American Journal of Neuroprotection and Neuroregeneration
Vol. 1, 1–7, 2009

REVIEW

Epigenetic Processes as Evolutionary Advanced Molecular Mechanisms to Cope with the Continuous Interaction Between DNA and the Environment

Silvia G. Ratti and Edgardo O. Alvarez*

Área de Farmacología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Laboratorio de Neuropsicofarmacología Experimental, IMBECU-CONICET, Mendoza, Argentina

The traditional concept that the phenotype of an organism is the resultant of the genetic code and the influence from the environment has recently acquired implications not previously suspected. Evidence has been accumulating showing that the once thought "static" interaction between gene coded information and its final gene expression is a complex dynamic process that continually updates in time. These processes are known as epigenetic mechanisms, where a heritable change in a gene expression is produced without modifications in the primary DNA structure. Diverse functional strategies have evolved for dealing with the existence of double content of DNA information or lack of allele gene information, such as the case of sexual chromosomes represented by the unequal X and Y partners. Processes such as gene dosage compensation and gene imprinting and the functional role of metastable epialleles are just a few of the many complex expressions of epigenetic mechanisms. In this review, these processes are discussed under the perspective of evolution, intending to show that they are "flexible" solutions to cover problems arising from the continuous interaction of DNA and the environment. Finally, these concepts are applied to HSR gene expression, where evidence from a study of our laboratory working with children of the province of La Rioja (Argentina) has showed that environmental factors can apparently modify the phenotype-attributable expression to this gene.

Keywords: Epigenesis, DNA Methylation, Retrotransposons, Gene Dose Compensation, Imprinting, Trace Elements.

CONTENTS

1. INTRODUCTION

It has traditionally established that the phenotype is equivalent to the sum of the genotype and the environment. Waddington proposed for the first time the term "*epigenesis*," which is defined as the interaction of genes and the environment, leading to the expression of the phenotype.[35]

But it was not until the 1990s when epigenesis is defined as a heritable change in the expression of a gene without modifying the primary DNA structure.[38] Thus, epigenetic processes represent the bridge between the genotype and the phenotype,[8] transferring outer signals to the genome, which will lead to a change in the gene expression. In spite of what is known about the existence of a developmental "program" inside the genetic material, this concept by itself does not explain the beginning of the differentiation leading to the cell specialization. It is reasonable to visualize that the existence of mechanisms acting on the DNA to control the execution of the program will permit to select genes to be or not to be activated in each cell at any moment. If this were not happening, all cells of an organism bearing the same genetic material will evolve in the same way. Also it is possible to think about mechanisms controlling the transmission of the patterns of activation and inactivation from one line of cells to the next ones. This transmission is necessary to keep the daughter cells

*Author to whom correspondence should be addressed.

maintaining and evolving the program of cell specialization. Processes controlling the execution of the developing program also are called epigenetics.[11, 12] There is evidence that the epigenetic changes are inherited not only during mitosis but also during transmission between generations.[23]

2. GENE DOSAGE COMPENSATION

A determined epigenetic mechanism arises at the same time as the evolution of species. In all those organisms where females and males differ in the number of sexual X chromosomes, processes have evolved eliminating the difference in the gene dosage linked to this chromosome. Thus, the products of these genes are expressed equivalently in females and males. This mechanism is called gene dosage compensation, and it has occurred via different mechanisms during the evolution of the species.[22] In the *Drosophila melanogaster*, both X chromosomes are active in the female. The dosage compensation is performed by hypertranscription of the genes of the single X chromosome present in males (Fig. 1). The sex determination and the dosage compensation is under the control of the gene Sex-lethal (Sxl). Modulation of the activity of this gene is determined by the ratio of the number of X chromosomes to the number of the autosome chromosomes (ratio X:A).[11] The Sxl gene operates by means of two sets of different genes: those acting on the sex determination and those acting on the dosage compensation. Mutations of the sex determination genes do not affect those of the dosage compensation, and mutations on the dosage compensation genes do not affect those of the sex determination. Genes responsible of the dosage compensation have been identified: the male-specific lethal genes (msl+s). Mutations affecting those genes with loss of their function cause specific male lethality, because of specific hypotranscription of its single X chromosome; while female viability is not affected, since transcription from any of the two X chromosomes are not altered. All four involved genes have been cloned: mle, msl-1, msl-3, and msl-2. The mle gene codes a protein containing characteristic domains of the family of the helicases. Gene msl-1 codes a protein containing an N-terminal domain typical of those proteins involved in the transcription and structure of the chromatin. Gene msl-2 codes for a protein having "zinc fingers" and a domain of the type of metallocyanines. This protein also has a group of amino acids, in zwitterionionic form, that would be domains possibly involved in the protein–protein interaction. The msl proteins are associated to multiple sites in the polythenic chromosome in males, but not to the same sites in the polythenic chromosome in females. The acetylated H4 histone in lysine at position 16 (H4Ac16) presents the same association pattern to the polythenic chromosomes as the msl proteins. This evidence, in addition to the observation that mutations in the msl+s do not give additive effects, supports the idea that proteins msl and histone H4Ac16 form multimeric complexes specific for the interaction with the X chromosome in males. Thus, this chromosome acquires a three-dimensional structure, permitting greater access to the transcription machinery and thus a double rate of transcription from the male DNA with respect to each of the two female chromosomes. Genes mle, msl-1, and msl-3

Silvia G. Ratti

Silvia G. Ratti, graduated as MD in the School of Medicine, Universidad de Buenos Aires, Argentina in 1993. After completing her residence in the "José M. Penna" Hospital, and "Carlos G. Durand" Hospital, she got the specialist degrees of Medical Clinician and Medical Geneticist. In 1998 she got the Master degree in Molecular Engineering and Molecular Biology from the Favaloro University, and later in 2005, Master of Clinical Research from the Faculty of Medical Sciences, Universidad Nacional de Cuyo. Her main research interest is the regulation of epigenetic mechanisms of imprinted genes related to cognition functions in the brain. Actually, she has joined as research member to the Laboratorio de Neuropsicofarmacología Experimental, Área de Farmacología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, and IMBECU-CONICET, Mendoza, Argentina.

Edgardo O. Alvarez

Edgardo O. Álvarez, graduated in Physiology from the Faculty of Science, Universidad Austral de Chile, Valdivia, Chile in 1976. Later he moved to the Universidad Nacional de Cuyo, Mendoza, Argentina. He got the position of Assistant Professor in the Faculty of Medical Sciences, and Independent Research Fellow from CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina). Actually he is Head of the Laboratorio de Neuropsicofarmacología Experimental, Área de Farmacología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo and IMBECU-CONICET. The main research interests are the brain mechanisms controlling learning, memory, motivation, emotionality and brain function laterality.

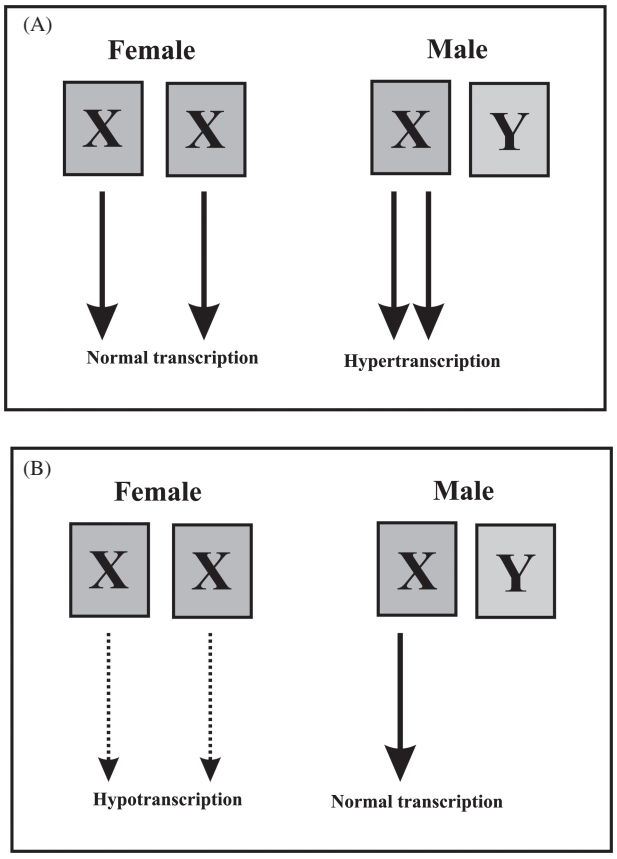

Fig. 1. Some observed mechanisms of the gene dosage compensation mechanism in living systems. In the case of the *Drosophila melanogaster* (A), genes of both X chromosomes are expressed equally in the female. In order to compensate gene dosage, genes in the X chromosome in the male are hypertranscribed. In the case of *Caeonorhabditis elegans*, genes in the X chromosome of females are hypotranscribed (B).

are expressed equally in female and male organisms, but msl proteins are functional only in males. In females, protein Sxl prevents the functions of the msl complex. These findings suggest that the msl-2 gene is controlled directly by the Sxl gene. Thus, in females there is not msl-2 protein, while in males the active msl-2 protein is possible because of the absence of the Sxl protein. Protein msl-2 gives functional specificity to the complex msl in order to be active only in males.[9]

In *Caenorhabditis elegans* gene dose compensation is performed in females by hypotranscription from its both X chromosomes[28] (Fig. 1). As *Drosophila melanogaster*, sex development and dosage compensation in *Caenorhabditis elegans* is determined by the initial genetic signal X:A. This signal determines the activity state of the sdc genes, which control the sexual determination and the dosage compensation genes. The molecular analysis of the sdc-3 gene shows that the protein sdc-3 has two different domains controlling dosage compensation and sexual determination. The *dumpy* genes (dpy) control the dosage compensation in *Caenorhabditis elegans*. Mutations affecting the function of these genes determine the specific lethality of the hermaphrodites, as a consequence of producing a hypertranscription of the X chromosome, while these same mutations have no effect on the viability of males, since the transcription of the single X chromosome is not affected. Gene dpy-27 has been cloned, and the product of this gene posses a very high homology with proteins important to the assembly and structural maintaining of chromosomes of *Xenopus*, and also to the chromosome segregation of the yeast.

In mammals, dosage compensation is produced by inactivation of one of the X chromosomes from the female.[22] There exists a dissociation of processes leading to sexual determination and those of dosage compensation. The sex determination is based on the presence of the Y chromosome and not on the X:A signal. Inactivation of female embryo is produced on a random basis, while in the extraembryo tissues the parental chromosome is always inactivated. The gene Xist has been identified as the inductor of the inactivation of the X chromosome carrying the gene (*cis*-inactivation effect). One model explaining the inactivation effect proposes that the specific transcription factor of the Xist gene should be in limited amounts, determining that only one of the X chromosomes can be inactivated. Gene Xist from the mother X chromosome should be regulated by genomic imprinting during the meiosis process in the female. This imprinting occurs by methylation mechanisms. When inactivation of the X chromosome in the extraembryo tissues is produced, gene Xist cannot be expressed because of the imprinting. Thus, the transcription factor only could activate the parental X chromosome, which will determine the selective inactivation of this chromosome. As development is progressing, the imprinting of the mother X chromosome begins to disappear. Thus, at the moment of the inactivation of the X chromosomes, both Xist genes, the one coming from the mother and the other one coming from the father, have the same chance to be activated by the transcription factor. However, the limited amount of this factor should be enough to trigger only one Xist gene and to inactivate that X chromosome bearing this gene. The Xist gene codifies RNA that joins to the X chromosome, acting as signals to proteins binding to produce a change in the chromatin leading to the inactivation. Later, DNA methylation remains in the inactivation state.

3. MECHANISM OF GENOMIC IMPRINTING

It is well-known that the genomic imprinting mechanism is defined as the reprogramming of the ovum and sperm genome to perform equal activation, unequal activation, or inactivation of certain genes, according to its parental origin.[27] Even though in mammals the constitution of the genetic endowment is identical, the functionality of the imprinted genes is not equivalent. Nevertheless, their

contribution to a developing organism can be complementary. The imprinted genes express functionality as hemicygotes due to silencing of their corresponding alleles. It is speculated that in mammals the imprinting mechanism arises from the impossible parthenogenetic occurrence, since it is necessary the genetic contribution be from both parents in order to complete normal development.[33] This type of transcriptional regulation gives an evolutive advantage to the individual genetic contributions to the next generations. In this way, both contributions are vital by themselves and at the same time crucial in their combination in order to complete development of the new organism. Interesting suggestions have been proposed regarding why this mechanism arises so late in evolution and affects the most advanced species. These theories emphasize the "conflict of interest" of parents to their progeny according to the different "genetic endowment" that each of the parents gives to their infants.[2] Experiments performed on chimaeras rats show the differential contribution of the parthenogenetic and androgenetic mechanisms to the descendants in the brain cells.[1, 14] Parthenogenetic endowments are expressed mainly in the frontal cortex, corpus striatum, and hippocampus, while the androgenetic endowment is expressed in the hypothalamus and the preoptic area. It has been proposed that the parthenogenetic distribution should be linked to the great biologic investment that the mother is performing during each gestation in order to selectively transfer genes that should contribute to support the qualitative properties to be expressed as higher brain functions. For example, in nonhuman primates the maternal expression of imprinted genes should give the descendants appropriate social cognition ability, and these genes are expressed in the frontal cortex.[13, 14] In the case of the androgenote, the genetic contribution appears to be linked to the capacity of males to fertilize many females at the same time, favoring the perpetuation of their genes by somatic growth, since the expression of these genes appears to occur in some brain areas such as the hypothalamus.[2, 20, 37] These interpretations support at a sophisticated level the concept that gene redistribution from sexual reproduction gives the species the capacity to survive in an environment that changes in unpredictably.

4. METASTABLE EPIALLELES

Programming of imprinted genes occurs during the gametogenesis and early developmental stages, determining an epigenetic "state" that remains constant throughout life. In order to differentiate this programming from epigenetic states that are labile, it has been defined as a metastable epiallele.[24] This refers to changes in the epigenetic state not depending on parental origin, like the mechanism of genetic imprinting causing the variability of the expression of certain genes in the absence of genetic heterogeneity. In order to establish changes in the epigenetic state, metastable epialleles use several resources that in the end produce hypermethylation or hypomethylation conditioning the expression of genes. However, other mechanisms are present. The epigenetic modifications in these alleles appear to be associated to retroviral elements.[34] There is evidence that in murine cells the intracisternal particles A and L1 elements provide a high methylation state with transcriptional inactivity of genes A^{vy} and $Axin^{fu}$.[16, 36] It is estimated that about 40% of the mammal genome is composed of retroelements.[10] Classically, active transposons have been associated to mutations on genes codifying proteins, slicing points in chromosomes, recombinations, and rearrangements in the genome. Also, transposons were able to influence neighbor genes, modifying the splicing, the polyadenilation patterns, or enhancers and promoter's functioning.[7] It is thought that the mammal genome defends itself to prevent possible damage caused by these transposonic elements by epigenetic silencing.[30] It has been proposed that this epigenetic silencing is performed by the activation of a series of elements that silence the transposon units by means of repressor RNA, histone modifications, DNA methylation, or alterations in the condensing and packaging of chromatin. Some observations, however, suggest that the transposon elements may perform beneficial functions to the genome. For example, constitutive heterochromatin is found in the telomeres and the centrosomes of chromosomes. Both elements in daughter cells are essential to duplication and reorganization cycles of the chromosome. The constitutive heterochromatin does not have transposons elements, but the centromere is surrounded of a pericentromeric region rich in transposons elements and retrotransposons.[29] It is speculated that because of these transposon units and the corresponding epigenetic silencing mechanism, the complete epigenetic context supporting the appropriate functioning of the centromere is given.[18] On the other hand, telomeres are composed of repetition lines assembled by a reverse transcriptase using RNA as template, which is the same mechanism used by retrotransposons to integrate them into the genome.[21] The subtelomeric regions nearby to the "on line repetitions" of the telomeres are made also by large fragments of transposonic elements.

5. PERSPECTIVE FROM ADAPTATION AND EVOLUTION: WHERE IS THE INFORMATION COMING FROM? WHO IS THE MESSENGER?

Initially, transposons were considered parasitic elements by many investigators. Then they were associated with alterations thought to be deleterious to the genome.[7] However, it has been found that transposons could be important to certain epigenetic mechanisms contributing to the normal regulation of genomic expression. It is known that survival of living systems depends on their adaptation to

changes in the environment. There is a growing belief that DNA is really a flexible molecule, using external elements to modulate its own functioning according to the modifications of the environment. The strongest support for this idea came from a simple and elegant experiment where, by means of a plasmid, a gene was inserted into a bacterium, and its functioning states (alternative, active, and nonactive) were regulated by environmental modifications of metabolism and temperature.[31] Classical explanations about variation in evolution processes have taken into account mutation of the genetic material, where natural selection favored those mutations that somehow improved adaptation of the organism to the environment. It is reasonable to think that mutations in some genes can give slow adaptation responses and in others they can give fast adaptation responses. One of the most strategic organs in a living system sensitive to these changes is the brain. However, it can be argued that relying only on mutation mechanisms could represent a very high risk to the system because the efficiency of those genes codifying, for example, for the rationalizing abilities in humans or learning responses in mammals might be seriously affected by adverse localized mutations. One possible way to preserve all beneficial effects gained by "appropriate mutations" in the system should be to improve mechanisms that leave the primary structure of DNA intact and activate or silence constitutive genes according to external signals to the genome, i.e., the epigenetic mechanisms. As mentioned earlier, the epigenetic mechanisms also has evolved. A good example, not considering pleiotropy, is the establishment of genomic imprinting in mammals. Most of the imprinted genes codify to key elements in the central nervous system. Thus, two complementary genomes are essential to the normal nervous system development and the absence or alterations in any one of them provokes dramatic deleterious changes to the whole system. In order "to explore" what is changing in the environment, the genome needs an "out-to-in-messenger," a carrier transporting in the external information by cytosol or nuclear receptor interactions activating or suppressing transcription factors. Thus, transposons that once were interpreted as "parasite elements" or deleterious factors to the DNA might be simply the "messengers" serving the genome and contributing the genome's power to adapt to changes in the environment (Fig. 2). It is possible to speculate that silencing mechanisms, such as acetylating or methylation processes, could have been defense strategies of the genome to the assumed invasion of the retrotransposons. In time, a joint adaptation could be evolved such as that found in the gene compensation dose mechanism.[17] Epigenetic mechanisms experienced evolution at the same time as the evolution of species.

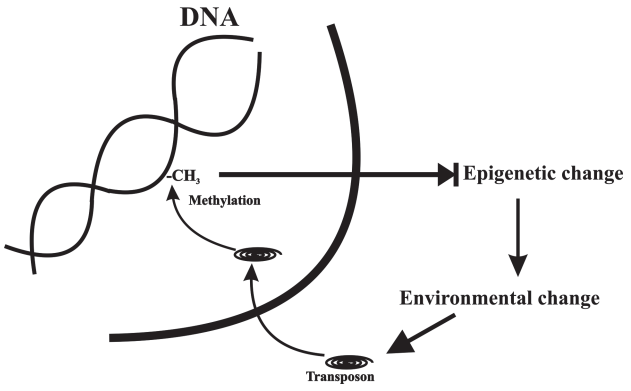

Fig. 2. Hypothetical mechanism whereby DNA may generate an epigenetic response after an inducing change is produced in the environment. It is assumed that changes in the environment are linked to transposons that by an unknown mechanism carry this information to the DNA molecule. Methylation processes inactivate expression of imprinted genes, generating a response that constitutes an epigenetic change.

6. THE EPIGENETIC PROCESSES IN ACTION: ONE EXPERIMENTAL MODEL

Considering that DNA in living systems can actively interact with its surroundings by epigenetic mechanisms, our laboratory was interested in examining possible candidates as epigenetic factors present in the environment. It is known that wherever man can live, he will always interact with an environment of some determined geological characteristics. Trace elements are present in soil and water around the world. It is known that when trace elements are incorporated into the organism in considerable amounts, important changes are produced leading to cell destruction and death. These are the toxic effects of elements and are the subject of toxicology. However, it has been determined that some of these trace elements in lower amounts in the organism serve important intracellular processes.[3, 15, 19, 32] These biological aspects of earth's elements and cellular function interactions are the subject of medical geology.[26] Our laboratory is interested in studying the HSR (Hand Skill Relative, OMIN 139900) gene. This gene has been proposed to be involved in hand skills, cognitive abilities related to hand writing and reading, and brain asymmetry.[5] What makes this gene particularly interesting is its easy phenotypic characterization, which was found to be imprinted.[4] On studying the phenotypic expression of the HSR gene in children coming from two regions of different geological characteristics, striking differences in the phenotypic expression were found.[25] The geographical region giving the anomalous HSR expression was that region where metal and nonmetal mine locations were abundant,[25] while in the region where these mines are absent, the HSR expression did not differ from that reported in the general population.[25] It is interesting to note that soil analysis of trace elements in some the region where the HSR was found modified showed concentration levels significantly higher than expected.[6] It can be speculated that some of the trace elements might be acting as epigenetic factors, inducing methylation processes

and thereby modifying gene expression. By examination of the methylation patterns of the DNA of children and comparison of those coming from the trace element rich zone with those of the control region, preliminary evidence suggests that there is an alteration in the DNA methylation patterns[39] (Fig. 3). However, further research will be necessary in order to establish a causal link between trace elements and the modified methylation patterns. Furthermore, some important questions about how a putative trace element can exert a possible direct action on the DNA molecule or an indirect action by transposons on DNA still need to be solved. Experiments are under way in our laboratory addressed to clarify these points, and it is hoped that with these new data it will be possible to understand part of the molecular mechanism whereby the genome is adapting to its environment.

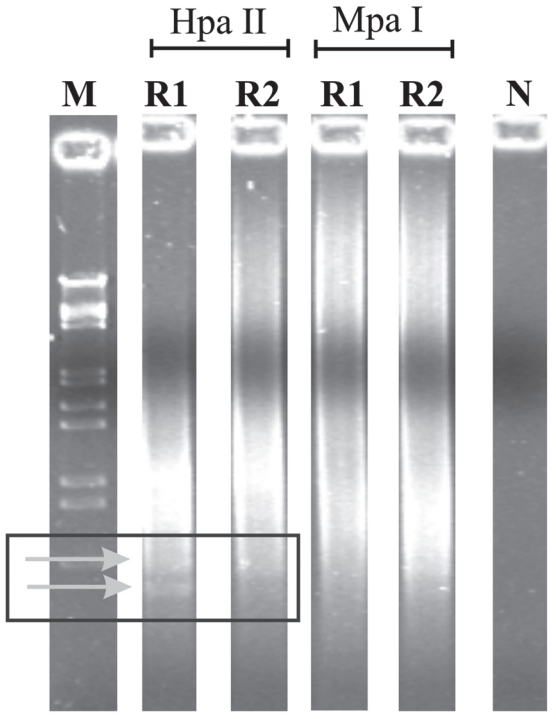

Fig. 3. Preliminary evidence showing that in the La Rioja study (24), the modified phenotypic expression of the HSR gene found in region 2 might have a molecular basis. The figure shows a representative electrophoresis pattern of DNA of children coming from region 1 and region 2. DNA was extracted from peripheral blood and subjected to incubation with HpaII and MpaI restriction enzymes. These enzymes can discriminate the methylated sequence 5′-CCGG-3′ in the DNA molecule. HpaII can cleave the nucleotide sequence when the bases are not methylated; while MpaI cleaves the nucleotide sequence whether or not bases are methylated. After incubation, electrophoretic runs can discriminate different fragments where comparison of bands provides information about methylation in the original DNA molecule. M = molecular marker; $R1$ = DNA from child of region 1; $R2$ = DNA from child of region 2; N = negative control. Comparison between DNA fragments of $R1$ and $R2$ shows that in $R1$ there are nonmethylated zones, which in $R2$ appear as methylated zones (arrows).

Acknowledgments: Experimental work of this article has been supported by grants from Instituto de Ciencias de la Salud, H. Barceló, sede La Rioja, and SeCyT-P, Universidad Nacional de Cuyo, Mendoza, Argentina.

References and Notes

1. N. D. Allen, K. Logan, G. Lally, D. J. Drage, M. L. Norris, and E. B. Keverne, *Proc. National Academy of Sciences (USA)* 92, 10782 **(1995)**.
2. M. Constancia, G. Kelsey, and W. Reik, *Nature* 432, 53 **(2004)**.
3. J. Chen and M. J. Berry, *J. Neurochem.* 86, 1 **(2003)**.
4. C. F. Francks, S. Maegawa, E. Z. McAuley, A. J. Richardson, J. E. Stein, M. Oshimura, and A. P. Monaco, Novel imprinted locus on chromosome 2p12 associated with relative hand skill in humans, Abstract. *XIIth World Congress of Psychiatric Genetics*, Dublin, Ireland, October **(2004)**.
5. C. F. Francks, L. E. DeLisi, S. E. Fischer, S. H. Laval, J. E. Rue, J. F. Stein, and A. P. Monaco, *Am. J. Human Genetics* 72, 499 **(2003)**.
6. J. L. Fernández-Turiel, A. López-Soler, J. F. Llorens, and X. Querol, *Environment International* 21, 807 **(1995)**.
7. L. Girard and M. Freeling, *Developmental Genetics* 25, 291 **(1999)**.
8. A. Goldberg, D. Allis, and E. Bernstein, *Cell* 128, 635 **(2007)**.
9. B. Granadino, L. Penalva, M. Green, J. Valcárcel, and L. Sánchez, *Proc. National Academy of Sciences* 94, 7343 **(1997)**.
10. J. B. Hollick, J. E. Dorweiler, and V. L. Chandler, *Trends in Genetics* 13, 302 **(1997)**.
11. R. Holliday, DNA methylation in eukaryotes: 20 years on, Epigenetics Mechanisms of Gene Regulation, edited by V. E. A. Russo, R. A. Martienssen, and A. D. Riggs, Cold Spring Harbor Laboratory Press, New York **(1996)**.
12. R. Holliday and T. Ho, *Methods* 27, 179 **(2002)**.
13. E. B. Kevene, F. L. Martel, and C. M. Nevison, *Proc. Biological Sciences* 263, 689 **(1996)**.
14. E. B. Keverne, R. Fundele, M. Narasimha, S. C. Barton, and M. A. Surani, *Brain Research* 92, 91 **(1996)**.
15. A. V. Kudrin, *J. Trace Elements Medical Biology* 14, 129 **(2000)**.
16. E. L. Kuff and K. K. Leuder, *Advances in Cancer Research* 51, 183 **(1988)**.
17. L. V. Matyunina, N. J. Bowen, and J. F. McDonald, *BMC Molecular Biology* 9, 55 **(2008)**, http://www.biomedcentral.com/1471-2199-9-55.
18. B. P. May, Z. B. Lippman, Y. Fang, D. L. Spector, and R. A. Martienssen, *PloS Genetics* 1, e79 **(2005)**.
19. J. H. Mitchell, F. Nicol, G. J. Beckett, and J. R. Arthur, *J. Molecular Endocrinology* 20, 203 **(1998)**.
20. T. H. Moore and D. Haig, *Trends in Genetics* 7, 45 **(1991)**.
21. T. M. Nakamura, G. B. Morin, K. B. Chapman, S. L. Weinrich, W. H. Andrews, J. Lingner, C. B. Harley, and T. R. Cech, *Science* 277, 955 **(1997)**.
22. A. Pannuti and J. Lucchesi, *Current Opinion in Genetic and Development* 10, 644 **(2000)**.
23. V. K. Rakyan, M. E. Blewitt, R. Druker, J. I. Preis, and E. Whitelaw, *Proc. National Academy of Sciences* 100, 2538 **(2003)**.
24. V. K. Rakyan, M. E. Blewitt, R. Druker, J. I. Preis, and E. Whitelaw, *Trends in Genetics* 18, 348 **(2002)**.
25. S. G. Ratti, P. Cordoba, S. Rearte, and E. O. Alvarez, *Int. J. Neuroprotec. Neuroregen.* 4, 52 **(2007)**.
26. S. Ratti and E. O. Álvarez, *Revista Médica Universitaria* 4, 1 **(2008)**, http://revista.medicina.edu.ar/vol04_01/index.php.
27. W. Reik and J. Walter, *Nat. Rev. Genet.* 2, 21 **(2001)**.
28. M. Reuben and L. Rueyling, *Dev. Biol.* 245, 71 **(2002)**.
29. M. G. Schueler and B. A. Sullivan, *Annual Review of Genomic Human Genetics* 7, 301 **(2006)**.
30. R. Slotkin and R. Martienssen, *Nat. Rev.* 8, 272 **(2007)**.

31. R. N. Tchuraev, I. V. Stupak, T. S. Tropynina, and E. E. Stupak, *FEBS Lett.* 486, 200 **(2000)**.
32. A. D. Toews, E. B. Roe, J. F. Goodrum, T. W. Boulding, J. Weaver, N. D. Goines, and P. Morell, *Molecular Brain Research* 49, 113 **(1997)**.
33. S. Varmuza and M. Mann, *Trends in Genetics* 10, 118 **(1994)**.
34. T. J. Vasicek, L. Zeng, X. J. Guan, T. Zhang, F. Constantini, and S. Tilghman, *Genetics* 147, 777 **(1997)**.
35. C. Waddington, Organisers and Genes, Cambridge University Press, Cambridge, UK **(1940)**.
36. C. P. Walsh, *Nature Genetics* 20, 116 **(1998)**.
37. L. S. Wilkinson, W. Davies, and A. R. Isles, *Nat. Rev. Neurosci.* 8, 832 **(2007)**.
38. A. P. Wolffe and M. A. Matzke, *Science* 286, 481 **(1999)**.
39. Ratti et al., unpublished results.

Received: 15 February 2009. Accepted: 19 May 2009.