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## HYPOTHESIS

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# Why Do Primordial Germ Cells Migrate through an Embryo and What Does It Mean for Biological Evolution?

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**Abstract**—An explanation of the role of primordial germ cell (PGC) migration during embryogenesis is proposed. According to the hypothesis, various PGCs during their migrations through an early embryo are contacting with anlagen of organs and acquiring nonidentical organ specificities. An individual PGC gets such an organ specificity, which corresponds to specificity of the first anlage with which this PGC has the first contact. As a result, the cellular descendants of PGCs (oocytes or spermatocytes) will express nonidentical organ-specific receptors, hence becoming functionally heterogeneous. Therefore, each clone of germ cells becomes capable of recognizing specifically the molecular signals that correspond only to “its” organ of the body. Such signals are produced by the body’s organ when it functions in an extreme mode. Signals from the “exercising” organ of the body are delivered to the gonad only via the brain retransmitter, which is composed of neurons grouped as virtual organs of a homunculus. Homunculi are so-called somatotopic maps of the skeletomotor and other parts of the body represented in the brain. Signals, as complexes of regulatory RNAs and proteins, are transported from the “exercising” organ of the body to the corresponding virtual organ of the homunculus where they are processed and then forwarded to the gonad. The organ-specific signal will be selectively recognized by certain gametocytes according to their organ specificity, and then it will initiate the directed epimutation in the gametocyte genome. The nonrandomness of the gene order in chromosomes, that is the synteny and genetic map, is controlled by the so-called creatron that consolidates the soma and germline into a united system, providing the possibility of evolutionary responses of an organism to environmental influences.

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Earlier, a concept was proposed that organisms should have a creatronal system, or creatron [1], as a system responsible for modification of the germ cell genome during the creation of evolutionarily essential adaptations. This modification is realized under the influence of environmental factors that force the soma to “exercising”, or wider, to actions executed under conditions extreme for a given species, i.e. similar to Lamarckian “exercising”. For instance, such actions include a systematic execution, in a series of successive sexual generations, by a group of muscles, tendons, and bones of such a profile of physical efforts that is unusual for organisms of their ancestral form. Other examples are presented by the forced existence of a series of generations under conditions of unusual salinity, extreme temperatures, co-existence with a new symbiont or a parasite, etc. Under such situations, each individual is compelled to deviations in its homeostatic systems, and such an activity deviating from normal is defined here as “exercise”. The role of

exercising is central to Lamarckism; hence, it is necessary to explain the likeness and fundamental difference of the proposed creatron theory of evolution as compared to it. The likeness is determined by an important role of activities of organisms themselves during evolution. The difference is determined by the absence of inheritance of the acquired characters; this erroneous basis of neo-Lamarckism is incompatible with the central dogma of molecular biology, which excludes transmission of information from proteins to nucleic acids. The creatronal system creates directed modifications of the germ cells’ genome but not the characters as they are; helpful characters are produced in descendants based on directed changes in the genome received from the “exercising” ancestors. Thus, the creatron is a system for processing adaptively significant information and its transmission from the soma to the germline cells’ genome. An adaptive success of the creatron’s work is a creation of directed epimutations in germline cells.

To directionally modify the germ genome, i.e. create within it nonrandom epimutations (such as strictly localized DNA methylation and modification of chromatin proteins), molecular signals, first of all as regulatory RNAs, must be delivered from the exercising part of the soma into a strictly determined part of the germline cell genome. And in solving this problem an important role belongs to the gonad–organ interface. This interface is a retransmitter, which receives signals from the organs and upon processing retranslates them into the gonads, is presented by neuronal projections of the body parts into the brain. For humans, such projections correspond to Penfield's homunculus; thus, motor and sensor homunculi are known. Signals from an "exercising" organ of the body (or from a part of this organ) enter not simply the homunculus but its strictly determined virtual "organ" (or a part of this "organ"). Just the "organ" which is represented in the homunculus by the neuronal projection of the "exercising" part of the body. The molecular signal entering this group of neurons of the homunculus is processed and redirected to the final target – the gonad. The organ specificity of the signals is retained due to their separate processing (in different parts of the brain homunculus). Therefore, if each gametocyte could selectively recognize molecular signals only from a strictly determined "organ" of the homunculus, the problem of specific delivery of signals from the soma into the germline would be solved. More detail about this will be presented a little below.

Three-dimensional neuronal projections of the body's organs in the central nervous system correspond to a grotesque image of a little human and for humans are called a homunculus, but for animals a more general term is required that is free of associations with humans. The term "avatar" fits such a role for the complex of somatotopic projections in the brain.

Signals from a certain "organ" of the homunculus/avatar must be recognized only by a definite fraction of the gonad's germ cells. This creates a key prerequisite for establishing the communication of the soma organs with the germline cells. The communication is realized under the control of the postulated creatronal system, or the creatron. The creatron is a processing–transporting system responsible for communication of soma with germ genome. Responding to influences from the environment, the creatron provides the generation and transportation of signaling molecules from the "exercising" parts of the soma via the brain homunculus/avatar to the gonads for creating, if necessary, novel evolutionary adaptations. The creatron is, in essence, the machinery for evolution. To the proper functioning of this system, it is necessary, in particular, to ensure an unobstructed transportation of all necessary signals (regulatory RNAs and proteins) along the following route. At first, the signal goes from a certain muscle (a bone, a segment of the intestine, etc.), "exercising" under extreme conditions,

to a strictly determined group of neurons; in the neurons the signal undergoes processing; then the signal as a molecular product of this group of neurons (also in the form of strictly determined RNAs and proteins) is retransmitted into the gonad.

Animals possess all transporting vehicles required for this. It has been established that in metazoans the intercellular communications can realize a long-distance delivery within the organism of macromolecules and even of macromolecular ensembles. These functions can be contributed by various combinations of: (i) the transneuronal transport of macromolecules (used even by such large ensembles as some viruses), (ii) transitory tunneling nanotubes, (iii) gap junctions, (iv) exosomes, etc. [2-7].

For the initial version of the hypothesis [1] there was a difficult problem – a specific targeted delivery of regulatory RNAs from separate groups of neurons to nonrandom groups of germinal cells of the ovary or testis. Although gonads have an innervating system, it cannot ensure the physical communication between "organs", e.g. of the motor homunculus, and nonrandom cells of the gonad. How to solve this problem? The response is prompted by consideration of the migrational fate of so-called primordial germ cells (PGCs), i.e. the early precursors of future mature gametes. Instead of attempts to build a highway for the signal delivery from each cell of the brain to each germ cell of the gonad (that is constructively impossible), an alternative variant can be used. Its essence is as follows: different clones of germ cells must be beforehand, during their development, endowed with a capability of receiving signals addressed to them and only to them.

If any signal from the brain homunculus "organs" which enters the gonad has, similarly to a letter in an envelope, a strictly determined marker, then such a marker helps the germline cell to recognize a signal addressed just to it. And this cell can recognize this signal independently of its concrete informational contents, just as it occurs in the case of the letter finding its addressee. And let us admit here that just for this purpose Nature has created an unusual phenomenon – wandering of primordial germ cells through the early embryo.

The migration phenomenon of every PGC through the developing embryo before the PGC settling the gonads is long known, but the role of this enigmatic phenomenon is not explained comprehensively. I suppose that this process is used for supplementing the PGCs and their descendants with the different organ-specificities: each PGC gets only such an organ specificity that corresponds to the specificity of the first anlage with which this PGC has the first contact. To reach this purpose, on contact with the nascent organ's anlage, a PGC receives from this anlage, at the direct cell-to-cell contact, a specific inducing signal (similar to an inducer of differentiation). Once having received such an organ-specific signal, the individual PGC gains a specialization, i.e. becomes a dif-

ferentiated cell. This PGC remains a germ cell with all inherent abilities, but from the moment of the above-mentioned specialization this PGC becomes unresponsive to new inducers of the embryo's other organs. The differentiation of this PGC is manifested by expression by this cell (and/or by its nearest cellular descendants) of a receptor, which allows the cell to recognize a strictly determined organ-specific signal, which can be delivered from neurons of the corresponding "organ" of the homunculus/avatar.

Thus acquired organ-specific specialization allows oocytes and spermatocytes to selectively receive signaling RNAs and proteins delivered from soma via the creatronal system during the whole reproductive life of the organism. This is possible if any somatic signal (as regulatory RNAs capable of initiating epimutations in the genome of germline cells) entering the gonad from the homunculus/avatar brings the regulatory RNA as a component of an obligatory complex with an organ-specific protein ligand. Due to the ligand, this complex is recognized by nonrandom organ-specific gametes, and the regulatory RNA entered the gametocyte together with the ligand can further participate in the epimutagenesis.

#### CREATRON AND PRIMORDIAL GERM CELL MIGRATION

The organism of every multicellular animal during its development is subdivided into two parts – somatic and generative ones. The generative part isolates itself by emergence of primordial germ cells (PGCs). Those early embryonal cells, which have succeeded in receiving, as a farewell wish from the oocyte, RNA molecules belonging to the so-called germ plasm, are converted into PGCs. As a result, a potentially immortal germline is maintained in every generation of multicellular animals. Other early embryonal cells participate in formation of the soma – the mortal part of the organism. This communication presents a fundamentally new explanation of the PGC behavior during the embryogenesis, which creates a prerequisite for the addressed delivery of molecular signals from the "exercising" soma to the germ cells; these signals can directionally modify the germ cell genome promoting an appearance in it of epimutations.

The emergence of germinal cells occurs differently in different groups of organisms [8, 9]. There is a general trend – due to an enigmatic reason, the more evolutionarily advanced organisms create their PGCs during the embryogenesis later than the less advanced ones. Nearly all plants and many multicellular lower animals (e.g. sponges) can produce germ cell during their whole life at the cost of somatic cells, i.e. in them the germ and somatic cell lines are not separated. However, the majority of evolutionarily advanced animals act otherwise. The line of germinal cells is separated especially early during the

ontogenesis in arthropods, nematodes, and some other invertebrates. *Drosophila* creates the germ cell line at the 13th division, cyclops – at the fifth division, and the nematode *C. elegans* – after the fourth division. In the tailless amphibian, PGCs isolate themselves later, at the blastula stage. In reptilians and birds, PGCs appear still later – at the gastrula stage, and in mammals the PGC appearance is the latest, during the neurulation and early organogenesis. PGCs emerged long before the formation of gonads populate them, only after complicated migrations through structures of the developing embryo. It should be emphasized that the later PGCs appear, the higher are the number and diversity of somatic embryonal cells, which form the background of their further migration. By the way, data of the birds' embryology show that all PGCs, being created outside the embryo itself, fail to penetrate into it for a relatively long time (as if waiting for complication of the embryo's architectonics) and only afterwards are transported with the blood flow throughout the embryo's body and migrate through it towards the gonad. And this must have a deep biological sense. But what? Sometimes the late self-isolation of the germ cell line is considered to be a way for accumulation by somatic cells of random mutations that later become a property of the gametes; this would be able to increase the genomic polymorphism but simultaneously to increase the load of unfavorable mutations. As a whole, the problem remains unsolved [8, 9]. In the present work a novel view on this problem is proposed, as well as on some associated problems of biology.

The migration of PGCs was first described by Nussbaum in the XIXth century [10], and his data were partially used by Weismann for his theories. In mammals, PGCs emerge from early embryonal cells outside the embryo; then PGCs migrate by different ways through the embryo during its early organogenesis. The migrations of PGCs result in colonization of the embryonal gonads. The diploid PGCs give rise to diploid oogonia in females and spermatogonia in males. Upon mitotic propagations, these cells create, respectively, oocytes and spermatocytes. The long chain of events, including two meiotic divisions, gametogenesis, finally results in formation of haploid gametes, oocytes or spermatozoa [9]. The migration of PGCs proceeds through the extracellular matrix, and the translocations are partially controlled by filaments, cell adhesion molecules, and individual glycoproteins of the extracellular matrix [11, 12]. The number and mobility of the PGCs are controlled, in particular, by the BMP family proteins [13]. Those PGCs which "have lost their way" and failed to enter the gonad in due time are normally subjected to apoptosis. If it does not occur during the stages of development, the ectopic PGCs undelivered to the gonad survive and can even cause a tumor: just this seems to be associated with emergence outside the gonads of about half of human pediatric tumors with germinal nature [14].

### THE MAJORITY OF OOCYTES IS LOST BEFORE SEXUAL MATURATION

Unlike male gametogenesis accompanied by divisions during the whole life, events of oogenesis, terminating the cell divisions even in embryo, are veiled with secrecy. By the birth or soon thereafter, mammalian ovaries are filled with so-called primordial follicles, each composed by an oocyte blocked at the end of prophase I and surrounded by a layer of specialized somatic cells. The reason for the still unexplained loss of cells during oogenesis is considered in many works [15, 16]. The majority of mammalian oocytes die still during the fetal period or shortly after birth [16]. Quantitatively, the oocyte population reaches, for example, its maximum of about seven million in a five-month-old human embryo. And then, surprisingly, the oocyte number begins to fall, and by the birth about one million oocytes remains. Moreover, the loss of oocytes, enigmatic in its functional significance, continues further, and to the sexual maturity a woman comes only with about 400 thousand oocytes, and their number continues to gradually decrease later (by the end of reproductive activity there are, on average, 1000 cells).

But why does the main reserve of female germ cells intended for sexual propagation disappear long before the propagation starts? There are two main hypotheses explaining the early death of oocytes: (i) a suggested local insufficiency for survival of growth factors and (ii) errors during the chromosome crossover. It is also supposed that only oocytes with a rather high density of growth factor receptors can escape apoptosis [17]. Corresponding facts have really been found, however they possibly can be only side processes, whereas the main process is an elimination of those gametocytes that could not execute orders of the creatronal system determining their organ-specific specialization and hence were forced to commit programmed cell suicide. It can be supposed that this is required as a part of creation in the female organism of a full-value creatronal system generating signals, which must selectively find their target – organ-specific clones of oocytes. Spermatogenesis, which continues during the whole life, also needs organ-specificity of clones. The absence in spermatogenesis of mass cell death similar to that observed during oogenesis seems to be associated with differences in the spatiotemporal organization of the female and male gametogenesis.

The adoption by different PGCs of some or other organ-specificity on their migrations during early organogenesis is necessary but insufficient for terminating their complete organ-specific specialization. The specialization of PGCs continues even after they have adopted organ specificities and reached the gonad where they have lodged having generated a great population of their cellular descendants.

The development of living beings is often accompanied by cell death – from the tadpole's tail to the human

brain formation. Thus, during embryogenesis of higher animals only those neurons usually survive which have received a trophic factor from the innervated target. About 70% of the human brain neurons are thought to die even before the birth. Studies on a center of the visual system of the chicken brain revealed that the number of neurons decreased by 27% before the hatching and by 7.8% after it [18]. Similarly, a great number of sensory neurons disappear in peripheral ganglia of mammals on the background of neuron competition for a limited resource of neurotrophic factors [19, 20]. It is known that oocytes also die on shortage of growth factors. Oocytes seem to die on insufficiency of two types of factors: (i) on insufficiency of growth factors common for various cells and (ii) in the absence or shortage of special organ-specific factors delivered from the central nervous system, or more exactly, from neurons of the homunculus/avatar. Here we suppose that these organ-specific signals are required by the oocytes to terminate the creation of the necessary organ-specificity. Those signals from the organ anlagen that the PGCs have received earlier are necessary but insufficient: additional organ-specific signals for completing the organ-specific maturation of the oocytes must be delivered into the ovary from the corresponding virtual "organs" of the homunculus/avatar. To catch all necessary signals is not a simple task. All those oocytes that have failed in this task and have not acquired an organ specialization are subjected to apoptosis. This explains the mass death of oocytes before the beginning of sexual maturation of the organism. By the way, spermatocytes also must acquire an organ-specificity and are also under the control of signals from the homunculus/avatar.

### DEEPENING OF ORGAN-SPECIFIC SPECIALIZATION OF GAMETOCYTES CONTINUES WITH THE ORGANISM'S MATURATION

Thus, it is postulated here that the organ-specific specialization of PGCs and their cellular descendants is a key event in establishment of the system of information transmission from the "exercising" soma (e.g. from the Lamarckian giraffe's neck pulling up) to the germline cells. However, the PGC migration lays only a foundation of the organ specificity of the germline cells, and this is only a start for their specialization.

The meeting of a "naive" PGC with a target, e.g. with a wing bud, will result in activation in the genome of this PGC of genes that will give the "wing" specificity just to this PGC and all its descendants including the oocyte clone produced by it later in the gonad. The mentioned organ specificity means the ability of a clone of individual PGC descendants to specifically apprehend in the future only a quite definite signal. Namely, just the signal which corresponds to the "self" organ – to the wing in our

example. The supposed organ-specificity of the germinal cells requires gametocytes to have some molecular differences. There are only few data on differences between the germinal cells; nevertheless, they are found, although without any relation with the concept proposed in this paper. Thus, in spermatogonia populations, a populational heterogeneity is revealed. These “undifferentiated” cells are shown to express different sets of proteins [21, 22].

It should be emphasized that if there were no need in formation of the organ-specificity of gametes, all PGCs could be created without the migration, immediately within the gonad as it is.

But if after all embryonal endeavors oocytes are created at last, what is the reason for their elimination afterwards, long before they could have a chance to realize their only destination – to participate in reproduction? The oocytes continue to die also after embryogenesis, and even those oocytes die that would seem to have got all substances required for their maturation: both nonspecific growth factors and organ-specific factors from organs of the homunculi/avatars. The answer to this question is determined by the features of ontogeny. Anlage, e.g. the wing bud in an eagle embryo, is little like the powerful wing of a bird soaring in the sky not only in the shape but also in the set of expressed proteins. With every step of the embryonal and then of the post-embryonal development, organs are complicating and growing, new syntheses are activated in them that were absent in that wing bud that once was met by a “naive” PGC during its wanderings. The development of the growing wing structures is accompanied by complication of the wing projection structure into the brain. There is a representation of wing, as well as of other organs, in the avatar of the bird’s central nervous system. Virtual organs of the maturing avatar represented in the nervous system as strictly defined groups of neurons are to retain the ability for communications with the real body organs during the organism’s maturation. That is, the physiological and anatomical maturation of the body must go in parallel with the congruent maturation of the avatar organs in the nervous system. Moreover, every organ of the avatar has to retain (during both the germinal cell maturation period and the whole period of subsequent reproductive activity of the organism) the ability to send specific molecular signals addressed to strictly determined organ-specific clones of germinal cells. Hence, these target clones (i.e. organ-specific clones of oogonia and oocytes in females and, respectively, clones of spermatogonia and spermatocytes in males) are also obliged to synchronously complicate their own organ-specificity during the development and maturation of the whole organism. On executing this task, every clone of germinal cells in the gonad is under the control of regulatory signals from the “self” organ of the avatar/homunculus, which are delivered into the gonad via axons and other systems of the

cell-to-cell communication. These signals are selectively recognized only by strictly determined groups of germ cells. Such an organization of the communication does not need every germline clone to be innervated. Such communication is a pathway, which during a long time was a sought for signal from the soma to the germline cells.

The complication of structures of a real organ, e.g. of the wing (with its details, from the blood supply and bone and muscle structure to feathering, undergoing the formation but which were absent during the early embryogenesis) must be accompanied by a coordinated complication of the avatar structures and clones of gametocytes that are addressees of the avatar signals. The necessary communication in the system “body–avatar–gametocytes” can function adequately during the whole reproductive life only if the gametocyte clones continue to specialize subdividing into subclones with the more detailed specialization with respect to the body structures along with the organism’s maturation. All germline cells, which fail to synchronously differentiate according to the complication and alteration of the physical and avatar organs, are forced to undergo apoptosis.

What occurs in the creatronal system if any physical organ of the body (e.g. the wing of a kiwi bird or an eye of a mole) loses its former activity because of specific features of the chosen ecological niche? If an organ of the body is used no more, the development of neurons of the appropriate avataric “organ” is reduced and even partially degenerates (as if denervated). Thus, the reduced avataric organ (in our example it corresponds to the wing or eye) ceases to maintain the former complicated specialization of subclones of the organ-specific gametocytes that means a functional simplification of the “wing” gametocytes in kiwi and of the “eye” gametocytes in mole. The participation of such gametocytes in the sex process will inevitably result in appearance of defective structures, in our case of the wing and eye deprived of their former functionality. This is the moving force of biological regress, which often accompanies biological progress during evolution.

Thus, scrutinizing some aspects of gametocyte functioning, especially of oocytes, results in some conclusions that present a new interpretation of reasons for PGC migration and for the mass loss of oocytes. However, these explanations concerning oogenesis can be expanded. For a long time, the following important question remains without answer: what is the reason for taking a decision what oocyte is permitted to mature for participating in fertilization and what oocytes, notwithstanding their viability, must remain at rest? According to histological estimations, only about 10% of all oocytes in a girl’s ovaries reach maturity during the reproductive period of her life [23]. If oocytes were chosen purely stochastically for maturation and participation in potential fertilization, the overwhelming majority of the epigenetic marks

caused by “exercises” would perish in vain. Therefore, it is reasonable to suppose that, on reception of any epimutation under the creatron’s influence, oocytes become more resistant to atresia also under the creatron’s control. These oocytes gain an advantage compared to other oocytes in the ability to participate in ovulation when a mature oocyte leaves its follicle being ready for a possible fertilization. It is reasonable to suppose that oocytes are permitted to participate in fertilization under the control of neurons and not stochastically. From the standpoint of evolution, it is advantageous to send for meeting with a spermatozoon that oocyte the ancestral cells of which were maximally receptive to all creatron signals delivered into them from the brain.

#### WHAT IS THE SENSE OF AN EXTREMELY PROLONGED ARREST OF THE FIRST MEIOTIC DIVISION?

The prolonged arrest of the oocyte cycle in prophase of meiosis I (at the diplotene and diakinesis stages) is a universally conserved feature of animal oogenesis [24, 25]. The meiotic arrest starts on the diplotene stage and smoothly transits to the diakinesis stage. The oocyte leaving diakinesis is induced by hormones and timed to sexual maturation. And what is the sense of the prolonged arrest of the first meiotic division (for years in women)? The cause of the meiotic arrest in diakinesis is traditionally explained by its predestination for the growth and store accumulation by the oocyte (vitellogenesis). These processes really occur and are very important, but for the cell growth, even for such a unique one as an oocyte, there is no need in years as they are for making stores.

The alternative explanation proposed here of the prolonged diakinesis is as follows. On the diakinesis stage, the oocyte is *on duty* expecting possible evolutionarily significant signals generated by the creatron only in the case of unusual (for the given biological species) “exercises” of any organ or system of the body; and these “exercises” are executed systematically and on a regular basis. The regular basis increases the probability of delivery into the oocyte of regulatory factors, and the higher is their concentration, the higher is the probability of emergence of an RNA-dependent epimutation. Various regulatory RNAs entering an organ-specific oocyte, e.g. from the avatic wing of bird’s brain, can be different not only in the concentration but also in the sequences, if the body organ undergoes different (mechanical, thermal, chemical, etc.) influences. The descendants will get modified sites of the genome expressed just in the wing, but the genes chosen for epimutagenesis will be different.

The prophase of the first meiotic division is cytogenetically subdivided into successive stages: leptotene, zygotene, pachytene (crossover is realized during these stages), then follow diplotene and diakinesis (with their

meiotic arrest), metaphase I, anaphase I, and, finally, telophase I. Every normal diploid cell, including gonias (oogonia and spermatogonia), has two homologous chromatids: the maternal and paternal chromatids together form one chromosome. However, during the whole prophase of meiosis I, including the diakinesis stage, an oocyte contains four homologous chromatids (the DNA content is 4C), because, previously to the start of meiosis, DNA replication occurs in the oocyte without chromosome segregation. However, in the meiotic I prophase all chromatids on the diakinesis stage (the end of prophase) still remain in their common house – in the I order oocyte [26, 27]. The presence in this oocyte of the doubled dose of the genetic material and the existence of chromosomal sets (inherited from both parents and still undelivered into different cells) create an especially advantageous situation for the reception by the oocyte of any new creatronic signals.

As already mentioned, creatronic regulatory RNAs from every “organ” of the avatar/homunculus enter only the “self” organ-specific gametocyte in a complex with the also organ-specific (i.e. targeted) protein marker. Due to the marker, the whole complex is recognized by the gametocyte specialized for a strictly determined organ or for its part. Each molecule of a creatronic RNA is aimed only at one sequence from the group of satellite regulatory sequences flanking the definite gene of the gametocyte. Hence, an individual epimutation will not affect functioning of the same structural gene when it is expressed in other organs under the control of another, unmodified, regulatory sequences. Every specific creatronic RNA chooses in the gametocyte, as its target, just that gene which functions under an extreme situation in the soma, in the “exercising” organ of the body. Various creatronic RNAs can work within the gametocyte genome using different mechanisms. But the most frequently used mechanism seems to be presented by initiation of RNA-directed DNA methylation when molecules of the creatronic RNA act as a guiding RNA.

The RNA-directed DNA methylation is realized with involvement of relatively small RNA molecules. And the insignificant length allows the same RNA molecule to find a complementary sequence within an inappropriate, occasional, region of the genome. This lowers the accuracy of the RNA targeting and would be able to unfavorably disperse epimutations throughout the genome instead of their necessary strict directing. The most effective way to improve accuracy of RNA-dependent epimutagenesis consists in selective transcription and exposure of only those organ-specific gametocytic sequences that are expressed in the congruent organ of a body (or in part thereof). Transcription of the gametocyte genome provides conditions for exposition of DNA segments promising for interactions with the creatronic RNA as a guiding one on the DNA methylation of the site, which after that becomes an epimutation. The genome transcription in

human germline cells is widely presented embracing, in particular, about 70-90% of all human genes [28].

The above-mentioned doubled content of DNA in the meiocyte, which is *on duty* during the diakinesis stage, only increases the creation efficiency of epimutations. In this case, the number of targets for the creatronic RNA is doubled, with alleles of all chromatids of both parents presented as potential targets. By the way, the earlier delivery of creatronic RNAs into a meiocyte, at the very beginning of crossover, seemed to be able to influence even the choice of those sites, which are drawn in recombinations, and such an intervention in crossover could be an essential factor in accelerating the generation of evolutionary novelties.

The gene-addressed creatronic RNA is often aimed at the gene regulatory sequences and not at its encoding part because it provides more chances for modifications. It is known that in different organs the same structural genes are often expressed but in different combinations, and the expression level of the same gene in different organs can differ according to needs of various differentiations of the cells. From the evolutionary standpoint, it is fundamentally important that “exercises”, for instance, of an eagle’s claw would be able to selectively modify in both oocyte and spermatocyte only that regulatory sequence of a definite gene (the same gene for the claw and wing) that would correspond to just the claw and not the wing.

Oocytes entering meiosis are in clusters or so-called “nests” and are believed to arise as clones, while each clone is thought to originate from the same dividing oogonium. The oocytes within the nest have intercellular bridges and are synchronous in their activities [15, 29, 30]. Such a structure of the nests is favorable for controlling the oocyte activities by the creatronal system: on reaching the nest, its signals use the bridges for penetration to all members of the clone. It is important for maintaining the organ-specificity of the clone cells by signals from the avatar/homunculus and also for delivery of specific creatronic RNAs initiating epimutations.

#### THE CREATRONAL SYSTEM SEEMS TO BE ABLE TO INFLUENCE THE SUCCESSION OF GAMETE MATURATION

Using the brain avatars/homunculi, the creatronal system, on the background of the organism’s extreme “exercises”, would be able to selectively delegate “to fight”, i.e. to accelerate the maturation and admit for fertilization the strictly determined groups of gametes. Enrichment of the population of spermatozoa just with the cells carrying epimutations would increase the probability of fertilization of an oocyte not by an accidental but just by a modified spermatozoon. The same is also true for oocytes. If the fertilization is systematically realized not by accidental gametes but by those modified isotopically (i.e.

in the homologous sequence), this will increase the tempo of changes in the appropriate character in the descendants.

It is quite probable that the high tempo of changes in the population under conditions of artificial selection is really associated just with the mechanism as follows: isotopically modified gametes are drawn in the reproduction more frequently.

It should be emphasized that the organ-specificity of germinal cells manifested itself in the ability to perceive organ-specific signals of the creatronal system does not prevent the creation of a full-value zygote by the male and female gametes, even if they originated from gametocytes with different organ-specificities. The cause of this important situation seems to be as follows. Although the organ-specificity of a gametocyte (as a specificity of chromatin configuration and its transcription) is maintained by organ-specific signals entering from the avatar/homunculus structures, these signals are inefficient for gametes already starting the fertilization: the organ-specificity is now abolished together with the alteration of former configuration of chromatin. Under ecologically placid conditions and on normal and not extreme functioning of biological species, an unobstructive formation of unions by both sex gametes is advantageous. In this case, the selection of gametes for fertilization based on their organ-specificity would only limit the prospects of the species. Therefore, the organ-specificity of mature gametes is not needed, and it disappears.

#### EPIMUTATIONS MUST NOT BE RETAINED FOR AN UNDULY LONG TIME

Epimutations must not be erased during the organism’s early development on the background of DNA methylation and demethylation. The first wave of the genome demethylation occurs during the PGC migration, and before the fertilization itself the genome remethylation is triggered. The early embryogenesis is characterized by the second wave of demethylation. Methylation increases again after the blastocyst implantation. This process is selective: many single-copy sequences in early mammalian embryos become demethylated due to both active and passive demethylation, whereas the imprinted gene methylation remains unaffected [31]. Existence of various 5-methylcytosine derivatives, including 5-hydroxymethylcytosine and other compounds, provides many possibilities for formation of epigenetic marks [32-37]. The transgenerational heredity without DNA methylation (as it is exemplified by *Drosophila*) can be realized [38], but its possibilities seem to be more limited.

How do germ cells choose what epigenetic marks are to be left and what eliminated? This question is still not clear even in the case of the genome imprinting. But epimutations created under the influence of creatronic

RNAs must be obligatorily protected against erasing. In the opposite case, transgenerational inheritance is impossible, except in relatively rare cases of the maternal epigenetic inheritance based on a direct delivery of signaling RNA into the oocyte. But such variant cannot exist for the epigenetic paternal inheritance. The protection of epigenetic marks against erasing seems to be provided by a selective binding of epimutations by protective proteins. In particular, these proteins could bind to a “nascent” epimutation during the RNA-directed DNA methylation with involvement of the creatronic RNA. The signaling domain of the creatronic RNA molecule could act as an attractor for binding the protective protein with an epimutation created.

Later, the creatronic guiding RNA molecule bound in an epimutational complex can be destroyed (“The Moor has done his work, the Moor can go”), whereas the emerged epimutation marked with a protective complex becomes selectively defended against demethylation. To maintain the epimutation defense in a series of subsequent cell doublings, the protective protein complex must be assembled anew on the replicated site but already without the guiding RNA. This can occur, if during the subsequent DNA replication, the protein component of the primary protective complex acts as a primer for the re-assembly of a new protective complex. Thus, the further maintaining of the epimutation defense against demethylation is reminiscent of the maintaining of the chromatin pattern during routine cell doublings. However, this routine process is not studied in detail, but a pathway is already established that allows the protein mark to retain its nonrandom position on DNA. It is shown, for example, that two histone-modifying proteins, namely H3-methyltransferases Trx and E(Z), on binding with certain DNA sites, can withstand (unlike the methylated histones themselves) the passage of the DNA replication machinery. This helps to maintain chromosomal epigenetic marks at proper DNA sites during every round of DNA copying [39].

During multiple re-assemblages of the protective protein complex sheltering the creatron-produced epimutation against demethylases, errors can gradually accumulate in the germline cells that lead to uncovering the DNA methylated sequence. The naked epimutation becomes available for erasing. In general, such a postponed destruction of the epimutation is favorable because it promotes the elimination with time (e.g. during several sex generations) those epimutations that have lost their urgency due to new environmental change. However, a too rapid abolishment of epimutations is unfavorable. The survival time of epimutations must be sufficient to allow a favorable epimutation to be fixed as a mutation. This could be promoted by alignment of epimutation-carrying sequences during crossover. The meeting of two homologous epimutations could provoke an emergence within the appropriate gene segment of the topographi-

cally nonrandom errors, i.e. mutations. Finally, this will result in a series of the gene variants, the most adequate of which to the environment will survive under purifying selection (without help of positive selection).

#### GENETIC MAP OF SPECIES AND ORGAN-SPECIFICITY OF GERM CELLS

The stability of gene positions relatively to one another in the chromosome is an important feature of genomes [40]. The synteny, or the same order of mutual positions of homologous genes in the chromosome, is specific for eukaryotes; however, this phenomenon is still not clear in detail. Due to an unknown reason the synteny is retained in chromosomes of different species as clusters of several genes or even of dozens of genes, and the order of gene succession remains unchanged during thousands of years, notwithstanding multiple chromosomal rearrangements. Closely relative species have a small number of huge syntenic blocks, whereas distant species have many shorter segments containing the same genes in the same order. Thus, 40-50% of genes that are neighbors in the fugu genome have the same order as in the human genome [41]. A pattern of conserved syntenic associations is revealed that extends back to ~360 million years ago [42]. Comparison of genomic sequences in drosophila has shown that the *Drosophila sechellia* genome contains about a hundred huge syntenic blocks of *D. melanogaster*, each block of about 120 genes. The genome of *D. grimshawi* consists of about 1400 small blocks of the *D. melanogaster* genome, each block including, on average, eight genes [43]. By hybridization of DNA from one species on chromosomes of another species (comparative painting), four human chromosomes – 14, 20, 21, and X – are shown to nearly completely coincide with the dog chromosomes – 8, 24, 27, and X, respectively. The chromosomes 8, 11, 12, 15, 16, 17, 18, and X of the swine *Sus scrofa* correspond, respectively, to human chromosomes 4, 13, 17, 2, 5, 20, 7, and X [43, 44].

The synteny phenomenon is usually explained as follows. The gene order emerged in ancient organisms is maintained because it is responsible for priorities of the gene expression during the early embryonal development of multicellular organisms and therefore is conserved [45]. But whether the stability of gene order can be brought only to this? No doubt, use by some genes of common regulatory elements is favorable for this phenomenon.

The proposed explanation is as follows. Structural genes surrounded by various regulatory sequences are used, in particular, on creation of the postulated here organ-specificity of the germline cells. The organ-specificity, upon its establishment in individual PGCs, is maintained further and even complicated during gametogenesis. Each organ of the body needs activity of definite combinations of structural genes, the regulatory



sequences of which require a coordinated expression (usually due to compactization and decompactization within chromatin). The required coordination can be realized more easily and economically due to modification of chromatin proteins, on the condition that the genetic sequences under control are located contiguously and thus can be subjected jointly to euchromatinization or heterochromatinization. Moreover, the contiguous regulatory sequences can be transcribed in coordination (e.g. from the common start of the transcription), and the transcripts, in their turn, can be used to coordinate the expression of syntenic genes (e.g. with involvement of RNA interference, etc.). The synteny manifests itself by existence of the so-called genetic maps of species.

The genetic map of chromosomes is a scheme of gene order and relative distances between the genes in a chromosome as a linkage group and also a scheme of gene distribution among individual chromosomes in a karyotype. The genetic map of any eucaryotic species is steadily maintained, certainly, even because the normal crossover could not occur in the opposite case. But the genetic map of every species is formed on the basis of heredity from the ancestors and its emergence is obviously nonrandom. As supposed here, the genetic map is organized based on specific features of the triumvirate consisting of three main components: (i) the body's organs, (ii) the avatar/homunculus construction corresponding to the organs, and (iii) the organ-specificity of the germ cells. The evolution of organisms occurs with involvement of the creatronal system uniting this triumvirate and providing the variability of the descendants according to requirements of the environment that existed during functioning of the ancestors. To efficaciously accelerate evolution by the creatron, it is necessary that signals successively processed and transported along the above-described triumvirate chain would be adequately recognized and taken for execution at every step of the process. This is impossible without coordination of organ-specificity of the triumvirate participants, although they are very different both outside and endogenously. The necessary coordination can be achieved only on using a strictly definite neighborhood of the corresponding genes permitting the propagation of the coordinating signals along strictly definite segments within the chromosome. Just this purpose can be achieved due to the unique structure of the genetic map of every species.

In addition to the ability of creating topographically directed epimutations, or modifications targeted for a certain genetic sequence, the creatronal system would be able, in principle, to perform also more radical actions. Their essence is as follows. Normally, signals of every "organ" of the avatar, i.e. organ-specific proteins and regulatory RNAs intended for "self" gametocytes, never enter other neuronal compartments, i.e. "non-self" organs of the avatar. However, in response to an abnormally high activity of the "exercising" organ of the body,

the corresponding "organ" of the avatar also triggers a high activity and accumulates an abnormally high concentration of the organ-specific protein and regulatory RNAs. The resulting stress situation can affect the usual isolation of neuronal compartments of the avatar. As a result, the regulatory RNAs and proteins from one organ of the avatar get a chance for expansion along transneuronal contours into other organs of the avatar. The regulatory RNAs of the different organs of the avatar form in the neurons of the off-schedule complexes with "non-self" organ-specific proteins. Therefore, these regulatory RNAs get a previously forbidden chance to penetrate from the brain into "non-self" gametocytes of the gonad. In these "non-self" cells the regulatory RNA can meet a complementary sequence that is not available for it normally (because of difference between the chromatin configuration and the transcription pattern in gametocytes with different organ-specificities) and initiate there epimutations. Some of these topologically off-schedule epimutations can be dangerous because they can bring in unwanted changes into occasional genes. Some other epimutations can induce during the crossover unusual rearrangements of chromosomes resulting in new variants of gene linkage. Finally, some descendant organisms can get a chance for survival but due to morphological, metabolic, or other changes. And then they form a new evolutionary branch that will be able to survive in an environment unacceptable for their recent ancestors, whereas their creatronal system returns to the placid regimen of functioning.

## CONCLUSION

Some biological phenomena, which are long known, can be explained in a new way from the standpoint of the proposed hypothesis. These phenomena include the migration of primary germ cells, the mass loss of oocytes before the start of reproductive activity, the synteny (the gene order in chromosomes), and biological regress. Sexual reproduction with widespread subdivision of animal organisms into males and females is another, and rather a curious biological phenomenon, which now could be interpreted otherwise. Why two sexes? – This question has been put by evolutionists for decades. Usually an obvious advantage of the population polymorphism maintained by sexual reproduction is mentioned. Moreover, the meeting of two epimutant alleles could promote an appearance of mutation during the crossover, i.e. a trait fixation. These are obvious advantages of sexual reproduction comparative to the asexual one. However, it seems to be still another cause for the wide spreading of two sexes, possibly, the most important one, but which has up to now escaped attention. It is as follows: the organ-specificity of germ cells creates a field for the directed modification and combination of any characters, morphological or biochemical. Just this creates a prereq-

uisite for the controlled, nonrandom, creation of various adaptations that often lead to speciation. Life can survive only by nonrandom changes.

The creatronal system as it is fails to act as an endogenous guide of phylogenesis. This is both fundamentally impossible and unreasonable. Instead of this, the creatron, partly like tuning fork or mirror, responds in its own way to external influences and, moreover, actively attempts to adapt next generations to them.

Being responsible for the directional variability of organisms, the creatron makes unnecessary the accumulation of random mutations due to positive selection that is the essence of neo-Darwinism. Having at their disposal the active mechanism for creating in the genome topographically nonrandom epimutations, the organisms do not need for their evolution rather an unpromising expectation for appearance of favorable changes. The creatronic directed variability can be incomparably more efficient than directional natural selection. The latter is the rarest exception in Nature, rather than a rule. In Nature purifying selection is acting that is capable of eliminating a possible spoilage of the creatron functioning. On such a background, the positive (Darwinian) selection is not only unlikely but quite needless. Thus, proposed ideas of creatron-mediated biological evolution are able to replace the explanation of evolution, which was earlier offered by Charles Darwin.

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