specific channels—calcium, zinc or, possibly other channels. Each ion channel is opened for a very short time interval during the interaction of the channel protein with the low-molecular-weight nuclear "fountain" RNA (fRNA). The channel opens for the time interval necessary and sufficient for altering the configuration of its protein components; it occurs when the channel protein forms a complex with the activated fRNA molecule. Specific molecules of fRNA themselves are activated due to complementary interaction with the specific DNA sites designated here as "fions". When fRNA is protected by the certain protein and exists as the ribonucleoprotein (fRNP), the above interaction leads to formation of the oligo-component complex fRNP–fion–channel protein, or "fountainsome". This structure creates, for a short moment, the ion fountain in the vicinity of the corresponding gene. The term "fion" is derived from the words *f*ountain and *ion*, since the fion participates in activation of the ion channel injecting a portion of ions, and their fountain-like shower is used as a means for the local shift of chromatin configuration. Fions located near various structural genes have specific distinctions in their nucleotide sequences, which are recognized by the complementary specific fRNA molecules. As a response to the formation of a fountainsome, the closed ion channel reshapes its configuration, injecting a portion of ions into the chromatin. Ions permeate from the perinuclear lumen into the chromatin only during this conformational reshaping of the channel, and before it closes again. As a result, one molecule of fRNA initiates the entry into chromatin of only a small number of ions. Specificity of the entering ion is controlled by the specificity of the fRNA and of the ion channel itself. Thus, the conformational reorganization of the ion channel protein is carried out only after its interaction with fRNP, which has already been activated by interaction of this fRNP with DNA of the fion.

The perinuclear lumen of the nucleus serves for this process as a reservoir for conditioning of ions, where maintenance of optimal concentrations of ions, relatively independent of their content within cytoplasm and karyoplasm, is performed. Dosed and short-term penetration of certain ions through fRNA-dependent channels, close to respective chromosome segments, remodels the chromatin configuration; this also changes the lifespan of mRNAs and levels of expression of structural genes.

The cardinal distinction of fRNA molecules from other ligands capable of opening channels consists in the ability of fRNAs to provide topographic selectivity of channels activation, because fRNAs bind only to those channels of the inner nuclear membrane which are found right up against the complementary fions rather than to any channels of the nucleus. Dosed entering of ions is able to radically remodel the configuration of chromatin which adjoins the corresponding sets of fions, providing in this way the selective compaction and decompaction of the genome depending on the nature of the ion and on the characteristics of those proteins that are bound to the specified segment of chromosomal DNA. It is necessary to emphasize that the fountain mechanism is effective only when the nuclear pore complexes neighboring the open ionic channels are closed. This feature of the fountain mechanism allows it to be widely utilized in the cytodifferentiations, which require for their implementation just adjustments of the patterns of compaction of genes. In a similar way, fRNA-

dependent ionic regulation of chromatin configurations can also account for the still enigmatic process of X-chromosome inactivation in cells of a female organism, as well as for gene position effects, and moreover, for the phenomenon of dominance of one allele over the other allele of the same gene, and some other epigenetic phenomena. I surmise that eukaryotes have invented the perinuclear cistern enveloping the chromosomes just to get the possibility of the strictly local and aimed ionic manipulations with their chromatin informotheca.

ON FACTS CONCERNING THE ROLE OF IONS IN THE NUCLEUS

There is a series of facts which could be interpreted from the point of view of the existence of the fountain mechanism. Kroeger and others pointed out the possibility of regulation of the polytene chromosome puffing by some ions [5-10]. There is evidence of the presence at the inner nuclear membrane of Ca^{2+} channels that can be activated by inositol trisphosphate or cADP ribose [11]. Longin et al. [12] have shown the capability of calcium and zinc nuclear channels to open and to close for short time intervals, within about 4-5 msec; they emphasized that the role of these channels remains unknown. Ca^{2+} channels were also reported in [13]. A relatively small volume of the perinuclear cistern increases if the metabolic activity of the nucleus is growing [14]. The properties of DNA can be altered depending on the ionic surroundings [15]. Ca^{2+} can increase the transcriptional activity and stability of mRNA [16]. For a review of data on ions in the nucleus see also [17].

SOME FACTS WHICH CAN BE INTERPRETED AS POSSIBLE EXAMPLES OF INTERACTION BETWEEN FIONS, fRNPs, AND NUCLEAR MEMBRANE CHANNEL PROTEINS

There are data suggesting the existence of hybrids of RNA and DNA sites belonging to DNA segments which are attached to the nuclear matrix [18]. When forming near the inner nuclear membrane, such RNA–DNA complexes could activate the ion channels providing their RNA is represented by fRNA. There is evidence suggesting a tight association of some RNAs with the nuclear membrane as well as data on the formation of complexes of RNA with satellite DNA that are resistant to nucleases [19, 20]. One of the annexin proteins can bind to Alu-sequences of DNA and RNP of chromatin, RNA of this RNP being the small nuclear Alu-like RNA [21]. It is of interest that this small annexin protein is endowed with a variety of biochemical activities: it can serve as a substrate for many protein kinases which are activated by growth and other factors, and it belongs to a family of calcium-dependent phospholipid-binding proteins; it as well is able to serve as a subunit of the protein complex recognizing the primer and increasing the activity of DNA polymerase alpha on the template of denatured DNA. This protein was presumed to provide conditions for initiation of DNA replication [21-27].

Alpha-RNP–Alu-DNA complexes were found in nuclei of cells of proerythroleukemic lines [27] when they are induced to differentiation along the erythroid pathway. The small RNA (alpha RNA), the member of this complex, belongs to the homologs of dispersed repeated sequences of SINE class (in particular, Alu-elements in rat cells). Alpha-RNP particles from cells induced to differentiation, unlike the control, have the ability to interact specifically with DNA Alu-sequences [27]; this perhaps points out the ability of these RNPs to induce the appearance of single-stranded DNA sites of the Alu-sequences capable of interacting complementarily with this RNA. Transcripts of some Alu-elements can apparently take part in repression of some chromatin segments [28]. In this context, it seems expedient to assume that the forming fRNP complexes, depending on the specificity of fRNAs and chromosomal proteins, should be able to elicit local compaction or decompaction of chromatin due to the influence of a number of factors. Among these factors are: a) the nature of chromatin proteins at the site of binding of fRNP to the complementary, accepting, DNA site, or fion; b) the nature of the ions (for instance, Ca^{2+}, Zn^{2+}, or other ions, or their combination created by functioning of channels of different specificity) that were locally ejected in a short-term mode from the perinuclear cistern into chromatin; c) the local concentration of ions attained nearby the given perimembrane chromatin segment.

It has been shown that some Alu-repeats can function, by some unknown mechanism, as hormone-dependent enhancers, making higher the level of transcription of genes transcribed by RNA-polymerase II [29]. It seems plausible to suggest that enhancers and silencers could function by means of the fountain mechanism when they, conjointly with the specific fRNPs, are involved in short-term acts of activation of ion channels near the perimembrane-located regulatory segments of chromatin and structural genes.

There are data indicating that small nuclear RNAs (snRNAs) can twofold or more stimulate transcription in isolated nuclei [30], the pattern of transcripts within these nuclei being like the pattern observed *in vivo*. It seems attractive to identify the above snRNAs as fRNAs, supposing that these RNAs were imported into the nucleus. Analogous facts are given in [31, 32].

The majority of quantitative traits are known to be controlled with the assistance of complex sets of satellite DNA sequences [33]. Polymorphism of microsatellites and minisatellites plays an undoubted though still not

explained role in the expression of many structural genes; and in the above context, this polymorphism might be interpreted as a participation of satellite DNA in coding and/or binding fRNP during intranuclear ionic regulation of chromatin.

Hereditary small growth of human beings, as well as a seemingly lowered resistance of the fetus to chronic alcoholism of the pregnant woman were found to take place, when in several chromosomes the lengths of pericentromeric spacers, which are constructed from satellite DNA and situated between the centromere and structural genes, were inherently shortened in one of the two chromosomal arms [34]. This may be related to two factors. First, satellite spacer of a chromosome, fastened via the centromere within the nucleus, presumably allows to the adjacent genes (and their fions) to stretch to the inner part of the nuclear envelope, organizing there, in norm, the salutary ion shower, which increases productivity of the corresponding genes. Secondly, the deleted part of satellite DNA could perhaps code (in the form of printomeres) for their own fRNAs, the shortage of which is pernicious.

FROM fRNA TO PRINTOMERES AND CELLULAR MEMORY

From the supposed fRNA-dependent ionic regulation of chromatin configurations, let us turn to the question of how the cell memorizes the obtained pattern of chromatin decompaction and how the cell transmits this memory to its descendants in spite of the necessity to condense chromatin in metaphase chromosomes. The enigma of the memory about a previous state of chromatin consists of the circumstance that tight condensation of chromosomes should erase the earlier pattern of their decompaction. How do daughter cells recall the strictly determined parental pattern of chromatin packing? A widely accepted answer is absent, though it is currently surmised that cellular memory could be related to such epigenetic factors as DNA methylation and preservation of regulatory proteins within the chromatin. Nevertheless, there are organisms totally devoid of DNA methylation; as to the regulatory proteins, the question arises concerning the above mentioned problem of regulation of regulators. The essence of the corresponding difficulty, as already stated, is in that the maintenance of the cellular memory, based on a distinct set of proteins, dictates the necessity for this set to be under control of other regulators of higher level, which should be memorized by other higher regulators, and so forth, thus generating a vicious circle. Taking this into account, it seems purposeful to try to solve the problem by admitting that a new function needs a new structure. In the given case, it is the set of printomeres represented by special DNA molecules coding for the above-mentioned fRNAs. Since printomeres, being DNA molecules, can be copied in the course of cellular doublings, this distinctive feature of printomeres is apparently a necessary and sufficient condition for the puzzle of cellular memory to be solved.

THE BIRTH OF THE PRINTOMERE AND ITS PROPERTIES

Each printomere arises in the course of local endoreduplication of a strictly determined small chromosomal segment that has in length perhaps from several kilobases to tens of kilobases and which codes for synthesis of fRNA molecules responsible for the corresponding cellular differentiation or determination. The act of cytodifferentiation of the eukaryotic cell is accompanied by appearance of a printomere of new specificity that codes for fRNAs necessary for modeling, by means of the fountain mechanism, the strictly defined specific chromatin configuration.

It is known that, on the preimplantation stage of murine development, i.e., in the period when the events of determination and first differentiations take place, singe-stranded breaks and/or breaches are revealed in the nuclear DNA [35]. If the breaches are present only in one of two strands of the chromosomal DNA, then under the first doubling of the fertilized oocyte the sister chromatids should be marked asymmetrically, and this is the very thing that is really observed [35]. It is possible to assume that such kind of DNA breaches should be used for forming the printomere as a special "perichromosomal" object. The tiny printomeres arise in the course of normal differentiations, and afterwards they firmly hold themselves on the huge body of their corresponding chromosomes, replicating and maintaining themselves in dividing cells without loss of contacts with their chromosomal DNA originals, namely with protoprintomeres. The linear double-stranded DNA of printomere has a single *ori* and lacks its own centromere. This is one of the cardinal distinctions of a printomere from the genuine chromosomes. The printomere is a copy of its protoprintomeric original. The printomere is placed on the chromosome laterally, side by side to its chromosomal original. Nucleotide sequences of the printomere and its chromosomal template are arranged colinearly and are connected via chromatin proteins, forming multiplicity of bridges between the printomere and the chromosomal protoprintomere. This resembles connection of in parallel stacked DNA molecules in polytene chromosomes, though with the distinction that each printomere is seemingly represented in its chromosome by a single copy. The printomere, after it has emerged on the protoprintomere's template, no longer uses the former chromosomal template for its further replications, inasmuch as the printomere can copy itself in successive cell

doublings as an usual double-stranded DNA molecule utilizing now its own *ori*.

The following scenario of the printomere birth is most probable. On one of the two strands of chromosomal DNA, there appear breaches, one breach at each terminus of the protoprintomere. Totally, two breaches spring up at this strand. The opposite strand of the protoprintomere DNA maintains its continuity. Free single-stranded termini of DNA at the boundaries of each breach are protected from nucleases by proteins. The 5′-termini of DNA at each breach are probably covalently bound to protein molecules that may be used, in particular, to terminate the DNA replication during the process of making the printomere at the chromosomal, protoprintomeric, template. To form on the protoprintomere its working transcribed copy, i.e., the printomere, apparently it is necessary to partially separate the protoprintomere from the remaining chromosomal DNA. The gaps located on the flanks of one of the two strands of the protoprintomeric DNA can serve for that. This temporarily disrupts the integrity of the chromosomal DNA, which, however, then can easily and reliably be completely restored owing to the participation of the enzymes of DNA repair synthesis.

For the protoprintomere's *ori* to be activated, the act of transcription of the initiating RNA that subsequently will serve as a primer to initiate the DNA synthesis within the *ori* is indispensable. The promoter for transcription of this initiating RNA is apparently activated under the influence of some specific inducer of the corresponding cellular differentiation. Replication of protoprintomeric DNA begins from the intrinsic *ori* that resides in the middle of a protoprintomere. Bidirectional DNA synthesis proceeds from this origin, when replicative forks are moving from the center of the protoprintomere synthesizing flanks, or arms, of the emerging printomere. The forks are intercepted near the termini of DNA, breaking in breaches.

Printomeres that control various cytodifferentiations in diverse species have a common principle of their action, but they may differ in the number and sequence of fRNA-coding repeats and with respect to the lengths of buffer spacers between these repeats, in the lengths of acromeres, i.e., tips of printomeres, and also in nucleotide sequences of acromeres. In those cytodifferentiations where telomerase is in action and where nucleotide acromere's sequences are similar or identical to the repeats of chromosomal telomere, unscheduled and unnecessary for the cell lengthening of the printomere's tips by telomerase appears possible. This is probably one of the reasons why telomerase activity can be switched off in the course of differentiations. Those cell types can evade this difficulty whose acromeres differ in their nucleotide sequence from telomeres.

Protoprintomeres are situated predominantly, if not solely, at subtelomeric, i.e., next to telomeres, and pericentromeric, i.e., next to the centromere, chromosomal regions. In the same places, that is in subtelomeric and pericentromeric heterochromatin, the proper printomeres should be situated.

For the printomere to be created, as mentioned above, DNA replication is of necessity. This requirement indirectly agrees, in particular, with the circumstance that cellular differentiation and specialization of plant meristem cells need nuclear DNA replication [36]. DNA breaks are found to accompany the processes of cell differentiations [37-40]. Single-stranded breaks, that are probably breaches, were revealed in the nuclear DNA in early murine development (from 1-cell stage to blastocyst). The most intensive incorporation of the label was observed near telomeres. It is of interest that clearly pronounced unequal, asymmetric, staining of sister chromatids was revealed in embryos at the one-cell stage [35]. This fact might be connected with the suggestion proposed here, that each new printomere should emerge only at one of the two protoprintomeric DNA strands. Moreover, all printomeres are possibly forming only on one of the two strands of the whole chromosomal DNA, at the above one-cell stage, in order to decrease the danger of two-stranded breaks of DNA.

According to the data of some authors, cellular differentiation and the appearance of double-stranded breaks in DNA are coupled processes, although the genuine significance of this connection is still not completely elucidated. For example, tumor necrosis factor causes both differentiation and DNA fragmentation in fibroblasts HL-60 [41], and in this relation the supposition was made that the process of DNA fragmentation does control, though in some unknown way, the expression of genes [41]. Other factors are also endowed with a analogous property: both DNA fragmentation and differentiation of some cell types are elicited, in particular, by gamma-irradiation [42] and by inhibitors of topoisomerases [43]. Erythropoietin induces not only differentiation in erythroid cells, but also double-stranded breaks in DNA [44].

Double-stranded oblique cuts with formation of DNA sticky ends, which ensure the maintenance of the chromosomal integrity, could arise, if two or more distinct printomeres, rather than one, are forming in a cellular differentiation. The above-mentioned DNA cuts can be a consequence of formation of two different but adjacent printomeres, originating and placing in tandem, one after another, however on the opposite strands of chromosomal DNA molecule, rather than on one and the same DNA strand. Correspondingly, one of the two protoprintomeres, located in a tandem, should in this case have its own flanking breaches in one of the DNA strands, whereas the flanking gaps of the second protoprintomere will be situated on the opposite strand of the same DNA molecule. As a result, chromosomal DNA inevitably gets the double skew incisions in this region.

The sticky termini in this region nevertheless do maintain the mechanical contact between the divided DNA segments during this critical for a differentiating cell period of time. As noted above, the breaches can be necessary for a stop of replicative forks on borders of each protoprintomere, when the forks are completing there the synthesis of flanks of the *de novo* creating printomere. The arrest of the replicative fork may be assisted by formation of the covalent protein–DNA complex at the 5′-terminus within a DNA gap. It is pertinent to note in this connection that covalent protein–DNA complexes are utilized in the course of appearance of controllable breaks and restorations of DNA strands, and that such complexes are found specifically in cytodifferentiations [45].

The ruptures within protoprintomeric chromosomal DNA that were generated at the onset of cellular differentiation to produce a printomere should afterwards be repaired by a cell. To ensure the chromosome's wholeness, this should be done before the cell cycle is completed. Other conditions being equal, it is apparently most favorable to create the printomere anew on its protoprintomere at the very end of S phase of the cell cycle.

The printomere cannot be attributed to the extrachromosomal DNA, since being laterally attached via proteins to the chromosomal body, printomeres share their fate with their chromosomes, being correctly distributed with their help and together with them in daughter cells.

Since printomere controlling the cellular memory has DNA, this cellular memory can hardly be regarded as an epigenetic phenomenon. Due to the singularity of the situation, I suggest that such memory be considered as a "paragenetic" phenomenon.

ON THE HIERARCHY OF REPACKINGS OF THE GENOME WITH THE PARTICIPATION OF fRNAs

It necessary to emphasized that there should be distinct kinds of fRNAs in the nucleus. Those fRNAs that are coded by printomeres themselves do immediately control the cellular memory. However, the printomeric fRNAs should not be obligatorily responsible for decompaction and compaction of literally all of the chromatin segments. Hierarchy in the operation of various fRNAs differing in specificity is quite allowable. To create and maintain cellular memory, it will be necessary and sufficient if fRNAs encoded in printomeres guide the unpacking of certain initial chromatin segments which will then "retransmit" this basic signal, converting it into a form of their own novel fRNAs. In its turn, this intermediate signal then goes, in hierarchical order, to successive sets of subordinated short repeats of the SINE families, e.g., to certain Alu-sequences. These SINEs code for those fRNAs that directly serve the structural genes of a given cytodifferentiation. Similar two-leveled or even multilevel regulation has some advantages. The printomere can be transcribed only either at the onset of a new cell cycle or at the end of the preceding cycle. Signals of the printomere are thus retransmitted and enhanced owing to the synthesis of those fRNAs that are transcribed from the appropriate SINEs, which are dispersed along the whole genome nearby many structural genes and other sites of chromosomes and which are ultimately unpacked by the order of the printomere's fRNAs. The above mentioned fions, in dependence on their specificity, bind either printomeric fRNAs or those fRNAs which are transcribed from the corresponding SINEs. This method permits repacking in series any necessary segments of the genome providing creation of any configuration of chromatin ordered by printomeres.

One of the most important roles of genetic LINE elements, which long transposones belong to, consists in that the transposones when moving into other genome regions are able to change the already existing pattern of fRNA-dependent compaction of chromatin. Being inserted into a novel region of the genome, the long transposone can serve as a mechanical spacer, and besides, having on its flanks, in LTRs, the elements of the fountain system of ionic regulation, transposone can create some novel, earlier non-existent, combinations of chromosomal segments that will be capable of becoming compacted or decompacted in new configurations when responding to the signals of the same, unchanged, printomere. Many mammalian species are known to have their own main families of long species-specific repeats. Possibly, this circumstance may be related to species-specific distinctions in hierarchic repackings of the genomes in the course of individual development.

Coordination of gene expression levels within a given cytodifferentiation could be achieved if some intermediate fRNAs are transcribed as a part of the premRNAs from some structural genes that are active in a given cytophenotype.

ON THE ROLE OF PRINTOMERES IN CELLULAR AGING

The nucleus enlarges during cell senescence, possibly due to attempts of homeostatic systems of the cell to increase to the maximum the number of elements that are unpacked and recruited into the operation of the fountain system, in order to intensify as much as possible the expression of genes under conditions of a deficit of fRNAs coded by shortened printomeres. The truncation of printomeres, due to DNA end underreplication and DNA end underrepair synthesis of linear print-

omere molecules, could lead to a gradual, rather than drastic, decrease of gene productiveness in a differentiated cell. The smoothness of this process, as far as it depends on printomere's shortening, is determined by the following circumstance. Providing the printomere contains several repeats which code for fRNAs, the loss of each in succession of them reduces the productivity of the printomere due to the decrease in its "gene dose". Shortened by one or more repeats, the printomere is still able to be transcribed and to remain resistant to nucleases. This is possible provided that the spacers located between the printomere's repeats are identical to acromeres in their sequence and if they are able to take the acromere's function of a cap, when any inner spacer appears at the absolute end of the printomere.

The acromere is a sequence containing a series of short repeats. Acromeres are localized at both termini of the linear double-stranded DNA of the printomere. Together with covering proteins, each acromere protects its printomere from nucleases and, in addition, it plays a role of a buffer which, like telomeric DNA, is shortening because of the DNA end underreplication and the DNA end underrepair. Acromeric buffer, sacrificing itself, preserves the safety of those informatively significant printomere sequences in which fRNAs specific for a given cytodifferentiation are encoded. The definition "acromere" is chosen for these telomere-like structures of the printomere in order to avoid confusion with the true chromosomal telomeres. Besides acromeres and a single *ori*, each printomere has, as it was already stated, sequences coding for fRNAs. These coding sequences are represented in the printomere by a series of repeats separated by the above-mentioned spacers whose sequence may be similar or identical to the sequence of acromeres. In such a case, the loss of an acromere and of the terminal informational repeat coding for the fRNA will not immediately lead to complete loss of the activity of the printomere, since the interior repeats of the truncated printomere still can retain their transcriptional activity. Nonetheless, the "gene dose" of the shrinking printomere persists gradually decreasing. This inevitably leads to decline in the concentrations of the corresponding fRNAs, that, in turn, should decrease the portion of the adequately decompacted chromatin and reduce the activity of transcribing genes. In this way, shortening of the printomere must inevitably weaken the activity of a cell and reduce its homeostatic potential. That is why the shortening of printomeres appears to be the primary cause of cellular senescence.

THE PRINTOMERE UNDER THE MICROSCOPE?

If the printomere exists as an autonomous unit in a complex with chromatin proteins, then, by analogy to the "ultramicrochromosome", it could be termed as the "printosome", and as such it could be identified by using some pertinent techniques. Whereas the methods of molecular biology in their application to printomeres is a task of the possible future, some observations of cytologists on chromosome morphology compel me to suppose that some structure—a candidate for the role of printosome—has already been revealed. Uruguayan researchers, visualizing results of T-banding, have discovered tiny cavities in chromosomes at the spots of some removed chromatin fraction. These unusual cavities were found in paracentromeric regions, i.e., in regions on both sides of the centromere and in subtelomeric regions [46, 47]. Printomeres could reside just in these regions, since these segments are most conservative and resistant to recombinations; and the conservatism of printomeres is favorable for preserving of species-specific properties. The intrachromosomal cavities from which the printosomes could be potentially extracted, were found in identical places of members of one and the same chromosomal pair; the cavities occurred to be totally free from chromatin. When, on the background of the preserved continuity of DNA, printomeres are removed from the chromosome, the protoprintomeres as chromosomal originals of printomeres should, of course, keep residing in chromosomes, being a part of the continuous chromosomal DNA.

CONCLUSION

According to the paradigm existing in this field, memory about the active and non-active state of genes is kept in cell doublings mainly due to preservation of some regulatory proteins at the active parts of the genome, even in entirely condensed metaphase chromosomes. This paradigm has never been completely proved, and here a new conception is proposed instead, based on the idea of the existence in the eukaryotic nucleus of a new genetic structure, the printomere. Specificity of transcripts, namely fountain RNAs (fRNAs), encoded in printomeres, could be sufficient for restoring in each successive cell cycle the necessary, directed by the printomeres, structure of chromatin compaction. The required and sufficient condition for this is complexation of printomeric fRNAs and fions, i.e., the sites of DNA complementary for fRNAs. Their interaction is needed for ion regulation of chromatin and the activity of genes. It is important to stress that the chromatin "recollects" its preceding configuration in a step-by-step mode, rather than all at once. There can exist several hierarchical subordinated fractions of distinct fRNAs, which can successively pack and unpack, each acting on a corresponding group of chromatin segments. The printomeric fRNAs have the highest position in this hierarchy, whereas the fRNA molecules,

which are lower in hierarchy, are coded for by subordinated chromosomal sites. Cellular memory is recorded only in printomeres and is stably retained throughout many cell generations because printomeres are able to replicate.

The above mechanism of the maintenance of cellular memory, besides its utility for ordinary cytodifferentiations, probably is quite widely exploitable by various eukaryotes. In particular, it can be in action during the course of selective inactivation of one of the two female X-chromosomes, as well as in genome imprinting, in gene position effects, in dominance of one of the two alleles of the gene, etc. All these phenomena became available for eukaryotes as soon as, at the dawn of their evolution, they invented the perinuclear cistern with its two membranes enveloping the nucleus, having had the opportunity for conditioning the ions in the perinuclear lumen in proper concentrations optimal for functioning of the printomere-dependent fountain mechanism.

The proposed hypothesis provides some novel interpretation of the role for short and long repeats widely represented in genomes which are getting now an original function, namely, ionic regulation of chromatin properties, i.e., the function that participates in organization of cellular memory and differential activity of genes.

On the whole, both the printomeres and the fountain system of ionic regulation of a genome, which is under the printomere's control, can be indispensable and sufficient elements to solve the problem of regulation of regulators, as well as to create and to maintain the cellular memory of nuclear organisms.

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