

Possible Mechanism of Sex Determination in Mammals

A. I. Ibraimov¹

¹ Institute of Balneology and Physiotherapy, Bishkek and Laboratory of Human Genetics, National Center of Cardiology and Internal Medicine, Bishkek, Kyrgyzstan

Correspondence: A. I. Ibraimov, Institute of Balneology and Physiotherapy, Bishkek and Laboratory of Human Genetics, National Center of Cardiology and Internal Medicine, Bishkek, Kyrgyzstan. E-mail: ibraimov_abyt@mail.ru

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Abstract

It is known that undifferentiated embryonic gonads (UEG) in embryos of mammals have a dual nature. They consist of an outer layer of cortex, from which in the process of differentiation develop female sex cells, and from the inner layer – medulla – develop male gametes. During the determination of sex, one of the layers of the gonad develops and the other is suppressed. Therefore, the sex of the future fetus depends on which of the tissue cells - medulla or cortex - survives in the UEG. However, almost nothing is known about the causes for the survival of the medulla or cortex.

We believe that, perhaps, the main cause for the development of one of the layers of the gonad and the suppression of the other is cell thermoregulation. The survival of the tissue cells of medulla depends on whether they can avoid heat death, which is determined by their ability to effectively remove excess metabolic heat from the cell nucleus. Since excess thermal energy is released into the cytoplasm through a dense layer of condensed chromatin around the nucleus, consisting predominantly of chromosomal constitutive heterochromatin regions (cHR), the fate of the medulla directly depends on the cHR in its cells.

Seemingly, the sex in mammals and human is determined by the cHR on Y chromosomes. It is important not so much the amount of cHR on Y chromosome as its localization in the interphase cell around the nucleoli. Thanks to this localization, excess heat is more effectively removed from the "hottest" point of the nucleus, where ribosomes are formed, which is also necessary for the production of proteins that inhibit the development of cortex cells. Otherwise medulla doomed to degeneration and a cortex tissue will remain in the UEG.

Keywords: sex determination, cell thermoregulation, constitutive heterochromatin, Y-chromosome, non-coding DNAs

1. Introduction

In many animals, including us, the genetic sex is determined at fertilization by sex chromosomes carried by the father's sperm, X in the case of female and Y in the case of male. In birds, moths, and butterflies, males are XX(ZZ) and females are XY(XW). However at the early stages of embryonic development a pair of undifferentiated embryonic gonads (UEG) and both rudimentary female and male reproductive system develops in the embryo. As result of this all embryos are potentially bisexual.

However, almost nothing is known about concrete mechanisms of sex determination (SD). The problem also becomes complicated especially as: a) the exact number, localization, products, and types of these gene interactions are not determined; b) the role of the sex chromosomes in the embryo SD remains not completely clear; c) there are no ideas as regards the possible role of a great amount of constitutive heterochromatin region (cHR) of the Y chromosome in the SD.

For the time being the mechanisms of the SD are not known. At present the balance hypotheses, worked out by Bridges (1939) and Goldschmidt (1955) are generally accepted. According to these hypotheses, the interaction of genes, located in the sex chromosomes and autosomes, underlie the SD. Thus, it is considered that sex is a polygenic feature. Another model is proposed – SD of eukaryotes in mammals is a result of cell thermoregulation and evolution on noncoding DNAs.

2. State of the Problem

The evolution and maintenance of SD is one of the central questions in modern evolutionary biology. Since the hypotheses for the origins of SD are difficult to test experimentally, most current work has been focused on the maintenance of sexual reproduction.

Indeed it is hard to believe that having impressive breakthrough in modern genetics and molecular biology the mechanisms of SD are still unknown. This probably has to do with the fact that in the basis of all hypothesizes and theories on sex biology lies idea on all-powered role of natural selection and genes in eukaryotic organisms' evolution. Although they help to explain reasonably and justify such widespread propagation of sexual reproduction in the world of eukaryotes; nevertheless, these approaches had little help in the development of theories and hypothesizes explaining sex determination.

Currently, two basic rules for sex determination in mammals are generally recognized. Classical embryogenetic studies established two rules for sex determination in mammals. The first of these was formulated in the 1960s by Alfred Jost on the basis of experiments to remove the rudiment of future gonads in early rabbit embryos: the removal of the genital ridges before the formation of the gonad led to the development of all embryos as females (Jost, 1970). It was suggested that the gonads secreted by the male hormones of the effector hormone testosterone responsible for the masculinization of the fetuses, and predicted the presence of a second anti-Müllerian hormone effector, directly controlling such anatomical transformations. The results of the observations were formulated in the form of a rule: the specialization of developing gonads in the testes or ovary determines the subsequent sexual differentiation of the embryo.

Until 1959, it was assumed that the number of X chromosomes is the most important factor in sex control in mammals. However, the discovery of organisms with a single X chromosome, developing as females, and individuals with a single Y chromosome and multiple X chromosomes, which developed as males, forced to abandon such ideas. The second rule for sex determination in mammals was formulated: the Y chromosome carries the genetic information required for sex determination in males.

In addition, it is recognized that along with genetic factors (the presence of sex chromosomes of one type or another), some of the vertebrates (fish, amphibians and reptiles) and some invertebrates are influenced by environmental factors (temperature, pH of the medium, availability of nutrients and other). Thus, the sex of an individual of a dioecious organism can be determined by genetic mechanisms, or under the influence of external environmental conditions.

3. The Proposed Hypothesis of the Possible Mechanism of Sex Determination

Earlier, we presented 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 chromosomes, mitosis, meiosis, cell thermoregulation and sex (Ibraimov, 2008; 2009; 2010; 2017). 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 nucleosomes to mitotic chromosome body and condensed chromatin. 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.

Our approach of possible mechanism of sex determination in mammals is relies on the hypothesis of cell thermoregulation (CT). CT is the process of removing excess metabolic heat from the nucleus into the cytoplasm and finally the whole cell. Structural basis of CT is the peripheral layer of condensed chromatin (CC) which is chromosomal cHRs. We assume that the thermal energy transfer between the nucleus and the cytoplasm is carried out through this dense layer of the peripheral CC, located inside the nuclear envelope. The density of CC depends on the amount of chromosomal cHRs in the cell genome (Ibraimov, 2003, 2008, 2009, 2017).

Since sex ultimately depends on whether or not the tissue cells of the medulla have survived, it is obvious that their preservation is crucial for the development of the male sex. Otherwise, all embryos will inevitably develop as females (see above). The question arises, how on the survival of the tissue cells of medulla can affect cHR on Y chromosome?

Within the framework of the CT hypothesis, the SD mechanism could be explained as follows. The likelihood of survival of the tissue cells of medulla depends on whether they can maintain the level of intracellular temperature so that the synthesis of the biologically active substances necessary for the active suppression of cell growth in the cortex tissue does not cease. Why was the ability of the medulla to survive intracellular temperature to be so important? The fact is that the tissue cells of medulla are in an unfavorable anatomical position compared to cortex

cells when it comes to removing excess heat energy. First, the medulla is under a layer of tissue of cortex and mesentery next to the aorta, which can make it difficult to remove heat beyond its limits. Secondly, by nature, the medulla cells seem to be more sensitive to the harmful effects of high temperature than cortex cells, as evidenced by the cases of cryptorchidism in boys. Therefore, the tissue cells of medulla, in order to survive with such an unfavorable anatomical environment, need an additional mechanism that would contribute to a more efficient release of heat energy. Otherwise, the cells of the medulla are doomed to heat death, thus allowing the cells to develop cortex with all the ensuing consequences.

4. Possible Mechanism of Sex Determination on the Example of a Human

The mechanisms of cell thermoregulation are already described in detail (Ibraimov, 2003, 2017). Briefly, the essence of CT reduces to the following. Internal sources of heat (thermogenesis) in endothermic organisms are well known: cellular metabolism, muscle contraction and active transport of ions. Thus, the thermal energy, including the excess energy, is produced inside of cells. As a by-product of cell activity, the thermal energy is not used to carry out biologically useful work, and therefore, should be timely removed outside the cells.

There is no known mechanism for a cell to actively dissipate excessive thermal energy. It is a rule to think that diffusion and possibly convection are the primary means to passively remove the heat generated inside the cell (Hochachka, 2003). This explanation raises a serious objection. The fact is, 'Inside the cell the molecules are mostly associated with polymeric structures (cytoskeletal polymers or membranes) and thus exist in very heterogeneous, solid state environments that alter their behavior dramatically compared to free molecules in test tubes' (Albrecht-Buehler, 1990). As such, highly localized heat sources are expected to create a subcellular temperature gradient. In other words, the interacting molecules in the cell do not float freely, as in a test tube with an aqueous solution. Therefore diffusion and convection may not be the primary means to remove the heat generated inside the cell. Therefore, it is necessary to seek other additional mechanisms of removing excess heat from the cell, and in particular of its organelles.

Hypothetically, we think of the CT mechanism as follows. For the known in the science reasons, at a certain stage of cell activity the thermal energy exceeding the optimal physiological level may be accumulated in the nucleus. Such excessive heat energy is required to remove outside of the nucleus, so it would not harm the normal work of cellular metabolism and of the genetic apparatus. As it is known, high temperature, among other things, has a strong mutagenic effect. Since the nucleus in terms of removing heat surplus, the choice is not great (increase of the own volume and/or density), it is forced to use a dense layer of CC, as the heat removal. Further, the thermal energy, using the cytoskeleton, membrane system and other dense structures of the cytoplasm as a "heat conductor", removed outside the cells in the intercellular space.

Now, we consider the question as surplus metabolic heat is removed from the nucleolus. Apparently, the role of nucleoli in CT is very large; as they are the most "hot" areas in the nucleus in virtue of performed their important functions – synthesis of rRNA. In this process, first of all, chromosomes participating in the formation of the body of the nucleolus are involved. In human these are the acrocentric chromosomes 13-15, 21 and 22 having nucleolar organizer regions (NORs), which contain ribosomal cystrones. As it is known HRs of acrocentric chromosomes, along with the NORs are involved in the formation of the nucleoli body in the nucleus (Ibraimov, 2017).

We think that in human the heat surplus removing role of the nucleolus is also performed by a big heterochromatin block on the Y chromosome in men. It is known for a long time that cHR block of the Y chromosome is associated with the nucleolus (Schmid et al., 1975). As the chromosomal cHRs Y chromosome and acrocentrics are found in the body and around the nucleolus, it is obvious that they are due to their high density can promote to remove excess heat from the nucleolus to the dense layer of CC around the nucleus. Therefore, it is important that is the presence of cHR on the Y chromosome in determining male sex in humans. Here it is relevant to recall that the cHRs block on the Y chromosome is larger in size, on average, than all cHRs acrocentric autosomes combined. Perhaps the same mechanism underlies the SD in other mammals.

5. Other Evidences Supporting this Idea.

We, a priory, believe that such a responsible process as gender determination cannot be based on a long chain of complex chemical reactions, where the probability of error or failure is very high at different stages of its implementation. The physical mechanism seems to be more reliable to us. Sex determination is too important to be trusted by the many genes that may have mutations during replication, transcription or translation failures.

Indeed, in mammals, it has been known for many years that the Y-chromosomal gene *Sry* ("sex-determining region Y") is necessary and sufficient for male sex differentiation, and many others genes have since been implicated in testis development. Expression screens such as microarray analyses have resulted in hundreds of candidate genes

that show sex-specific expression patterns. However, it has been difficult to place these genes into a network of gene regulation and function. Even for *Sry*, it is still not known how its expression is regulated, what proteins might interact with it, and which genes it regulates. Although *Sry* was discovered 15 years ago, no *in vivo* target gene has been identified. It remains controversial whether *Sry* has multiple target genes or just one that carries out all functions necessary for initiating male sex development. The identification of an SRY target gene(s) remains one of the greatest challenges in the field. Given the fact that *Sry* is regarded as the pivotal factor necessary and sufficient for mammalian male sex determination, it is surprising that there are mammals that determine male sex without the *Sry* gene (for more details see the review of Wilhelm et al., 2007).

The possible role of cHR on Y chromosome is not limited only with SD in the early stages of embryonic development. He seems to be directly related to both sex differentiation (Ibraimov, 2008; 2009; 2017) and to the maintenance of gamete production in men throughout his life. The importance of maintaining the intracellular temperature at the optimal level for the production of male gametes is directly evidenced by the anatomy of the location of the testes. They are usually outside the body cavity, where the temperature level is maintained at 2-3 °C lower than the core temperature. Therefore, we believe that to maintain a relatively low temperature in the testes is not enough only its anatomical location. There seems to be a need for more active intervention. In this case, the effective heat removal from the tissue of male gonads by cellular thermoregulation.

And finally, the phenomenon of freemartinism is known in cattle. At the birth of a heterosexual twin in a cow, in some cases the internal organs of a heifer acquire the signs of a male, although the outer genitals are arranged according to the female type. Their appearance is explained by the fact that the male hormones of the steer, developing normally, begin to be allocated earlier than the female hormones of the heifer, and redefine the sex of the female embryo. Therefore, to survive the medulla in the early stages of embryo development, it is very important to avoid thermal degeneration of the cells of its tissue in order to have time to develop male hormones that suppress the development of cortex cells.

6. Discussion

Within the framework of the problem discussed here, there are issues that need additional coverage. First of all, we are talking about the assumed role of a large segment of cHR on the long arm of the Y chromosome in the determination of sex. Regardless of whether our assumption is true or not, it remains an open question why on this sex chromosome there is a large cHR block that occupies almost half of its entire length? What is its biological meaning? Does it relate to determination, sex differentiation and development of secondary sexual characteristics? If so, what is its mechanism: chemical or physical? There is no scientifically sound answer to these questions.

However, even without the necessary experimental data in such cases, it can be postulated that cHR on the Y chromosome should have some relation to the development of sex in males. But what kind? The difficulty of the matter is that in the chromosome Y region that occupies cHR, there are no known for science genes. Whereas, on the euchromatic half of the Y chromosome, genes are found, including the above-mentioned *Sry* gene.

Nevertheless, there is evidence that at least one of the types of constitutive heterochromatin – Q-heterochromatin region (Q-HR) – on the Y chromosome is not a neutral element in the human genome. This is evidenced by the results of two extensive comparative population studies, obtained in different years in the framework of other programs. In the first case, it was found that the Q-HR on the Y chromosome, being the largest Q-heterochromatin segment in the human genome, somehow 'restricts' the amount of Q-HRs on autosomes in males (Ibraimov et al., 2000). The second study showed that, irrespective of sex, age and racial-ethnic characteristics, in the genome of females the amount of autosomal Q-HRs is significantly larger (Ibraimov, 2014).

Approximately the same data were obtained when analyzing the amount of cHR on the fruit fly chromosomes. For example, the total amount of the cHR with a normal diploid female of the *D. melanogaster* is 20% less than in a male. The same ratio is observed in so called super-females and super-males, and in triploid females and males. Thus, in all cases the females have the cHR approximately by 20% less than the males, irrespective of the ploidy of the autosomes and sex chromosomes number in the karyotype (Ibraimov, 2008). These results seem to prove that the SD in the animals is most likely determined by the total amount of the cHR in the embryo genome, but not by the gene balance, which is located in the X chromosome and autosomes. Apparently, for the same reason, amount of autosomal Q-HRs increases in females compared to males (Ibraimov, 2014).

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I apologize to those authors, whose works were not cited, or were cited only through reviews, owing to space limitations.

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