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Noncoding DNAs and Origin of Sex

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ABSTRACT The data that probably sex and sexual reproduction of eukaryotic organisms are the result of the long evolution of noncoding DNAs (ncDNAs), which led to the origin of mitotic chromosome, mitosis, meiosis, sex determination and differentiation mechanisms, is presented. It is supposed that sex and sexual reproduction were a direct consequence of the origin of unicellular eukaryote, but not a result of emergence of some specific structural genes determining the sex development. Genes pertaining to sex appeared later, but again not for determination and differentiation of sex, but for development of the secondary sexual characters with the help of hormones. The possibilities of experimental check of certain stages of the sex origin are discussed.

INTRODUCTION

The overwhelming majority of modern biologists associates development and life evolution with structural, functional and frequency changes of genes. If the role of non-informative, i.e. noncoding, DNAs (ncDNAs) in these processes is admitted, then only as a secondary one. The possible role of ncDNAs in the development and the evolution of eukaryotes, except for maybe satellite DNAs (satDNAs) and chromosomal heterochromatic regions (HRs), is not considered seriously. Mostly, they are considered as "surplus" DNAs.

Earlier, we presented data on the possible role of ncDNAs in the generation of eukaryotic cell, multicellularity, thermoregulation, as well as hairless skin and large neocortex of a human being (Ibraimov 2003, 2004, 2007, Ibraimov and Tabaldiev 2007). In the present work was gathered data that probably sex and sexual reproduction of eukaryotic organisms are the result of the long evolution of ncDNAs, which step by step led to the origin of mitotic chromosome, mitosis, meiosis, sex determination and differentiation mechanisms.

As we suppose, so complicated evolutionary changes were the consequence of an amazing ability of ncDNAs to provide the very different forms of DNA organization: from nucleosoma to mitotic chromosome body. Apparently, the basis of the ncDNAs' potential for different forms of self-organization is formed by their common capability of mutual nonspecific attraction – "stickiness", – which is connected to the presence of short repeated sequences of nucleotides in them. Though, the modes of DNA packaging into interphasic cells do not influence on the contents of the genetic information of a nuclear genome, nevertheless, they are essential factors in a vital activity of not only single cells (Ibraimov 2003), but of the whole organism (Ibraimov 2004, 2007). Hereby, we do not assert that ncDNAs are capable of specific reactions. Their nonspecific molecular composition does not allow this. We just want to say that nonspecific reactions can serve as the basis for the creation of specific forms of response to different environmental changes, and this circumstance can be related to the sex origin of eukaryotic organisms.

As it is known, sexual and asexual reproductions are just the different ways of adaptation of organisms of that species to living conditions and ensuring material continuity between generations. Sex and sex differences are adaptive mechanisms, ensuring the process of intraspecies combinative variation, as well as its genetic isolation. The biological advantage of sex and sexual reproduction consists in the ability for recombination of the best inherited characters of both parents, as a result of which the progeny can be more viable than either of the parents.

The same cell of a sexually reproducing unicellular eukaryote represents both a body cell and a germ cell. In multicellular organisms there is a specialization, as a result of which the special cells, serving for asexual and sexual reproduction, isolate. The whole organism of the unicellular creates gametes and that is usually preceded by series of its particular progamic states. Gametes of protozoa have the same size and structure (isogametes); gametes of metazoa differentiate to male and female anisogametes (heterogametes).

Hence, the sexual reproduction assumes merging of two gametes, which is formed owing to meiosis. However, since meiosis represents a special type of mitosis, and mitosis is not possible without mitotic chromosome, then the solving of the sex origin problem is probably to be started with the mitotic chromosome origin examination.

THE MODEL OF MITOTIC CHROMOSOME ORIGIN

The following model of mitotic chromosome origin seems highly probable. At a certain stage of "bare" ring chromosome evolution of some lines of prokaryotes the sites with ncDNAs started to emerge (Ibraimov 2003, 2004). This has led to: a) the increase of the length of such chromosomes; b) the delay of separation of already replicated DNAs because of the mutual attraction of chromosome sections with ncDNAs (chromomere). To divide such ring chromosomes, at the least they need to be shortened to the maximum. This can happen only owing to ncDNAs according to the principle, which has a place at mitotic prophase stage. When the thickness of such "cylinder" reaches the certain limit, «sister ring chromosomes» will start to repulse from each other and finally will divide in two.

Formation of nucleosomes is the first step in DNA packaging into a minor metaphase structure. We believe that it is connected with the availability in eukaryotic genomes intervening sequences of ncDNAs, which has the ability to attach to histones (by DNA-protein recognition mechanisms). Lack of nucleosomes in prokaryotes in spite of the availability in the

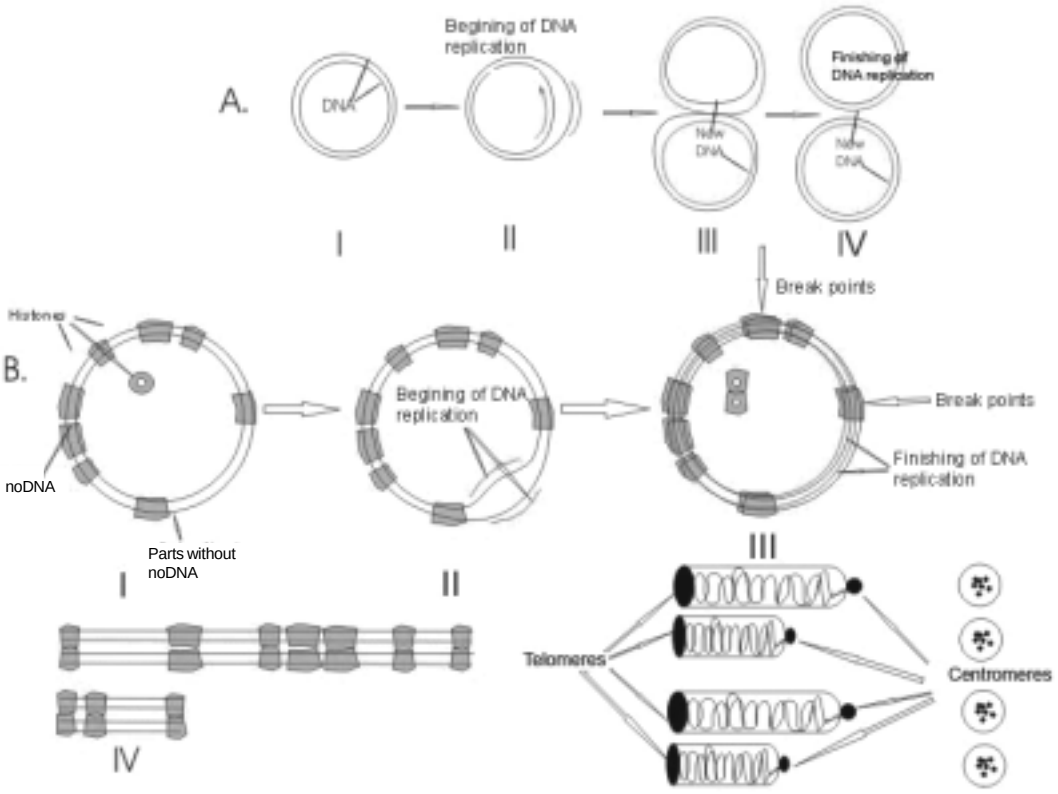

Fig.1. Origin of the mitotic chromosome

cells the histone-like proteins is possibly attributed to this important reason. In other words for formation in nucleosomes, chromomeres, chromosome bands and condensed chromatin (CC) it is necessary that in the DNAs should be nucleotide sequences with anchorage dependence features, due to which they will be inside the nucleus (in more detail see Ibraimov 2003, 2004).

Virtually, a gap between S and M phases in meiosis and the fact that sister chromatids are tied together relate to the origin of the mitotic chromosomes. In the cases when this division mode becomes difficult, ring chromosomes break. Perhaps, more favourable outcomes expected those ring chromosomes where breaks happened in the sections with the considerable amount of ncDNAs. In due course, these ends could be transformed into centromeres and telomeres, ensuring additionally the closeness of those sections of the former ring chromosomes (Lima-de-Faria 1983). Thus, could be originated the eukaryote genetic linkage groups in the form of telo-, acro- or metacentric chromosomes. There appeared possibilities for the endless combination of genes in the population of eukaryote organisms through meiosis, having opened yet unknown prospects for their further development (Fig. 1).

A. Replication of the ring chromosome in prokaryotes.
B. Replication of the ring chromosome with ncDNAs sections and its transformation in the mitotic chromosome. After breaking at the sections with the ncDNAs (III), pieces of the chromosomes (IV) turn to closed systems by way of transformation of the ncDNAs remains at the ends in the centromeres and telomeres (Lima-de-Faria 1983), i.e. in the mitotic chromosomes (V). The sister chromatids (IV) diverge owing to retraction and merging of the chromosomes sections with the ncDNAs into two parallel cylindrical bodies when the contact surface area between two chromatids becomes minimum (see below).

NONCODING DNAs IN MITOSIS AND MEIOSIS

Despite the fact that there is a vast body of literature about microscopic transformations and molecular processes at mitosis and meiosis, still there remain quite many puzzles concerning the chromosome behaviour during cell division. The two, which are somehow connected with ncDNAs, stand out against them: à) why sister chromatids remain stuck together till the end of anaphase; b) what makes them stuck together regardless of the fact that their DNA replication was completed at the S stage in interphase? It is hard to explain these phenomena by gene activity, because they do not transcribe in metaphase chromosomes.

The ability of eukaryotic cells to delay segregation of chromosomes until long after their duplication distinguishes their cell cycle from that of bacteria, in which chromosome segregation starts soon after the initiation of DNA replication. Furthermore, mitotic chromosome condensation, without which large genomes cannot be partitioned between daughter cells at cell division, would not be possible if chromosome segregation coincided with DNA replication. Despite its importance, the mechanism, by which sister chromatids are tied together, is poorly understood.

FISH shows that most sister DNA sequences separate from each other (at least a short distance) soon after DNA replication. Nevertheless, sister chromatids usually do not acquire morphologically separate axes until prometaphase, well after the onset of chromosome condensation. Human chromosomes, for example, appear as undivided "sausages" during prophase even though they are already highly condensed (Sumner 1991). The robust cohesion at centromeres may be due to more to their heterochromatic nature than to their ability to form attachments to the mitotic spindle. Other heterochromatic chromosome domains, like the entire Y chromosome in flies, also remain tightly stuck together during mitotic arrest. A relation between heterochromatin and stickiness is also seen during normal mitosis. Human chromatid pairs move to the poles during anaphase with different kinetics, and the laggards are invariably chromosomes with the greatest amount of centromeric heterochromatin (Vig 1987). The only established effect of heterochromatin has been an influence on chiasma number and distribution in meiotic chromosomes of certain species (John 1988; John and Micloš 1979). However, Lica et al. (1986) have proposed that satDNA is necessary for holding sister chromatids together. This proposal was strongly reinforced by the morphological observations made by SEM. In particular, Sumner (1991) was the first who discovered that the centromeric

heterochromatin remains undivided when the chromosome arms have split into separate chromatids. On the basis of these direct observations he assumed that "the function of centromeric heterochromatin is to hold the chromatids together until their anaphase separation".

As it is known, meiotic division having much in common with mitosis, nevertheless has a number of peculiar properties: 1) one homologous chromosome attracts another and they settle themselves side by side, in pairs. At the same time, each chromomere draws together with homologous chromomere, and centromere – with centromere; 2) in mitosis each centromere settles itself separately from its homologue, but in meiosis they lie together. At mitosis centromeres divide and sister chromosomes, connected to them, now move towards the opposite poles. At meiotic division paired centromeres do not divide, but each one moves separately from others, carrying one chromosome from each pair to the opposite poles; 3) during the first meiotic division each of the paired homologous chromosomes is presented by two chromatids, connected to the common centromere, as at mitosis. Bivalents contain four chromatids, the two of which are connected with maternal and the two – with paternal centromeres. During the second meiotic division centromeres divide and the daughter centromeres, each with its chromatid, head for the opposite poles; 4) crossing over, in which the homologous sections of paired maternal and parental chromosomes exchange materials. Hence, to answer the question why meiosis is carried out by two divisions, but not the one, it is required also to ascertain the nature of forces conditioning attraction and repulsion of centromeres and chromatids.

Apparently, this required force is ncDNAs' ability for mutual attraction. Probably, the attraction of homologous chromosomes to each other at the prophase stage of the first meiotic division, which is distinguished by an exceptional duration (up to 50 years in the case of human oocytes), is caused by chromomere structure of eukaryotic chromosomes. Walker (1971) was the first who suggested that satDNAs take part in recognition of homologous chromosomes during meiotic conjugation. What concerns a human being, the role of satDNAs apparently is quite insignificant. For example, they constitute just about 4% of DNA in a human being's genome (John 1988). Perhaps, it would be right to attribute this force to repeated DNAs in the whole, but not just to satDNAs in chromomeres of prometaphase chromosomes.

It ought to be admitted that we know almost nothing about the mechanism of chromosome pairing in meiosis, due to which homologues appear to be so tightly brought together that there can start synapsis with the formation of synaptonemal complex (SC). As it is known, the formation of SC does not need homology as a mandatory condition when pairing, though the homologous pairing is a more usual occurrence. Usually, synapsis is considered as an extremely accurate mechanism of pairing of homologous chromosomes. The SC formation is an essential part of this process. However, in several cases during meiosis there observed a non-homologous pairing, during which SC is formed too. There are many examples proving that SC formation is not in need of homology when pairing (Bostock and Sumner 1978).

The SC's structure and composition do not give us a clue to the solution of its role in crossing over. It is known only that SC consists of proteins. SC is intended rather for keeping chromosomes at a certain distance from each other, than for bringing them to an intimate contact (Bostock and Sumner 1978). Moreover, the presence of SC is not yet a guaranty that crossing over will happen. As it was showed by John and Lewis (1965) crossing over and chiasmata formation represent the same phenomenon. The time coincidence of SC disappearing with chromosome length shortening and the fact that the SC formation happens when chromosomes undergo a prophase shortening testify the above as well.

The fact that crossing over is not possible during DNA replication by means of a "copy selection" mechanism or yet after DNA synthesis by breakage and further cruciform reunion of non-sister chromatids in homologous sites testifies once again that at first chromosome conjugation must happen. First of all, here is required the attractive force for pairing homologous chromosomes, and apparently this force is connected with ncDNAs in interphase chromosomes. As it is known, homologous chromosomes are equal not only in size and form, the same chromomeres ("knobs") are located along them, and probably they contain identical classes of ncDNAs (Bostock and Sumner 1978).

One more phenomenon is known – in meiotic metaphase I centromere regions are pulled aside to the poles. Homologous chromosomes start to divide, but still hold out in chiasmata formation sections. In metaphase of mitosis we can observe the opposite situation. As Sumner (1991) has showed here chromatids start to separate from the telomere end of metaphase chromosome and only at the very end separate the centromere regions. Why this happens? As we assume, ncDNAs play the important role in both mutual attraction and repulsion of chromosomes in chromosome bands.

In mitosis, sister chromatids separate without the help of mitotic spindles having the dividing cells treated with colchicine ("C-mitosis"). Apparently, the separation of sister chromatids in "C-mitosis" is also caused by a complete fusion of chromomeres and chromosome bands along the chromosome into one homogeneous body at the end of metaphase. When chromatids turn into short "thick" cylindrical bodies, the contact area between sister chromatids becomes so small (see Fig. 1) that they are not in the position to remain tied together in the "boiling" cytoplasm. Here, the attractive forces between chromatids, even if remained, depend mainly on the quantity and the quality of repeated DNAs. Probably, that is why centromeres are the last to separate in mitosis and distal chromosome regions are the first. Thus, the role of ncDNAs can be significant both in attraction and repulsion of mitotic chromosomes. For that it is necessary that in the respective chromosome sections was a sufficient amount of repeated DNAs.

And finally, following their attachment to the spindle, kinetochores oscillate and the chromosome migrates to the spindle equator, a process termed "congression". Once all of the chromosomes have congressed, the cell is in a metaphase and the chromosomes are said to lie on the metaphase plate. Kinetochores control the metaphase-anaphase transition by inhibiting sister separation until all the chromosomes are properly attached and aligned (Heald 2000). With their complex and coordinated behaviour and their key role in regulating entry into anaphase, kinetochores are considered the brains of chromosomes in mitosis, directing their movement and orderly segregation. At present, the bulk of the evidence seems to indicate the kinetochore does contain some form of chromatin and may possibly be derived from (or organized by) the centromere DNA (for review see Pepper 1988).

Hence, at the stage of the cell division, ncDNAs participate: 1) in shortening and dense packaging of the chromatin fibres for formation of the body of the metaphase chromosomes; 2) in keeping the sister chromatids up to the end of anaphase together; 3) in repulsion of sister chromatids from each other at the stage of anaphase; and 4) it gives chromosomes the necessary strength and flexibility so that they can pass the mitotic cycle.

THE ROLE OF ncDNAs IN SEX DIFFERENTIATION

There is a good reason to assume that the role of the ncDNAs in the cell differentiation may be significant. Though for the time being we do not know the concrete mechanisms of the ncDNAs influence on the cell differentiation, nevertheless the listed below facts justify to their possible participation at this important stage of development:

a) the specialized cells, tissues and organs appeared only after appearance of the cellular nucleus, i.e. the eukaryote organisms;

b) there are good reasons to believe that the eukaryote cell itself is the result of a long-term evolution of the ncDNAs (Ibraimov 2004);

c) with appearance of a nucleus isolated from the cytoplasm, the genes in the eukaryote chromosomes are no longer easily accessible to the transcription machinery, as in the prokaryote cells;

d) the eukaryote chromosomes unlike the "bare" ring chromosomes of prokaryotes are closely connected with the proteins, and their genes in addition to the "mosaic" structure (exon-intron) occupy only a small part of the nucleus genome, where the main bulk of the DNA constitutes the ncDNAs;

e) apparently, for the differential activation of genes it is necessary that not all the coding DNAs be available to the transcription machinery of a cell. For this it is necessary, somehow, to isolate the genes from the direct influence of the inductors in the cytoplasm. Apparently, such an isolating means is the nuclear envelope with a thick layer of peripheral CC of cells (Ibraimov 2004);

f) as a rule, the DNA of mitochondrions and chloroplasts in eukaryotes are outside the nucleus, and this situation, seemingly, is of an extraordinary importance. If they were inside the nucleus, then the energy supply of the eukaryote cells would be seriously under the threat, as these

coding DNAs may be influenced by the condensed forms of the ncDNAs with well-known consequences (as in case of the position effect variegation);

g) ncDNAs in the nucleus are not an amorphous mass or simple bulking agents: they have an aptitude for higher forms of physical organization of DNA, starting with nucleosomes and finishing with mitotic chromosome bodies (Ibraimov 2004).

Germ cells in multicellular organism body belong to specialized cells. Sex development, as in the case of all other organism features, is defined with the help of genotype and environmental factors. Nevertheless, the role of specific structural genes and sex chromosomes in sex determination and differentiation is not clear yet. However, recently there has appeared information about the possible role of ncDNAs in individual development and evolution (Ibraimov 2003, 2004, 2007, Ibraimov and Tabaldiev 2007), including the sex differentiation of higher eukaryotes (Ibraimov 2008). In particular, on the basis of the cell thermoregulation concept there was proposed a hypothesis of a possible sex differentiation mechanism. Seemingly, the sex differentiation (SD) in animals and human is determined by the amount of constitutive heterochromatin region (cHR) in the chromosomes of the undifferentiated embryonic gonads (UEG) via cell thermoregulation. It is assumed the medulla and cortex tissue cells in the UEG differ in vulnerability to the increase of the intracellular temperature. If the amount of the HR is enough for efficient elimination of heat difference between the nucleus and cytoplasm in rapidly growing UEG cells the medulla tissue survives. Otherwise it doomed to degeneration and a cortex tissue will remain in the UEG (in more detail see Ibraimov 2008).

THE ORIGIN OF SEXUAL REPRODUCTION

Above, we already discussed the possible role of ncDNAs in mitotic and meiotic cell divisions. On their basis the following possible model of sexual reproduction origin is proposed. For that it is required the availability of the following:

a) mitotic (asexual) reproduction;

b) chromomere in mitotic chromosomes with sufficient amount of repeated ncDNAs capable of mutual attraction;

c) changing conditions of external or internal environment influencing on the mitotic prophase stage duration. Apparently, the importance of availability of the first two conditions does not require additional argumentation. We will focus on the third one alone.

When at the stage of prophase a long delay of mitosis occurs under the influence of yet unknown factors of environment, sister chromatids often conjugate with chromatids of homologous chromosomes, forming bivalents. Obviously, at the same time there can have a place the chiasmata formation with well-known genetic consequences. As they are shortening and thickening, the centromere regions will start to separate first, and then the arms of homologous chromosomes will do (see above). Thus, in the anaphase of the first "meiotic" division there separate the homologous chromosomes of each pair, but not the sister chromatids of each chromosome.

Probably, in the process of evolution such haploid cells have been merging into diploid organisms. Inasmuch as this mode of reproduction involves two individuals quite often with various sets of genes, then this could not help influencing on viability of such organisms. The advantage of this reproduction mode was proved by further evolution.

Thus, the sexual reproduction was an immediate consequence of meiotic division origin, but not a result of some specific structural gene emergences determining sex development. Sex related genes emerged later with higher eukaryotes, yet not for sex determination, but for stimulation of the secondary sexual character development with the help of hormones. The sex, even one of higher eukaryotes, develops from indifferent primordial gonads without gene interference (Ibraimov 2008).

COULD CERTAIN STAGES OF SEX ORIGIN BE EXPERIMENTALLY CHECKED?

On the basis of the foregoing we assume that probably the key role in sex origin and sexual reproduction of eukaryotic organisms belongs to ncDNAs, but not to structural genes. Apparently, sex and sexual reproduction have occurred as a result of the long ncDNAs' evolution, which successively led to the origin of mitotic chromosome, mitosis, meiosis, and sex determination and differentiation mechanisms.

Fortunately, the certain stages of the supposed sex origin account can be checked experimentally. For example, in principle the proposed model of mitotic chromosome origin can be considered to have been already checked. Hereof testify the experiments on generation of satellite DNA-based artificial chromosomes for use in gene therapy. For example, it is demonstrated that the short arm of human acrocentric chromosomes, which contains tandemly repeated ribosomal DNA genes and different satDNA sequences, is an optimal chromosomal region for inducing *de novo* chromosome formation (Hadlaczky 2001).

Evidently, with sophistication of cell cultivation, cloning and *in vitro* fertilization methods there has come the time for experiments on carrying out meiotic division of somatic cells – *in vitro* meiosis (IVM). The main point of IVM is to expose any type of somatic cells to meiotic division in order to receive haploid cells ("gametes") for *in vitro* fertilization (IVF). As we believe there already exist methodical and theoretical prerequisites for realization of IVM:

a) availability of culture of Sertoli cells;
b) techniques of germ cell transplantation;
c) the demonstration that spermatogenesis can be successfully carried out in a testis of different species. The finding that mouse Sertoli cells in the rat into mouse transplantation experiments can fully support rat spermatogenesis, in spite of the ten million years or so evolution that separate the two species;
d) fertilization has been achieved even when sperm motility and morphology is poor. Sperm recovered from the epididymis or from the testis can also be used in introcytoplasmatic sperm injection (ICSI), and a few live births have been reporting following the injection of spermatidis. For men whose ejaculates contain even a few sperm, ICSI, in which a single sperm is injected into the cytoplasm of the egg, has proved unexpectedly successful, giving pregnancy rate equalling that normal IFV;
e) there have been no published reports of primordial germ cells entering meiosis *in vitro*, when maintained as isolated cells. However, if mouse germ cells do indeed have a cell-autonomous tendency to enter meiosis irrespective of the urogenital ridge, then theoretically somatic cells can also enter meiosis in an environment of a tissue or an organ culture system;
f) it has managed to show that the diploid spermatogonia progressing *in vitro* to haploid spermatidis involves coculture with an immortalized Sertoli cell line (in more detail see Rassoulzadegan et al. 1993; McLaren 1998; McLaren and Southee 1997).

Hence, we believe that inasmuch as all somatic cells are pluripotent, then at least some of them (e.g. less specialized cells, like fibroblasts) can experience the meiotic division, if the respective *in vitro* conditions to be created. Schematically, for realization of such experiments it is required to:

a) prepare a culture of fibroblast cells from a donor as a source material for receiving haploid cells – "gametes";

b) have a culture of Sertoli cells as supporting tissue to nourish and regulate the development of somatic cells from diploid to haploid stages;

c) prolong the mitotic prophase stage as much as it is required to make prophase homologous chromosomes conjugate as they do during the ordinary spermatogenesis. For that, carry out IVM at a temperature 2-3^{O}C lower than the core temperature of a corresponding type of mammals (see below);

d) separate donor cells with haploid sets of chromosomes;

e) use nuclei of such "gametes" for further IVF or intracytoplasmic injection.

Virtually, there is nothing unexpected in an attempt to realize IVM. It is founded on the long-term researches on conversion of undifferentiated cells to fully differentiated cells within laboratory. The main problem in realization of IVM – can somatic cells be "dedifferentiated" or even redifferentiated *in vitro*, so that they undergo artificial "meiotic division".

However, recently there has occurred one more circumstance assuring the possibility of IVM realization. It concerns the lately discovered cell thermoregulation (CT) phenomenon, the essence of which is in the following: the peripheral layer of condensed chromatin (CC) of nucleus, being the densest domains in a cell, apparently conducts heat between the cytoplasm and nucleus when there is a difference in temperature between them. The assumed heat conductivity effect of CC is stipulated by its

principal features: condensed state during the interphase, association with the lamina and the inner nuclear membrane, replication at the end of the S period of a cell cycle, formation of the chromocenter, genetic inertness, and wide variability in the quantitative contents both within and between species (Ibraimov 2003). The reality of the CT existence is shown at the organism level. In particular, it turned out that the individuals in the population differ from one another by the heat conductivity of their bodies (BHC). At that the BHC value depends on amount of the constitutive heterochromatin (cHR) in their genomes (Ibraimov and Tabaldiev 2007).

The matter is that the role of CT may be important in gametogenesis as well. It is known that spermatogenesis of mammals with the rare exceptions (elephants and whales) happens outside the body cavity at a temperature 2-3^{O}C lower than the core temperature. Apparently, for several specialized cells it is extremely important to maintain intracellular temperature at a certain level to realize entrusted functions. Perhaps, germ cells belong to that kind of cells too. If that's the case, then it is easy to imagine that Sertoli cells in addition to already well-known to science functions (mechanical, support, protection and nourishment) also execute the thermoregulation part, namely they withdraw the metabolic heat excess from germ cells. To make sure that this is true, it is enough to remember the histology of testicle tissues of mammals: on every chart showing the structure of part of the wall of a seminiferous tubule and interstitial cells it can be seen that spermotocytes become embedded in the many invaginations in the lateral margins of the Sertoli cells where they develop into spermatids before passing to the edge of cell bordering the lumen where they mature as spermatozoa. Moreover, the Sertoli cells secrete the fluid carrying spermatozoa through the tubules. Probably, this fluid also takes part in cooling germ cells, involved in continuous mitotic and meiotic divisions. It is obvious that the maintenance of a relatively low temperature in testicles of mammals, the core temperature of which is rather high, has also a deep genetic sense – to reduce the possibility of probable errors at replication, reparation and recombination of DNA during spermatogenesis.

The very history of HRs chromosome discovery testifies an exceptional sensibility of noncoding part of genome to the changes of environmental temperature. Heitz (1928), being the first to discover chromosomal HRs, developed a new inexpensive cytological method (the boiling technique, "Kochmethode"). Boiling in aceto-carmine had produced the first C-banding patterns (Passarge 1979). The first successful experiments designed to make heterochromatin segments visible in metaphase chromosomes were completed in the late 1930s when Darlington and La Cour (1940) discovered differential chromosome regions in the lilaceous plants *Paris* and *Trillium*; these regions, after cold treatment, appeared thinner and less stained than the rest of the chromosomes. In interphase nuclei the same segments were visible as positively heteropycnotic chromocenters. This phenomenon has been observed in plants and animal species. We assume that during temperature reduction the slow down of HR compactization takes place (one of the types of ncDNAs), and as a consequence formation of metaphase chromosome body detains, thus giving time to prophase homologous chromosomes to "know" each other. That is why, our suggestion to use low temperature as a means of mitotic prophase stage prolongation to receive IVM is unlikely to astonish.

We repeatedly indicated the possible participation of cHR in the composition of peripheral layer of CC in the interphase nucleus in CT (Ibraimov 2003; 2004; Ibraimov and Tabaldiev 2007). Here, apparently, the role of cHR on Y chromosome during the mammal sex determination and differentiation should be especially underlined (Ibraimov 2008). That is why the participation of the largest cHR block on Y chromosome of mammal karyotype in elimination of the heat excess from spermatocyte nuclei during spermatogenesis seems to be a fairly expected biological process. The maintenance of relatively low and stable temperature is provided by: CT with the help of cHR on Y-chromosome; testicle position outside the abdominal cavity; Sertoli cells; blood supply peculiarity due the vascular plexus formed by the spermatic artery and vein acting as a countercurrent heat exchange system, and the dartos muscle of the scrotal sac moves the testes towards or away from the body according to the outside temperature. However, the primary meaning of the low temperature is that it assists in prolongation of the mitotic prophase stage for

there take place conjugations of homologous chromosomes, without which the meiotic division is not possible.

Hence, ncDNAs take part at all stages of sex development and sexual reproduction, starting with sex determination and finishing with mature gamete formation. Of course, we can not exclude totally the role of structural genes in these processes. Probably, they are really important. However, it is obvious that their role in sex origin is of the secondary importance, and in the evolution they appeared later, when the sexual reproduction became inherent for eukaryotic organisms.

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