

Towards the quantitative traits regulation: fountain theory implications in comparative and developmental biology

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ABSTRACT A fountain mechanism of quantitative regulation of gene expression level during development is proposed. The mechanism is based on postulated ability of a special class of RNA molecules, so called fountain RNAs (fRNAs), to induce passive and selective ionic channels in the internal nuclear membrane. Ions diffuse via channel from the nuclear lumen into the chromatin compartment. An RNA-dependent battery of ion channels is assumed to produce «a fountain» of ions in close vicinity to the corresponding genes. An ion atmosphere, in its turn, locally changes the chromatin configuration and effectiveness of transcription and processing of transcripts. Hence this mechanism can be used to change genes productivity. It is a basic mechanism of quantitative traits regulation. A passive selective ion flux periodically stops after a threshold ion concentration induces local chromatin compactization and arrests the activity of ion channels in a given chromatin compartment. This process serves as a basis for many cellular biorhythms that are relatively temperature-independent because of the passive nature of ion channel. It is postulated that eukaryotes became eukaryotes just to obtain this fountain mechanism that allows them to perform gradual quantitative modulation of corresponding genes expression levels. The fountain mechanism is partly responsible for dominance and heterosis, X-chromosome inactivation, gene position effects, and some other epigenetic events. It plays an important role in embryonic and post-embryonic development. A significant portion of the former «junk» DNA can be referred to as fDNA involved in the proposed mechanism functioning. Genomic rearrangements of fDNA could lead to micro- and macroevolutionary changes in the animal and plant kingdoms. The pivotal evolutionary function of transposons could reside in their ability to contain and relocate fDNA along the chromosomes.

KEY WORDS: *ion modulation of gene expression, quantitative traits, biorhythms, transposons, position effects*

Introduction

The mainstream of current molecular developmental biology concerns the roles of individual genes in development, including elucidation of their transcription factors and mRNAs processing and translation at corresponding stages of development. Attention to molecular mechanisms of extracellular, intracellular, and nuclear matrix is also gradually increasing. This fruitful approach does yield tremendous expansion of our knowledge. Nevertheless, the main question, that was put forward by eukaryotes existence itself, still remains unanswered. They have a nucleus. What for? If the nucleus protects long chromosomes carrying eukaryotic «redundant» DNA, then one could ask what this redundancy is for, whereas prokaryotes perfectly survive without redundant DNA. Why is it not possible for eukaryotic chromosomes to contain only the coding genes with a minimal excess of DNA needed for recombination and segregation? Why does a

nucleus have two membranes and a nuclear lumen between them? What is the aim of a spatial order of the chromosomes location inside the somatic nucleus, if there is no need for the chromosomes to search each other and recombine, since fertilization does not take place there? If the spatial arrangement of chromosomes is maintained to regulate genes and gene clusters, it is necessary to reveal a mechanism requiring the existence of such 3D architecture.

In an attempt to attack this problem or, in other words, to try to grasp the very essence of «eukaryotism», it seems expedient to take into account that perhaps the eukaryotes quantitative traits are the most fundamental distinction between the eukaryotes and

Abbreviations used in this paper: fRNAs, fountain RNAs; frDNA, fountain DNA encoded fRNA; fiDNA, single-stranded DNA targets; fdDNA, fountain DNA for total frDNA + fiDNA; ATP, adenosine triphosphat

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prokaryotes. During evolution of the eukaryotes, in contrast to the prokaryotes, the variability of quantitative traits is broadly represented. For example, there are significant differences in sizes of different organs and organisms within any eukaryotic population, as well as in the rate of their growth and, more generally, in the intensity of functioning, whereas prokaryotes have a great handicap in the rate of mutations of the structural genes, thus obtaining many qualitative rather than quantitative differences. If one assumes that distinctions in quantitative traits among eukaryotes should be somehow related to their possession of a nucleus, then it seems pertinent to propose a link between the ability of eukaryotes to modulate the activity of genes or gene clusters and, hence, the levels of expression of quantitative traits, on the one hand, and peculiarities of the eukaryotic nucleus with its spatially ordered long chromosomes and «redundant» DNA, on the other hand. One can suppose that elucidation of the above link might help to solve the enigma of «eukaryotism». The aim of this work is to try to find this link and, hopefully, to solve the old enigma of eukaryotes.

Elucidation of a possible developmental role of the so called junk or egoistic DNA comprising the major part of the eukaryotic genome is in itself an important but still unsolved problem of current developmental biology. The mechanism of quantitative traits regulation in ontogeny also remains elusive, despite enormous success in deciphering the molecular properties of strong and weak promoters, numerous transcription factors, enhancers, etc. In an attempt to solve both problems, that may be interconnected, a new molecular mechanism is proposed which may be very important both in embryonic and post-embryonic development. According to the proposal, the major part of the eukaryotic genome is involved in ion-dependent regulation of gene expression. More specifically, modification of the local ion surrounding of any gene could be a powerful factor of modulation of its expression intensity, thus enabling any gene in any cell to change the mRNA productivity that might be determinant in ontogeny and phylogeny of animals and plants. Therefore, it is proposed that a significant part of the eukaryotic genome, termed here «fountain DNA», should be involved in quantitative regulation of the gene activity, and this issue is a central topic of the present paper. The other, smaller, part of the former egoistic DNA, called location DNA, has recently been proposed as an important participant of biological morphogenesis (Olovnikov, 1996).

A new mechanism of regulation, namely quantitative modulation of eukaryotic genes expression levels, is proposed, and some pertinent biological consequences of its existence are considered as well. This mechanism is based on the activity of special passive ion channels that originate in the inner nuclear membrane under the influence of the so called fountain RNAs (fRNAs). The mechanism is here defined as an RNA-dependent ionic mechanism, or shortly fountain mechanism, since it produces a flux of a particular ion along the concentration gradient from the nuclear lumen into the local chromatin compartment.

fRNA molecule forms a specific complex with complementary single-stranded DNA site, the latter being anchored to the inner nuclear membrane in close vicinity to the corresponding gene or chromomere. It is assumed that a passive ion channel is formed in this place. The ion channel activity alters the ion environment of a gene. This leads to changes in the local transcription process productivity. The proposed channels are formed non-randomly in the genome. The usage of specific fRNAs, which are transcribed

from other parts of the genome does open a possibility of selective quantitative regulation of gene expression. The proposed modulation of gene expression should not be erroneously confused with already known means of regulation via strong or weak promoters, etc.

The involvement of fountain mechanism in regulation of quantitative traits, such as weight, height, rate of growth, blood pressure, etc., is proposed. Therefore elucidation of this mechanism and its artificial regulation could have potential important practical sequences. The fountain mechanism may be involved in allele dominance and it is probably a key mechanism of heterosis. It may play a pivotal role in some epigenetic processes, such as X-chromosome inactivation and position effects. Since RNA-dependent ionic channels are able to function with some rhythmicity, as will be discussed below, the fountain mechanism can underlie the system of cellular biorhythmicity. Due to its ability to change quantitative expression of many genes, the fountain mechanism could be recruited into modification of adaptive characters during development and evolution.

Chromatin configuration and ions

Chromatin contains various ions. It is known that Ca^{2+} is mainly associated to proteins, while Mg^{2+} is mainly bound to DNA (for a review of studies of the ion environment influencing the DNA structure and function see, Jayaram and Beveridge, 1996). It was thought for many years that alterations of ion contents of the nucleus is an important regulator of its activity (see Kellermayer, 1981). A structured distribution, seemingly related to G/Q-banding patterns, was observed in the Mg^{2+} and Ca^{2+} maps of chromosomes (Levisetti *et al.*, 1996). Kroeger (Kroeger, 1963, 1977; Kroeger and Troesch, 1974) proposed that changes in ion environment of the chromosomes play a role in gene activity by inducing the puffs during action of some hormones. Specifically, puffs were observed to appear in the isolated nuclei with changes in NaCl concentration. Mg^{2+} is able to induce a Balbiani ring. However, it has so far been unclear how to use this approach for highly selective regulation of genes (for criticism and discussion of this question history see Kroeger and Troesch, 1974; Ashburner and Cherbas, 1976; Kroeger, 1977; Lezzi and Richards, 1989; Zhimulev, 1992). Most monovalent cations such as Na^+ stabilize the duplex. Divalent cations, Mg^{2+} including, on the opposite, do decrease DNA stabilization (Lee *et al.*, 1993). Increasing the ionic strength, particularly with divalent cations, encourages the formation of left-handed DNA from alternating pyr-pur sequences. Many DNA-binding proteins require specific cations for their activity. Changes in local pH can also influence the chromatin configuration. Counterions, including Na^+ and Mg^{2+} , compete for binding and altering the DNA configuration (Bloomfield, 1996). As to the nature of ions that are involved in the fountain mechanism, firstly, let us take into account K^+ . In the nucleus of an oocyte, concentration of Na^+ is three times lower than in the ooplasm. The nuclear content of K^+ , on the contrary, was approximately 1.6 times higher than that in the cytoplasm (Century *et al.*, 1970; Paine *et al.*, 1981). It is meaningful that the perinuclear lumen is enlarged with the increase in nucleus metabolic activity. This may probably be connected with supposed increase in concentration of solutes that are necessary for fRNA-dependent ion channels (for review see Zbarsky, 1988).

On fRNAs structure and on designation of DNA pertinent fractions

In order to discuss the mechanism of gene regulation by means of RNA-dependent ion channels that are spatially ordered, it is pertinent to introduce some new terms. The following designations are used here: fRNAs for fountain RNAs, frDNA for fountain DNA that encodes fRNAs, fiDNA for single-stranded DNA targets to which fRNAs are binding in order to induce an ion channel, and merely fountain DNA (fDNA) for total frDNA plus fiDNA.

A fRNA molecule has two domains: the so-called chromosomal domain and envelope domain. The former, DNA-recognizing, or d-domain, recognizes its single-stranded DNA target. The latter is responsible for interaction with protein(s) of the nuclear envelope and, hence, it is designated as an envelope domain, or en-domain. The en-domain is necessary for induction of ion channels in the inner nuclear membrane. Both d-domain and en-domain differ in different fRNAs. d-domains should recognize various targets within the chromosomal DNA and, hence, this is a reason for d-domains variability. En-Domains may be responsible for induction of specific ion channels. Therefore, d-domain is less conservative in comparison with en-domain.

Protein(s) of ion channel are activated only by signals from conformationally activated en-domain of fRNA and/or protein of fRNP. The fRNA itself, after having been hybridized with single-stranded DNA target, changes its conformation in the en-domain. Single-stranded targets in DNA are maintained in this unusual form through binding to the single-strand-binding proteins (SSB proteins). These proteins are like common free SSB proteins, though they are possibly fixed in the periphery of the inner nuclear membrane. This single-stranded form of target DNA probably originates during DNA anchoring to the nuclear envelope.

These RNA-ionic channels should be physically tethered to the corresponding chromosomal target. This very unusual requirement is based on the ability of proteins to drift in the membranes. Without strong linking, the newly formed channels may float away in an undesirable direction. To avoid this, fRNA probably forms a very unusual structure when some covalent cojoners DNA-RNA are produced. Structurally, they are like those which are known for RNA-DNA single-stranded cojoners similar to Okazaki fragments or to RNA-DNA complexes that are specific for retroposons or retrons. The fDNA-RNP covalent link adds more flexibility to this construct, because it additionally allows it to adapt to changes in the distance between the target DNA and nearest target protein floating among lipids of the inner nuclear membrane. Therefore, fRNAs should participate in several events. They hybridize with a single-stranded site of target DNA, then form a covalent RNA-DNA product, and, at last, stimulate formation of specific ion channels via interaction with the corresponding proteins of the inner nuclear membrane. As to the above mentioned conjoined RNA-DNA covalent complexes, it is important to emphasize that, being similar to Okazaki fragments which are in abundance within the interphase nucleus, they may still be elusive for experimenters.

Where are fRNAs encoded?

It is assumed here that fRNAs of different specificity should be encoded mainly in subtelomere regions of the chromosomes and, to a much lesser extent, in some sites of the chromosome arms. A

temporal coincidence between the appearance of a subtelomeric deletion on *P. berghei* chromosome and the loss of the ability to produce viable gametocytes was observed (Birago *et al.*, 1994). It was also noted that the growth retardation in children and low height of their parents are clearly correlated with drastically diminished content of C heterochromatin in the pericentromeric and peritelomeric regions (Podugolnikova *et al.*, 1994). There are similar observations for the subtelomeric regions. Individuals with a ring chromosome and those with sites of chromosome breakage close to the telomere have short stature, developmental delay, triangular face, and clinodactyly (Rogan *et al.*, 1996). It is possible that these phenomena are connected with a shortage in DNA that encodes fRNAs.

The subtelomeric motifs are dispersed among the chromosome arms. These sequences could serve as potential targets for fRNAs. The well known *Drosophila*-like and *Xenopus*-like types of genomes may correspond to the two distinct strategies of design of the fountain systems. The components of this system can be combined into big blocks or, on the opposite, they can be dispersed as very short but numerous clusters. It is claimed here that these two strategies might be adapted to two opposite ecological requirements for the rate and duration of the corresponding developmental stages. For example, the animals with demands for a prompt certain stage may gain a benefit from large ion batteries and acceleration of growth rate at this stage, while the others need retardation of this tempo and will choose the opposite variant of fountain system.

Some fRNAs may be also components of 3'-tails of mRNAs. The latter possibility could open another dimension in the fountain mechanism, since it leads to a gene network of the eukaryotic genome. As it is discussed elsewhere in this paper, genes of some fRNAs are probably inserted in transposons.

The subtelomere is convenient as a region coding for fRNAs owing to its following properties. It is permanently located very close to the nuclear membrane due to the telomere's anchoring to the nuclear envelope. The subtelomere itself can be under the influence of a local ion influx. Besides, having a high population polymorphism due to the high rate of local recombination events, the subtelomeric regions could provoke high quantitative polymorphism of many traits in populations, what potentially does increase adaptability of the population as a whole.

For complementariness of the subtelomerically encoded fRNAs and their targets in the chromosome arms in vicinity of the structural genes to be ensured, it is necessary to perform, at the population level, an exchange of genetic materials between the corresponding regions of chromosomes. It can be proposed, therefore, that the reason for extensive Robertsonian re-organizations, that are chromosomal structural changes due to centric fusion or centric fission, should be based on the necessity to maintain permanent population polymorphism within the genome portion that is responsible for the fountain mechanism functioning. It is especially important for survival of those populations and species which occur in highly changeable habitats. In such conditions, only highly heterogeneous populations, which pay for their survival by those individuals whose quantitative traits occur to be inadequate to the present ecological situation, can survive. Other, non-Robertsonian, intra- and inter-chromosomal duplication events could thereafter transfer the newly gained genetic material of the telomere-associated region closer to the necessary structural genes. Thus, the above

necessity may be the pivotal cause of permanent and still enigmatic chromosomal rearrangements widely observed in plants and animals, though in different intensity that may probably correlate with the readiness of species to survive in case of intense ecological cataclysms.

Some current exchange of genetic materials between the subtelomere and the other part of genome, or vice versa, can be exemplified by the appearance of some pseudogenes both in the subtelomere and chromosomal body. Comparison of the helicase gene sequences with available databases indicates that a large portion of these genes, including exons encoding two functional domains of the carboxyl-terminal region of these proteins, has been duplicated as part of a larger human telomeric repeat sequence found on many human chromosomes (Amann *et al.*, 1996).

This has resulted in the creation of numerous pseudogenes as parts of a subtelomeric repeat. The presence of these helicase pseudogenes, as well as pseudogenes for other genes such as *interleukin-9* receptor, within many subtelomeric regions supports the possibility that the spread of this region should be subject to exchange between different chromosomes (Amann *et al.*, 1996).

Activity of passive ion channel

Selective ion channel that belongs to the fountain mechanism is probably formed by using some membrane proteins that have been a part of the inner nuclear membrane even before the molecule of fRNA arrives. After arrival into surrounding of the corresponding chromomere, fRNA, as such or in the form of fRNP, first of all creates a RNA-DNA complex with local single-stranded DNA. After some conformational changes of fRNA in this complex, another domain of the same fRNA molecule (or fRNP) becomes activated. Now it can combine with the corresponding inner-nuclear-membrane protein which, in turn, becomes able to transform itself into an ion channel, e.g. due to opening a previously closed gate. Selective diffusion of the corresponding ion (its nature depends on selectivity of the gate of the above mentioned passive channel) from a perinuclear cistern into a local chromatin compartment is based on concentration ion gradients. The ions spout from the channel like «jets of a fountain». This process does not require ATP. The energy of ATP is necessary only for pumping the perinuclear lumen with corresponding ions rather than for functioning of the passive ion channels themselves. Ion battery can probably work for a long period of time, from several minutes to several tens of minutes. Then it stops its activity due to several reasons, including the destruction of fRNAs and proteins, equalizing ion concentration on both sides of the inner nuclear membrane, or possible influence of the membrane potential and other electrochemical events. To the point, intranuclear ion in flux is not possible under too low ion concentration within perinuclear lumen.

However, another cause of subsequent cessation of the ion channel activity is especially important. Too long work of a battery of ion channels in close vicinity to a chromomere should lead to local increase of a given ion concentration which is already non-optimal for the activity of structural genes of a chromomere. The local condensation of chromatin will prevent access of new portions of fRNA molecules to their targets to maintain the normal activity of ion battery. As a result, new ion channels can not be built, while the old ones are soon destroyed if individual ion channels have a short half-life. Hence, the chromatin of a given compart-

ment, formerly decompactized, now begins, on the contrary, to condense. Hence periodic local switch off of the ion fountain is not only possible but even quite necessary.

Nuclear compartments and biorhythms

Many details of the nuclear matrix organization correspond to the «nuclear matrix as a system of channels» (Razin and Gromova, 1995). According to this scheme, the scaffolding element providing a surface for the attachment of DNA loops is not a filament but a large three-dimensional channel like a tube. The chromosomal DNA domains are assembled around this tube in the same way as a modern multi-storey building is assembled around a tower with elevators. According to Razin and Gromova (1995), this tube serves as a channel to transport mRNAs to the nuclear pore complex. However, these facts can also be interpreted as the ability of chromatin to form a closed tub-like compartment that is turned over to contact with the inner membrane of the nuclear envelope. In other words, chromatin probably forms a cave the inner part of which could serve as a chromatin compartment. The bottom part of this compartment is formed by the inner nuclear membrane with its passive ion channels. To the point, in this context, the terminological intermingle when using the term channel should be avoided. The postulated RNA-dependent ion channels, which cross the inner nuclear membrane of the nuclear envelope, have no common features with those channels that could be used to transport high molecular weight substances between the nucleus and cytoplasm (Kramer *et al.*, 1994; Razin and Gromova, 1995). As to the role of nuclear skeleton meshwork in organization of nuclear compartments, this question is still open (for current data on the nuclear envelope and possible nuclear skeleton-dependent nuclear compartments see: Kramer *et al.*, 1994; Razin and Gromova, 1995; Hozak 1996; Yoneda, 1997).

Role of fountain mechanism in organization of biorhythms

To isolate a nuclear compartment a container is needed. A dense nuclear matrix with a special fraction of a highly condensed chromatin may play the role of its walls. This «container» is formed when the corresponding fRNA-dependent battery of ion channels has been already made. Otherwise, the container walls will prevent access of fRNAs to their membrane-bound DNA targets. Therefore, this container should periodically appear and then disappear under the command of excessive concentration of a certain ion. Correspondingly, the synthetic activity directed by local genes will firstly be enhanced under the influence of stimulating ions, and thereafter transcription will be decreased and even stopped owing to too excessive ion concentration. After the container walls are destroyed, the synthesized mRNAs have a possibility to get out of the compartment and they move as RNP granules to the nuclear pore complex to be exported into the cytoplasm. This means the end of a cycle of the events related to the life of passive ion channels battery. After restoration of the initial ion environment that may be performed by intranuclear transport of counterions and/or by ventilation with the help of neighboring nuclear pore complex or even by destroying nuclear envelope as a regular event of cell cycle, the fountain cycle can be reiterated. These localized fountain cycles can play the role of pacemakers of many biological rhythms.

In some cases, a common compartment for several gene clusters belonging to different chromosomes is built. These genes take an «ionic shower-bath», one for all, from a single large battery of ion channels. Probably, just this circumstance is responsible for that chromosomal behavior when certain segments of the interphase chromosomes have specific spatial arrangements in interchromosomal clusters (see Hilliker and Apples, 1989; Markova *et al.*, 1994).

As was stated above, the fountain mechanism could probably serve as a pacemaker of biorhythmicity. In numerous chronobiological studies, the so called endogenous component of biorhythms was revealed, which persists in the absence of environmental cues. Other conditions being equal, the period of any endogenous rhythm was found to be almost independent from the body temperature and the level of metabolic activity. Exactly this effect should be expected, if some, so to say pioneer or primary, rhythms are based on the cycling fountain mechanism. Since the pivotal mechanism of biorhythms still remains elusive in spite of great progress in isolating periodicity genes, it seems appropriate to suggest a fountain mechanism for this vacancy.

Products coded by various genes are interacting with each other. That is why the fountain-directed cyclicity of some pioneer biorhythms must involve those genes in its periodic activity which normally does not need immediate contact with ion fountains. As a result, the whole cellular activity and functioning of the multicellular organism, as a whole, inevitably become involved into performing rhythms whose properties are already dependent on intergenic interactions rather than on periods and other peculiarities of the above mentioned pioneer rhythms of fRNA-dependent ion channels themselves.

Rhythms with more prolonged periods can be based on interactions of rhythms with shorter periods, namely on the above pioneer fountain-associated rhythms. Biological clock demonstrates many physiological parameters with periodicities close to 24 h. Complete suppression of some circadian rhythms can lead to their replacement by ultradian rhythms (Depresbrummer *et al.*, 1995). Ultradian rhythmicity is characterized by periods from several minutes or more to several hours. Ultradian rhythmicity shows significantly different periods among mouse strains (Mazzucchelli *et al.*, 1995). Periodicities and rhythmic parameters, including studies on maintaining and altering their characteristics, such as period, phase, amplitude and wave-form of various oscillator(s), have recently been reviewed (Takahashi and Hoffman, 1995).

It is interesting that biorhythmicity can be considered as an example of quantitative epigenetic regulation of gene expression since one and the same gene at the same genetic background is capable to provide, in various time intervals, for a high, middle or low productivity of a cell.

The possibility to regulate biorhythms through ions was a subject of active discussion, in the course of which it was suggested that many hormones should somehow interact with the nuclear membrane in order to induce ion channels in it (see Njus *et al.*, 1974; Goodwin, 1976). It seems, however, that utilization of specific RNAs, like fRNAs discussed here, could provide much more versatile and effective mechanism in comparison with the hormone-dependent induction of ion channels. However, fRNA can bind only to single stranded complementary DNA while the latter normally does not exist in free form in most genomes. This is achieved due to the SSB-like proteins, as was discussed above.

Transposons as participants of fountain system and their role in evolution of developmental mechanisms

It is proposed here that an important and probably the pivotal function of most of transposons is their supposed participation in the fountain system. Assuming that transposons could transfer genes of fRNAs (and targets for these fRNAs) within the eukaryotic genome, we can deduce that these mobile elements could enormously diversify the fountain system and, hence, additionally modulate different quantitative traits in the course of evolution. Transposons could transfer genes of fRNAs closer to the target chromomere. They may also introduce targets for binding fRNAs closer to a certain gene, in both ways increasing the efficiency of transcription. On the opposite, if a certain chromomere becomes condensed by a given ion channel, the transposon can induce transcriptional silencing of a gene, instead of its enhancing.

The idea of transposons as quantitative regulators ascends to McClintock. Though the molecular mechanism of their action remains mostly unknown, it is tempting and very appropriate to suggest that transposons could modulate quantitative traits via the fountain mechanism.

Transposons infest the genomes of eukaryotes and have undoubtedly played an important, though still elusive, role in their evolution. Probably transposons participate in evolutionary changes of the fountain systems in several ways. They could transfer genes for fRNAs and their DNA targets into new positions. But in addition, the activity of transposons could lead to shortening or lengthening of telomere and centromere spacers of any chromosome arm. This aim may be achieved via deletion or insertion of the corresponding mobile elements within these spacers. Telomere and centromere spacers can hold away the genes of a chromosome arm at a proper distance from a dead zone of reinforcement shield of the nuclear envelope (for more details see below). Therefore, terminal spacers enable the corresponding genes to gain a direct contact with that portion of the inner nuclear lipid membrane which, in contrast to a reinforcement zone of an envelope, can be involved in ion channels formation. In addition, insertions and deletions of transposon copies could change distances between the adjacent ion batteries what may be important as a means of insulation and separation of neighboring ion fountains serving for different gene clusters. This permits to avoid undesirable interference of these batteries. It is known that some mobile elements have highly preferred target sites, tiny islands in the vast ocean of the target genome (Craigie, 1992). Probably, non-randomness of such hot spots hints at the possibility that transposon-dependent reorganizations of the fountain systems in evolution of different organisms could also proceed non-randomly.

Regulation of quantitative traits is very important in development and evolution. After being transferred into the appropriate part of a giraffe genome, transposons could intensify, for example, growth of the neck and front extremities, whereas its rear extremities do not receive such fountain-dependent enhancers. In this case, the growth of those limbs will be drastically modulated within the same organism, in spite of the fact that the front and rear extremities receive the same quantities of various hormones and other growth factors. The same is also true for different length of our fingers, etc. Using the fountain mechanism, transpositions and chromosomal recombinations, it is possible to create an immense variety (both in morphological structures and in functions) in the animal and plant kingdoms.

In the present context, transposons may play an important role in appearance of mosaicism. For example, mosaicism of maize kernels and fruit fly cells in case of position effects could have a general nature based on the same fountain mechanism. Depending on the local concentration and nature of ions provided by a local fountain system, gene expression produces variegated phenotypes and many mosaic variants observed in diverse species.

Nuclear fRNA-dependent fountain mechanism without nucleus

There are many data on the pivotal role of a nucleus in biorhythms. Many decades standing mystery of biorhythms was even deeper immersed into enigmatic clouds by the discovery that the genome of a cyanobacterium, blue-green alga, that photosynthesized but had no eukaryotic nucleus, did also have rhythms (see Kondo *et al.*, 1997). Rhythms in cyanobacteria are probably based on a fountain mechanism that uses the intra-cytoplasmic thylakoid (a system of photosynthesizing membranes). It has a form of closed sac with the nucleoid inside, a peculiar analog of the normal nuclear envelope of eukaryotes. Hence, the fountain mechanism could operate at the contacts of cyanobacterium nucleoid and thylakoid. Probably, some analog of this mechanism could be revealed in isolated chloroplasts and mitochondria.

3D organization of nucleus and ion fountains

The reason for certain 3D organization of chromosomes within a nucleus (Kurz *et al.*, 1996) may be explained, at least in part, through necessity of existence of the proper 3D organization of fountains within the nucleus in order to avoid their undesirable interference and to design, when necessary, their coordinate functioning to serve the joint big ion shower-bath for several gene clusters located at distinct chromosomes.

Chromosomes are normally attached to the matrix structures of the nucleus and they are non-randomly located in the nucleus, as it was firstly concluded by Rabl (for review see Prokofieva-Belgovskaya, 1986; Zbarsky, 1988; Hilliker and Apples, 1989; Zhimulev, 1992; Razin and Gromova, 1995; Zalensky *et al.*, 1995; Dernburg *et al.*, 1996; Hozak, 1996; Kurz *et al.* 1996; Romanini and Fraschini, 1996). The mitotic chromosomes are attached to the nuclear matrix via telomeres (DeLange, 1992). Some attachment sites of the chromosomes are strings of satellite DNA that were found to be complexed with RNA. These complexes are unusually resistant to nucleases (Markova *et al.*, 1994). It has been revealed that, at mitosis, the sequences of the attachment sites in Friend-S cells have been revealed both in telomeric and centromeric regions of the chromosomes, while at interphase, the additional anchoring sites are distributed into 9-13 well-defined clusters (Markova *et al.*, 1994). At least some of these clusters are potential candidates for fountain DNA (fDNA) that is localized in the regions of large ion-channel batteries of the inner nuclear membrane in the cells with high synthetic activity. These clusters have RNA-DNA complexes which are possibly responsible for initiation of ion channels.

It should be emphasized here that gene clusters, being activated with the help of the fountain mechanism, could then continue to transcribe already without direct contact with a periphery of the nucleus. Therefore, they already may not possess those RNA-DNA complexes which were necessary for them during launching

the passive ion channels. Nevertheless, these genes may continue to gain benefit from having the optimal ion atmosphere that is maintained owing to the fountain mechanism. According to this, only a small fraction of active cells may have the above-mentioned RNA-DNA nuclease-resistant hybrids at the nuclear periphery at any given moment, as was observed for erythroleukemia cells (Markova *et al.*, 1994).

As to chromatin compartments within which ionic fountains are at work, the corresponding caverns in the chromatin are the simplest variant. The walls of each cavern, like cupola of circus, are above the real fountain that is splashing on the circus ring.

There is sufficient evidence that RNA molecules are bound to the nuclear envelope (Feiferman and Pogo, 1975; Markova *et al.*, 1994). Some of these data concern those RNA molecules that represent the focal sites of transcription and RNA splicing near the nuclear periphery (Jackson *et al.*, 1993; Xing *et al.*, 1993). At least some of genes located at nuclear periphery could operate under the cupola of the «fountaining circus ring» in close vicinity to the nuclear envelope. The end of this performance coincides with the formation of condensed granules containing a portion of newly made transcripts.

These newly formed small nuclear ribonucleoprotein particles then rhythmically, in accordance probably with circumbiorhythmic rhythms (they have periodicity of approximately one hour), are exported to cytoplasm. This type of periodicity was shown both for RNP production and protein synthesis (Brodsky and Nechaeva, 1988). As was formulated above, the fountain mechanism cannot operate non-rhythmically, and this is a pivotal reason of periodicity in protein synthesis, as well as in all other circumbiorhythmic, circadian and many other endogenous oscillations known in chronobiology. A newly formed RNP particle then moves from its ionic fountain-dependent place of origin to the nuclear pores in the interphase nucleus to be rhythmically exported to ribosomes (for spatial distribution of such particles see Spector, 1990).

On chromomere gradient observed by Lima-de-faria

The higher the fRNA concentration, the higher the probability that all corresponding DNA targets would meet their fRNAs, and *vice versa*. The concentration of fRNAs, that are encoded mainly near the chromosome ends, is directly proportional to the distance between the end of an interphase chromosome and target chromomere. Hence, the longer this distance, the more fDNA targets should be on a given chromomere for its genes to get an adequate amount of RNA-dependent ion channels. Chromomeres, or bead-like compacted segments of chromatin in mitotic chromosomes, are known to have different sizes depending on whether they are near telomeres or centromere. Lima-de-Faria observed the so-called chromomere size gradient. The chromomeres are generally larger near the centromere and their sizes become progressively smaller towards the chromosome ends that is to telomeres. The slope of this gradient is a function of the chromosome arm length (Lima-de-Faria, 1954). The above mentioned fountain model can apparently explain the chromomere size gradient. It should be added to the above explanation that any segment of the chromosome arm that encodes the fRNAs independently of subtelomere, could introduce local distortion into the chromomere gradient. Some unexplained changes in such gradients were observed (Lima-de-Faria, 1975).

In related species, some specific genes reside in specific positions within individual chromosome arms in relation to the centromere and telomeres and to each other, in spite of variable lengths of the corresponding arms. This finding was called «Chromosome Field» (Lima-de-Faria, 1980). The proper explanation of the effect of non-random order of genes is the same as for chromomere gradient. This order is dictated by concentration gradients of fRNAs.

Terminal heterochromatin as a spacer that allows genes to avoid «dead zone», where chromosome end is anchored and where it is impossible to induce ion channels

It is now firmly established that chromosomes, including the interphase ones, are anchored via the centromere and two telomeres underneath the nuclear envelope, at the opposite poles of a nucleus. It is assumed here that terminal heterochromatin segments at both sides of the chromosomal arm are spacers necessary for normal work of the fountain mechanism. They allow genes (and accompanying fiDNA, that is a target for fRNAs) to avoid a «dead zone» in the nuclear envelope. This dead zone is a big sector of the envelope that is covered by reinforcement components of the nuclear envelope and skeleton. Owing to the local presence of these mechanical shields, the genes cannot contact directly with proteins of inner lipid membrane. Hence, it is impossible to induce ion channels in these areas. This is why the genes should be held away from these shields of reinforcement in order to permit origin of necessary ion channels. If this suggestion is true, one can predict that shortening of these terminal heterochromatic chromosomal spacers should lead to changes in the normal activity of «periterminal» genes. One can note that there are data fitting this prediction. For example, after shortening of such spacers in the yeast telomere, some genes change their activity what may be explained through the loss of possibility to reach the common inner membrane beyond the board of reinforcement shield to which the telomere is anchored. Similar results were obtained when telomeric satellite DNA was lost. Again, the corresponding gene which is too close to the nuclear reinforcement can not be assisted by ion channels. Hence, steric hindrance can be an important epigenetic factor.

Thus, it is claimed here that the genes located near the ends of chromosomal arms should have relatively long spacers to hold away themselves from the above mentioned dead zones in order to have ion showers.

The same may be true for age-dependent alterations of the activity of some genes if they are close to telomere. The telomere shortens due to end underreplication (for review see Wright and Shay, 1992; Kipling, 1995; Counter, 1996). As a result, the telomere of a senescent cell, as a terminal spacer, in accordance with the fountain theory proposed here, becomes too short to permit normal activity of those ion fountains which are at service of genes located near the chromosomal ends. The reinforcement shields of the envelope are now too close to some genes which therefore are devoid of necessary ion shower. As a result, they become heterochromatized and their transcription activity gradually ceases or decreases. Also known are some clinical syndromes connected with the shortage of satellite DNA and constitutive heterochromatin at the ends of chromosomes (Prokofieva-Belgovskaya, 1986; Podugolnikova *et al.*, 1994). Probably such clinical effects are stipulated by a mechanism similar to the just considered one.

Thompsonian grid and ionic regulation of quantitative traits in development

From the proposed point of view, it is possible to suggest a mechanism for Thompsonian observations of results of morphological transformation using «elastic grid» (Thompson and D'Arcy, 1961). Thompson found that it is possible to transmute contours of animals from relative species, belonging to the same order or class, by transforming coordinates of the corresponding drawings. The functioning of fountain mechanisms can provide for gradual and selective acceleration or retardation of local growth of the corresponding body regions and organs during development. Thompsonian transformations in the frames of deformed coordinate grid may really mirror the gradual local increase or decrease of growth rate in the course of ontogeny.

Conclusion

According to the proposed viewpoint, the «redundant» sizes of eukaryotic genomes are not a luxury, but a harsh necessity. Eukaryotes became eukaryotes by establishing a solute-containing lumen around the chromosomes. This perinuclear cistern is needed as a reservoir for maintaining concentration gradient of ions diffusing through passive ion channels to certain gene clusters. To achieve this aim, a direct contact with the cytoplasm is not admissible since its ionic content is situationally changed.

On the basis of above propositions, the fountain mechanism may be largely involved in differentiation and determination. It seems reasonable to envisage the formation of a specific pattern of ion channels around the corresponding structural genes in the course of cell differentiation. It is very likely that various repackaging of euchromatin and heterochromatin regions occur partly for the sake of fountain mechanism.

Invention of the nucleus and fountain mechanism permitted eukaryotes to obtain an easy way of modulating the expression of an endless list of quantitative traits. Among such traits are high milking capacity of cattle, rate of growth of girl or rose-bush, and difference in lengths of fingers of our hand. More generally, creation of the whole stunning variety of morphological and physiological traits in the plant and animal kingdoms is based chiefly on modulations of their quantitative traits rather than on little distinctions between their structural genes, though an evolutionary significance of novel and modified structural genes is self-evident.

Biological rhythms are a fundamental organizing feature of living organisms (Pittendrigh, 1981). The fountain mechanism may explain biorhythmicity of many cellular and organismic activities and, hence, this mechanism favors the adaptability of eukaryotes to the various environmental periodicities.

The list of processes, where the fountain mechanism may be involved, also includes some important epigenetic events. Probably, it participates in some cases of epigenetic inheritance, including X-chromosome inactivation, genomic imprinting, position effects such as mosaicism of transgenes (Dobie *et al.*, 1996), etc. However in many similar cases, the fountain mechanism is apparently assisted by other mechanisms, the consideration of which is beyond the scope of present consideration.

In addition, the fountain mechanism could be involved in regulation of such an important genetic phenomenon as dominance: an allele devoid of ion channels in its close surrounding has all

chances to be recessive. Moreover, synergistic functioning of ion batteries belonging to homologous chromosomes of a hybrid can be responsible for heterosis, characterized by significant quantitative differences between the offspring and parents. The variety of sets of ionic channels at different distances from each allele may lead to a complex picture of allele expression. It is important to stress that, depending on the nature of ion and chromatin, the fountain mechanism can enhance or decrease the activity of corresponding chromosomal regions.

As follows from the above considerations of the fountain mechanism involved in development, it may be indispensable also for many micro- and macroevolution processes, since it provides a relatively simple means for modulation of the functioning eukaryotic genes. Changes in many morphological and physiological traits are mainly based on quantitative rather than qualitative changes. It is often more important to decrease or enhance some syntheses and, on this basis, to gain larger or smaller organs, rather than to entrust the fate of species only to the mutation of structural genes, though the endless games with qualitative modifications of biopolymers are abundant in evolution.

The evolutionary history of eukaryotic genomes is punctuated by the proliferation of various repetitive elements. Significant portion of them may now be attributed to the mechanism that regulates quantitative traits in eukaryotes. It is assumed here that these changes could partially be explained by rearrangements of genetic elements of the fountain mechanism. If so, a bulk of former «junk» DNA may prove to be the fountain DNA, rather than junk.

The fountain mechanism requires a great amount of genetic material, including DNA (the so called frDNA) for coding fountain RNAs (fRNAs), as well as for coding targets (fiDNA) for these fRNAs. Moreover, a great bulk of DNA is spent to build spacers between distinct ion batteries for the purpose of separating incompatible fountains and structural genes. Additionally, a significant portion of DNA is needed for spacers that help to hold the corresponding chromomeres away from the ends of chromosomes where it is impossible to arrange the ionic fountains because of the local presence of reinforcement structures covering the inner nuclear membrane.

As our knowledge about the nucleus widens, more paradoxes related to the nucleus become evident (see for reviews Georgiev, 1969; Gall, 1981; Davidson, 1986; Goldberg and Allen, 1995; Dernburg *et al.*, 1996; Romanini and Fraschini, 1996). Among these paradoxes are: (1) well-known C-paradox of Cavalier-Smith, concerning the disproportionate amount of nuclear DNA content in comparison with the amount of DNA potentially able to code for proteins; (2) elusive role of the DNA sequences with different degrees of repetition; (3) compartmentalization in the nucleus and how it relates to transcription and processing, and (4) mysterious long-distance chromosome interactions, including participation of non-coding chromatin, that are revealed in gene position effects, such as stochastic transcriptional silencing of a gene positioned adjacent to heterochromatin, etc. It seems that some key aspects of these paradoxes could be interpreted now from the viewpoint of the proposed fountain model of modulation of gene expression in the nucleus, including both increased and decreased gene productivity, as well as short- and long-distance interactions of gene and their ion fountains. For example, the longer the spacers between the distinct fountains, the lesser the possibilities of their undesirable interference. Hence, a significant

fraction of genomic DNA, e.g. satellite DNA, should be spent for such «inter-fountain» spacers.

There is a wide inter- and intrataxonal variability of DNA content in plants and animals. But it does not correlate with their evolutionary progress or complexity of their organization. Nevertheless, correlates of repetitious DNA content in many species, including repetitious DNA abundant in eukaryotic genome, have been noted for a range of features, cellular to geographic (Price, 1976; Bharathan, 1996). Probably this is caused by selection for maximal fitness which, in turn, is based on the corresponding quantitative traits, while these traits are to a great extent under the control of corresponding sets of repetitious fountain DNA.

Many different biological phenomena dependent on nuclear activity could probably correlate with the fountain system activity. For instance, the reason of existence of supernumerary chromosomes, the so called B chromosomes, which presumably do play some still unknown role in adaptability, can be explained through their involvement in the fountain system. It seems plausible to explain the appearance of B chromosomes as an additional source of genes both for fRNAs and targets for these fRNAs. One could also assume that in the course of chromatin diminution in lower animals, great portions of fountain DNA might be eliminated. This fraction may be useful for intensive syntheses in germ cells of these organisms, whereas in their somatic cells, this DNA is unnecessary and even may be undesirable.

A seemingly great excess of nuclear DNA, without drawing on the above ideas, does really sound like an egoistic or junk DNA, whereas in reality it is a genuine «draught-horse» of the nucleus.

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