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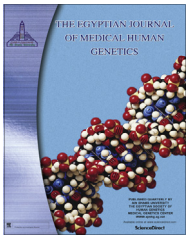

REVIEW

Biological imprinting: Some genetic considerations

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Abstract Genetic imprinting represents one of the most puzzling, still unexplained, phenomena in genetics. Changing some agreed upon concepts and redefinition of some common traditional terms in classical genetics seems imperative for understanding the nature of imprinting, as well as for interpretation of possible mechanisms implicated in its occurrence.

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1. Introduction

Genomic imprinting refers to differential expression of chromosomes, parts of chromosomes, single genes or sets of genes dependent on which of the two sexes they are inherited from, i.e., their parental origin. Following the establishment of imprinting in the male and female germ lines, respectively, the two parental genomes exhibit functional differences at fertilization [1]. Some sex differences in expression of inherited traits may result from genetic imprinting. To achieve imprinting, some genetic materials can be modified during gamete production or early embryonic development in one of the two sexes, so the traits determined by the imprinted genes are expressed differently than would be expected under typical Mendelian inheritance. A growing body of evidence points to methylation of cytosine residues in the context of cytosine–guanine (CpG) dinucleotides as the mechanism of imprinting. Such methylation, especially if it occurs in the promoter regions of genes, can nullify the ability of the genes to be transcribed. Certain genes that can be imprinted will be methylated in the production of sperm, others in the production of ova, and they can be reactivated by demethylation when they pass through gametogenesis in the opposite sex. It is still not known why certain alleles are subject to imprinting while others are not, and why they are more likely to be imprinted in one sex than the other.

Amplification of genes with functional overexpression, rather than inactivation or silencing, might result as a consequence of imprinting, that is, as the gene passes through gametogenesis in one of the sexes, sections of it become duplicated and the gene thereby gets amplified and shows abnormal copy number increase. This is seen in neuroblastoma, where an increased number of DNA segments containing the paternal N-myc protooncogene are detected. A similar phenomenon occurs in Huntington's chorea where amplification of segments of DNA in the gene is limited, exclusively, to the paternal HC genes inherited from the fathers.

Though genomic imprinting, which results in parental-specific silencing or suppression of gene expression especially during early development, is proposed to be the major mechanism that prevents occurrence of parthenogenesis in mammals, it can, also, result in development of many genetic diseases if detrimental mutations affect the other active expressing allele. Genetic diseases resulting from this particular pathogenetic mechanism are referred to as imprinting disorders and include many diseases like Beckwith–Wiedemann syndrome, Silver–Russell syndrome, Prader–Willi syndrome and Angelman syndrome [2].

2. Genetic mutations

The classic definition of genetic **mutation** entails any structural change in the genetic material at any of its organizational levels, nucleotide/gene/chromosome/whole genome, leading in most instances to deleterious functional alterations. At the single nucleotide level, mutational changes, referred to as point mutations, comprise structural changes of the nucleotides, or bases, of the gene by deletion/addition/replacement leading to pathogenetic defects that include **frame shifting** (change of the base sequence due to addition or deletion of one or two bases with consequent shifting of the codon-frame of the gene and the amino acid frame of the defined protein), **missense** alteration (change of the amino acid defined by the original code comprising the original base to another different amino acid defined by the new code comprising the new base), **same-sense** alteration (change of a base of a codon to another base forming a new codon that defines the same amino acid due to degeneracy of the genetic code) and **non-sense** alteration (change of one base of a functional codon that specifies a particular amino acid to another base leading to the formation of a stop or termination codon that does not code for, or define, any amino acid). Change of a stop codon to a functioning codon leading to aberrant continuation of translation and synthesis of longer polypeptide chains will be referred to, arbitrarily, as **re-sense mutation**, an abbreviation of regaining sense, mutation.

3. Epigenetic alterations

The corresponding classic definition of **epigenetic** changes entails structural changes of the bases, affecting neither their number nor their sequence along the affected region, that can alter gene expression. For example, methylation of cytosine bases along the gene promoter will not change the number of methylated bases or their sequence along the epigenetically altered region but can alter their expression. Methylation of bases is a reversible mutational change mediated by specific enzymes that required S-adenosyl-methionine as methyl donor, methyltransferases, and reversed by specific demethylases. Similarly, methylation of specific regions of the DNA-associated proteins, primarily histones, is mandatory for maintaining vital functional aspects of the genome. For instance, maintaining a proper balance of histone methylation, by the opposing activities of lysine methyltransferases and lysine demethylases, is critical for genomic stability, cell cycle progression, gene regulation, DNA replication, and cancer prevention [3]. Modulations of gene expression induced by epigenetic changes of the gene, e.g., by methylation, and/or epigenetic alterations causing structural modifications of gene-associated proteins, e.g., by methylation/acetylation/phosphorylation/, can be attributed to many causes including, for instance, promotion of heterochromatin formation [4], changes of protein function and protein–protein interactions [5].

Epigenetic changes, irrespective of their nature, are better considered as a specific subcategory of genetic mutations, since structural alterations induced by these changes, e.g., DNA-methylation/histone modifications, can result in functional disturbances, like suppression of transcription. Postulations, based on hypothetical mechanisms as well as on some

observations, that suggest the possibility of transfer of epigenetic changes from mother cells to daughter cells, or from mothers/fathers to their offspring, make this redefinition of epigenetic changes as a subcategory of genetic mutations a reasonable suggestion.

Though genetic defects induced by epigenetic changes in DNA structure are easily interpretable, genetic disturbances caused by corresponding changes in DNA-associated proteins, e.g., acetylation of histones, necessitate redefinition of some arbitrarily used terms in genetics, like gene silencing. It also points to the underestimated role played by DNA-associated proteins in causation of genetic disorders. The intimate and numerous spatial/temporal structural/functional relationships between the genome and the proteome make it quite unreasonable to explain pathogenesis of genetic disorders as consequences induced exclusively by mutational events of genes. Similarly, attributing pathogenetic mechanisms underlying the development of genetic diseases solely to these mutational events ignores the critical roles played by the different compartments of the proteome, comprising DNA-associated proteins/transcription factors-associated proteins/post-transcription modifications regulatory proteins/signal transduction regulatory networks/DNA methylating–demethylating enzymes/etc, in modulation of nearly all aspects of gene function starting with detection of the need for gene activation and ending with regulation of all post-translational modifications including structural modifications/targeting and trafficking of synthesized proteins as well as their localization to their final intracellular/inter-cellular or extracellular destinations.

Interpretation of significance of imprinting comprises many theories some of which are far away from being considered as scientific evidence-based assumptions. For instance, the conflict theory in placental mammals assumes that paternal alleles in pregnancy cannot detect or comprehend their relatedness with future paternal alleles in offspring of the same mother and function in a way leading to optimal extraction of maternal resources through promotion of fetal growth, hence the need for their partial/relative suppression via methylation/acetylation/histone modification to ensure normal development of the offspring. The theory also postulates that maternal alleles, on the other hand, depict their equal relatedness to future maternal alleles and try to conserve maternal resources for the future via restraining fetal growth, hence their demethylation and overexpression during development. This widely accepted theory, apart from being a philosophical point of view based on the core concept of pragmatism that has nothing to do with scientific thinking, assumes a wild-life barbarian behavior attitude of the human genome during development, a novelist postulation that could never be considered seriously in this regard.

4. DNA methylation/demethylation

DNA methylation may keep a specific microRNA profile that mediates, through regulation of gene expression, imprinting. An important function of genetic imprinting in protection and maintenance of integrity of the genome during development has been attributed to different epigenetic processes, including DNA methylation, which silence overexpression and activities of transposable elements (TEs), and recent advances in genome-wide profiling suggest that during reproduction DNA methylation patterns are at least partially transmitted or even enhanced in the next generation to ensure stable silencing of transposons [6]. However, elucidation of the critical role played by piwiRNA, subtypes of microRNAs composed of RNA-piwi protein complexes, in gene silencing, most specifically the silencing of transposons during development, by acting as antisense structures to transposon sequences [7] reveals the presence of other unique genomic regulatory mechanisms more palatable for obvious explanation than assuming ambiguous postulations like imprinting and transmission of epigenetic changes to daughter cells during development and, later on, in post-natal life.

5. Parthenogenesis

The occurrence of parthenogenesis in living organisms, where a female can reproduce asexually with her own genome leading to the development of a normal offspring, imposes the need for revealing and redefining the true nature and significance of imprinting. Parthenogenesis, whether it be facultative or obligate, has been detected and documented in approximately 70 species, including both **invertebrate** animal species (including nematodes, water fleas, some scorpions, aphids, some bees, some phasmida and parasitic wasps) and a few **vertebrate species** such as some fish, amphibians, reptiles and very rare birds [8]. Facultative parthenogenesis, where a female undergoes parthenogenesis if a male is absent from the habitat or if it is unable to produce viable offspring, has been documented in sharks, Komodo dragons and a variety of domesticated birds [9]. Likewise, imprinting is not essential for normal or early development in many species showing this phenomenon. For instance, development of viable and fertile gynogenic *Drosophila melanogaster* as well as androgenic flies has been reported by many workers [10,11]. Likewise, the finding of viable parthenogenic and androgenic zebra fish animals reveals that imprinting need not involve developmentally essential genes [12,13].

These findings oppose the current interpretations of the well-known facts regarding normal mammalian development which requires maternal and paternal contribution, and is attributed to imprinted genes. Experiments showing that maternal uniparental embryos (gynogenotes or parthogenotes) develop into tissues predominantly of embryonic origin with a failure of the extraembryonic lineages, whereas paternal uniparental embryos (androgenotes) develop into conceptuses derived of extraembryonic lineages, have been reasoned assuming that the absence or overexpression of imprinted genes exclusively expressed from either the maternal or paternal genome causes the developmental failure and that expression of imprinted gene is the main barrier to parthenogenetic development in mammals [14]. Though parthenogenesis may be looked at as a selective advantage in some endangering or stressful conditions, e.g., absence of males, its occurrence in lower species and its absence, or rarity, in higher species, directly contradicts the long-held allegations regarding evolution, where acquisition, rather than loss, of selective biological advantages constitutes the core concept of evolution.

Though the phenomenon of genetic imprinting in males might be, partially, interpretable based on genomic and proteomic differences imposed by differential regulation and/or expression of sex influenced/related/modified/regulated genes

on the X and Y chromosomes, no reasonable explanation exists, yet, for occurrence of imprinting in females. Postulations regarding epigenetic reprograming, a vague term used to denote preimplantation epigenetic modifications of parental genomes whereby active demethylation of the paternal genome within a few hours after fertilization and passive demethylation of the maternal genome by a replication-dependent mechanism after the two-cell embryo stage happen [15], need verification taking into consideration the marked complexity of the structural organization and functional capabilities of the human genome. A base line specific programed state of the genome during development has not been revealed, and use of the term reprograming within this context seems unjustified. In addition, the cycle of epigenetic processes including methylation/erasure of imprinting signals during development need not be exclusively considered as an absolute evidence of occurrence of imprinting, since it can be equally interpreted as being one genomic regulatory mechanism of temporal/spatial mass expression/suppression of sets of genes involved in particular functions during specific periods of development.

6. DNA methylation and protection of microRNAs

The elucidation of a possible role played by DNA methylation in protection of microRNAs from degradation triggered by uridylation in plants [16], probably, represents a major advancement in this regard because methylation and uridylation are conserved processes in small RNA pathways in plants and animals. This important key finding might, possibly, help in understanding the relation between DNA methylation and imprinting, at least in males. DNA methylation, per se, cannot function as a sex differentiating mechanism between male and female genomes. A more sensible postulation would assume a characteristic genome-determined or proteome-determined sex profile for this differentiation. A specific sex-differentiating transcriptome profile, as a link between assumed genome-based and proteome-based profiles, and also as a mechanism mediating functional correlations between both profiles, seems possible in this respect since it will reflect both qualitative and quantitative sex differences between males and females based on genomic differences. Though this postulation is applicable for XY male zygotes, it is not applicable for XX female zygotes, since neither qualitative nor quantitative differences are, apparently, detectable between both genomes.

7. Position-effect variegation (PEV)

The phenomenon of position-effect variegation (PEV) in plants, whereby silencing or inactivation of gene(s) results from abnormal juxtaposition with heterochromatin, results when a gene normally in euchromatin is juxtaposed with heterochromatin by rearrangement or transposition. When heterochromatin packaging spreads across the heterochromatin/euchromatin border, it causes transcriptional silencing in a stochastic pattern. In position-effect variegation, genes become silenced by heterochromatization. Genetic fine structure studies revealed unique dosage dependent effects where the evolutionary conserved histone, H3 lysine 9 methyltransferase SU(VAR)3–9, plays a central role in heterochromatic gene silencing [17]. The key role exerted by chromatin proteins in regulation of gene expression has been reported in Drosophila by [18] who postulated that both position effect variegation and Polycomb-dependent regulation of homeotic gene expression are phenomena in which genes are inactivated in a clonally inherited manner. In both processes inactivation involves proteins that interact with the chromosome at or close to the position of inactivated genes [19].

The phenomenon of position-effect variegation would, probably, have a critical role in explaining many molecular aspects of imprinting and the role(s) played by DNA-associated proteins in its initiation and/or regulation since it clearly points to the distinctive cooperative roles of the genome and the proteome in initiation and/or maintenance of imprinting.

A third key finding that reveals possible relationship between causation of position-effect variegation and subsets of RNA has been reported in the fruit fly, *D. melanogaster*, where mRNA level of a specific dominant transacting gene, Modifier of white or (Mow) gene, decreases in the presence of one dose of the Mow gene in larvae and adults, but the reduction is greater in females than males. A quantitative analysis of steady state transcript levels revealed that the Mow mRNA level, and two other functionally related genes, brown and scarlet, also exhibit a similar sexually dimorphic alteration in expression, mediated by Mow. In the mid-pupal stage, by contrast, the level of white and brown mRNA is increased by Mow. In addition, Mow acts as a weak suppressor of position effect variegation (PEV). These observations suggest a connection between dosage modulation of gene expression and suppression of position-effect variegation [20].

However, these findings suggest that genetic factors that modify position-effect variegation can alter the level of somatic expression of involved genes but do not establish or induce the genetic imprint. The chromatin structure, probably, plays an important role in maintenance of the imprint, but a separate mechanism may be responsible for its initiation. In other words, altered chromatin structure is involved in conserving the somatic memory of the imprint, but does not necessarily determine it, and initiation of imprinting may be initiated by different mechanisms under independent genetic control.

8. Role of chromatin in imprinting

The above findings point, clearly, to a central role played by specific subsets of the proteome, the chromatin structure comprising both euchromatin and heterochromatin, in regulation and/or maintenance of imprinting of certain components of the genome. More importantly, they might suggest a similar role played by specific subsets of the transcriptome, mRNA and microRNA, in modulation of certain stages of the process of imprinting. Additionally, they clarify the potential role of chromatin modification in epigenetic programing. Proper interpretation of occurrence of imprinting, specifically in females, necessitates, and awaits for, many hypothetical postulations. Some of these postulations might comprise, for instance, the need for elucidating a specific Y chromosome independent sex profile, because the biological functions of the Y chromosome, probably, far exceeds its role in sex determination. It might, also, include the need for revealing a differential profile of the transcriptome in both sexes. In males, due to the presence of two different sex chromosomes, there are two distinctive genomes, two distinctive transcriptomes and two distinctive proteomes, each derived from the germ cell participating in

fertilization, and attributing occurrence of imprinting to differential X–Y sex-dependent within this environment might be acceptable because of the major qualitative and quantitative differences between the genomic/transcriptomic/proteomic profiles of males and females imposed by the Y chromosome. However, despite the corresponding existence of two distinctive genomes, two distinctive transcriptomes and two distinctive proteomes each derived from the germ cell participating in fertilization in females, the absence of the Y chromosome dependent genomic/transcriptomic/proteomic profiles necessitates a different approach for interpretation of imprinting in females.

9. Post-fertilization genomic imprinting

A peculiar state of normal genomic imprinting is observed in immediate post-fertilization period and might help in disclosing some of the molecular mechanisms underlying imprinting. Following fertilization, the male pronucleus and the female pronucleus migrate toward each other, the pronuclei disintegrate and their chromosomes gather around a common metaphase plate. In mammals, the genome of the ovum shows maximum expressive activity immediately following fusion with the genome of the sperm which remains repressed for nearly the first few (4–5) post-fertilization days. This state of genomic imprinting, or genome-wide demethylation, of the sperm genome results from action of putative active demethylases in the oocyte cytoplasm on paternally derived sequences. Maternally derived sequences of the ovum are protected from this reaction [21].

This specific pattern of differential genomic imprinting in the post-fertilization period has no clear explanation and disturbances in this pattern of differential genomic expression can lead to arrest of development or aborted abnormal development at very early stages. However, skipping these drastic consequences of defective genomic imprinting, e.g., due to sub lethal quantitative threshold effect, in XX embryos may lead to maintaining this state of disturbed genomic imprinting all through development and can lead to establishment of a Y chromosome independent specific sex profile in females. Disturbed regulation of this profile, e.g., by defective chromatin modifications, defective regulation of transcription by microRNAs or defective DNA methylation/demethylation, might represent one pathogenetic mechanism that underlies the development of specific disease phenotypes caused by defective genetic imprinting.

New insights into the real nature and true significance of genetic imprinting are confronted by the vague and conflicting findings regarding most aspects of this mysterious biological phenomenon. Assumptions regarding the significance of genetic imprinting stemmed principally from its postulated role needed for avoiding parthenogenesis in most mammals. Apart from this essential biological function, all other functions attributed to imprinting can be, and are actually, mediated by other genetic regulatory mechanisms that do not hamper the genome or expose it to detrimental risks if they get disturbed by single mutational events as it is the case with imprinted genes, an observation which turns genetic imprinting into a selective disadvantage for cells or organisms subjected to its consequences.

Other enigmatic aspects of imprinting add more complexities to hypotheses aiming at disclosing its nature. For instance, analysis of data concerning the spawning migration of Pacific salmon from the open ocean to their correct coastal home area revealed a significant link between this enigmatic phenomenon and the geomagnetic field drift along this migration route and proposes an empirical evidence of existence of geomagnetic imprinting [22].

10. Mitochondrial genome (mtDNA) and imprinting

Since the presence of a Y chromosome in the sperm and of mitochondria in the ovum are the major genomic differences that differentiate between developing male and female offspring, speculations regarding potential roles of these two components in initiating and/or maintaining imprinting seem reasonable and cannot be ignored. A major obstacle in this respect returns to scanty understanding of the very little information available regarding the actual roles of paternal and maternal genetic/transcriptomic/proteomic components in regulation of embryogenesis. Paucity of information about the programmed balance between nuclear and mitochondrial genomes and the relative participation of each in regulating all structural and functional aspects of development, including imprinting, allows for a great deal of theoretical hypotheses and postulations most of which are in need of much experimental research.

A possible role attributed to mitochondrial genome in imprinting has not been considered seriously because of many observations. **First**, apparent absence of any appreciable role of sperm mitochondria in early stages of embryogenesis due to the very small number of sperm mitochondria, nearly 75 mitochondria, compared to nearly 100000 mitochondria present in the oocyte at fertilization. **Second**, inheritance of mitochondria exclusively through the oocyte as selective dissolution of mitochondria of the sperm by proteolysis happens in early embryonic development at the 4–8 cell stage [23]. **Third**, theoretical assumptions that consider exclusion of paternal mitochondria at very early stages of development an important process that probably serves to minimize lethal cytoplasmic gene competition and to prevent the inheritance of sperm mitochondrial DNA that has been subjected to degradation by free radicals during spermatogenesis [24]. **Fourth**, theoretical postulations assuming intolerance of organisms, including humans, of mitochondrial heteroplasmy which can cause progressive and lethal bioenergetic or neurological disease [25]. **Fifth**, presence of various lines of evidence indicating that mitochondria in the oogonia and the early embryo are quiescent and hence relatively unlikely to engender damaging reactive oxygen radicals since replication of mtDNA in the embryo does not start except after the first few days (4–6 days) after fertilization [26]. **Sixth**, absence of any mitochondrial genes that can be causally related to functional alterations implicated in epigenetic mutations and/or imprinting mechanisms. **Seventh**, absence of any mitochondrial genes that have mutual interactions with, or can affect, nuclear genes apart from mitochondrial genes participating with nuclear genes in mediating oxidative phosphorylation processes. **Eighth**, absence of any mitochondrial genes that have any roles in critical regulatory mechanisms responsible for maintaining genomic identity/integrity/stability including mechanisms of imprinting, e.g., chromatin modifications and DNA methylation.

Some of the abovementioned postulations rely on mere theoretical speculations that lack valid experimental proofing. For instance, interpreting exclusion of paternal mitochondria as a protective mechanism serving to hinder participation of heavily mutated sperm mtDNA in fertilization is shaky because the mitochondria possess efficient base and nucleotide excision repair pathways capable of repairing oxidative damage to mtDNA [27]. Additionally, this interpretation ignores the fact that the sperm nuclear genome is equally exposed to the same detrimental mutagenic events during spermatogenesis as well. Also, presumptions regarding the importance of avoiding dual parental inheritance of mitochondrial genome have neither apparent nor acceptable reasoning in view of the necessity of dual parental inheritance of the much larger and more diverse nuclear genome for normal development.

Similarly, assumptions regarding quiescence of mitochondrial genome in the oogonia and the early embryo cannot be accepted unless existence of alternative sources of energy needed during this critical stage of development, characterized by exceedingly active cellular metabolic activities and energy dependent and demanding processes like cell division and cell differentiation, is revealed.

The vague unsupported assertion that exclusion of parental mitochondria from participation in fertilization is a critical mechanism aiming at protecting the zygote from being colonized by heavily mutated mitDNA of the sperm has been, probably, postulated within the context of hypotheses trying to reveal the actual mechanisms that prevent dual parental inheritance of mitochondrial genome. This assumption has been postulated as a mechanism serving to avoid lethal genomic conflict among subservient yet essential organelles. These conflicts are attributed to the role of ultra-selfish genes in evolution of sex which, in turn, creates a series of new conflicts which may explain the existence of sexes and uniparental inheritance of cytoplasmic genes which sets up a new set of conflicts over the sex ratio which, in turn, may influence the evolution of sex determining systems, sex allocation systems and post-zygotic isolating mechanisms [28].

These assumptions, similar to novelistic hypotheses, cannot be taken seriously because fanciful ideas and imaginary concepts of conflicts/challenges/struggle between two parental genomes each comprised of innumerable diverse components including structural/functional/regulatory mechanisms that exert strict and persistent control over the organization and functional integration of endless, mostly still unrevealed, biosystems/biomolecules/cellular organelles during development, seem quite unrealistic simply because normal development and maintained existence depends entirely on accurate and programmed cooperation and complementation, not conflicts, between both genomes.

Evidence of occurrence of programmed imprinting of mitochondrial genome at fertilization and/or during early post-fertilization days of embryogenesis has been deduced from many observations including: strict down-regulation of replication of mtDNA from the fertilized oocyte through the preimplantation embryo, onset of mtDNA replication exclusively in cells of the trophectoderm at the blastocyst stage and restriction of mtDNA replication in cells of the inner cell mass until they receive signals to differentiate to specific cell types [29]. Additionally, observations suggesting possible roles played by mitochondria, as a key regulator of energy metabolism in the cell, in pathogenesis of Prader-Willi syndrome and revealing imprinting of many mitochondrial gene products, e.g., mitochondrial complexes IIIII enzymes, as evidenced by their differential expression in different organs and tissues of Prader-Willi syndrome patients, adds more support to hypotheses postulating contributory roles of mitDNA in mediation of genomic imprinting [30].

However, a possible role of mtDNA in imprinting based on these observations necessitates reconsideration of findings concerning marked dilution of the very few numbers of sperm mitochondria by the huge numbers of maternal mitochondria in the zygote which nullifies any roles of sperm mitDNA in regulating basic and critical post fertilization processes including imprinting. Mitochondrial fusion and fission are normal in oogenesis and embryogenesis [31], so rare fusion events involving sperm mitochondria may be possible. In most mammals microtubule formation catalysed by the sperm centriole serves to bring the male and female pronuclei into apposition and to form the first cleavage spindle apparatus [32]. One or more of these early-departing mitochondria might evade proteolysis by fusing with an oocyte mitochondrion to establish a heteroplasmic founder line [23]. Accordingly, the possibility of establishing a heteroplasmic mitochondrial population in early development with consequent occurrence of dual parental mitochondrial genomes and parallel expansion of both genomes during development cannot be excluded.

Though resorting to philosophical presumptions and logical concepts is of prime importance in configuring the starting framework of search and reasoning, reliance on this attitude must be considered very cautiously in fields of knowledge based exclusively on facts deduced from accurate experimentation and strict conformable validation. This applies most for biological sciences dealing with a great deal of organisms sharing basic features imposed by the common building units of life but widely differing in most other structural and functional aspects as revealed by the boundless spectrum of inter species differences. Application of experimental observations or analytical findings of life activities in some organisms to humans should be considered cautiously even for common shared aspects due to the uniqueness of human race among living creatures and because of some of the illogic theories resorted to in this respect like evolution, genomic conflicts and the like. In addition, it must be kept in mind that exceptional events in biology probably represent still unexplained or unrecognized indispensable basic processes that happen at much lower rates compared to classic, regular non-exceptional processes.

11. Conclusions

The previous aforementioned findings regarding the different roles of chromatin modifications and microRNA in initiating and/or maintaining imprinting, probably, under the control of the imprinting regulatory centers allow us to draw many speculations about the true nature of imprinting and the mechanisms underlying its establishment. However, in spite of the considerable wealth of information regarding this biological process, the major task within this context, represented by the need to identify the real mechanisms capable of recognizing, and differentiating between, male and female genomes at fertilization remains totally unsolved. Obviously, this critical, development determinant function is, and must be, mediated by master higher order genes responsible for defining and

maintaining genomic identity of the developing offspring. Attributing this pivotal function to DNA methylation or to chromatin modifications is an unjustified over simplification. Attempts at hypothesizing or postulating existence of sex differentiating mechanisms controlled by global and comprehensive genomic regulatory systems have to take into consideration the roles of microRNA species and chromatin compartments in this regard. The biological concept of life summarized in the central dogma of molecular biology which defines the sequential priority of biosystems and biomolecules in controlling life activities within cells in a specific defined pattern, i.e., genome–transcriptome–proteome, compels us to impart to specific transcriptome compartments, e.g., microRNA, regulatory roles that control the functions of specific compartments of the proteome, e.g., chromatin and heterochromatin modifications. In view of these biological facts it seems quite reasonable to formulate a hypothetical sequence of biological processes starting with higher order master genes responsible for maintaining species-specific genomic identity through transcription of specific transcriptomic structural/regulatory sequences which mediate and control the ordered synthesis of characteristic proteomic profiles. In view of the absence of any clues as to the postulated roles of master genes in this respect, it might be plausible to attribute the establishment of imprinting to specific regulatory sequences of the transcriptome, notably subsets of microRNA.

The relationship between siRNA subsets and pyknons allows us to extend these hypothetical postulations further to assume a role played by pyknons, as a link between coding and non-coding sequences of DNA, in regulation of specific transcriptome compartments involved in defining specific proteome profiles, including mechanisms responsible for mediation and maintenance of imprinting. The peculiar characteristics of human pyknons impose their inclusion within any assumptions concerning the role of the genome in defining the transcriptome. Pyknons might represent the genomic compartment underlying the establishment of imprinting, in addition to many other important biological functions. However, these postulations assume a Y chromosome independent sex-specific imprint profile imparted separately by the specific pyknon constitution to male and female genomes. The specific pattern of pyknons would result in a sex-specific sequence profile of microRNA transcribed by pyknons. DNA methylation/demethylation would, then, exert its regulatory epigenetic effects through either suppression or enhancement of microRNA degradation leading to a sex-specific imprinting pattern.

Imprinting probably represents one, among many, species-specific innate genomic mechanisms responsible for the establishment of the cardinal fundamental aspects of genomic identity including the formulation of characteristic species-specific profiles of the transcriptome and the proteome and the development of sexually distinctive phenotypes. Though current observations point to the possible roles of microRNA and chromatin components in mediation and maintenance of imprinting processes through biochemical molecules/networks, imprinting might be mediated by other different unexpected mechanisms apart from the currently identified conventional biochemical mechanisms, e.g., the geomagnetic imprinting postulation.

Although these assumptions might solve some of the conflicts and uncertainty regarding the underlying regulatory mechanisms leading to establishment of genetic imprinting and the pathogenetic alterations leading to development of genetic diseases caused by imprinting defects, they remain as mere theoretical postulations that need much research for validation.

Conflict of interest

The author declares no conflict of interest.

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