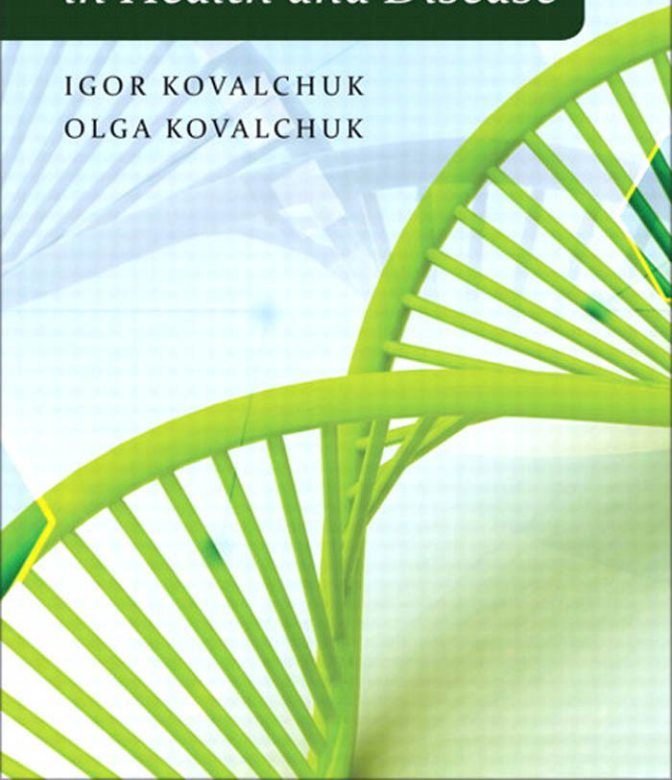
EPIGENETICS

in Health and Disease

IGOR KOVALCHUK
OLGA KOVALCHUK



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Dedicated to Anna.

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1

Historical perspective

Genetics can be broadly defined as the science studying the mechanisms of inheritance in general and genes in particular. *Epigenetics* can be, in part, defined as the branch of biology dealing with the mechanisms of inheritance. In contrast to genetics, epigenetics involves the control of gene expression that is not accompanied by any changes in DNA sequence. Epigenetics deals with the mechanisms of heredity which do not involve modifications of DNA sequence and are reversible in nature.

Success of a certain population depends on the fine balance between the ability to retain a given genotype in the stable environment and the ability to evolve by modification in response to substantial environmental changes. Changes in the genome can be dual in nature; they might deal with stable physical changes in DNA sequence leading to mutations and reversible chemical modifications of nucleotides or chromatin structure leading to epimutations. Mutations are the basis of genetic changes.

This book introduces you to the concept of epigenetics and epigenetic regulation. The book discusses processes of evolution in light of current understanding of the role of epigenetics and describes the role of epigenetic regulations in the growth and development of somatic cells, tissue differentiation, and the maintenance of epigenetic states in various cells of the same organisms. Furthermore, the book provides an introduction to an in-depth understanding of the role of epigenetics in the mechanisms of inheritance and interaction with the environment. The chapters also describe the role of epigenetics in health and disease. Finally, the book introduces you to the

concepts of silencing, co-suppression, and paramutations, and discusses the role of epigenetics in these processes.

This book is aimed primarily at students beginning to study epigenetics, whether at the undergraduate or graduate level. It may also be essential reading for research scientists in the field of epigenetics, genome stability, stress tolerance and adaptation, transgeneration effects, genome evolution, and other related fields, as well as anyone who simply wishes to know more about the field of epigenetics.

The mechanisms of environmental influences on the phenotypic appearance of organisms and inheritance were developed nearly two centuries ago and represented a prominent part of the descriptive work performed by Jean-Baptiste Lamarck and Charles Darwin. Although their ideas were often viewed as too preliminary and naïve, it was those ideas that laid a solid and important foundation for the development of the field of epigenetics. Epigenetics has a lot to do with an organism's interaction with the environment; therefore, it is important to review how our understanding of the interactions between the organism's genome, surroundings, and phenotype has developed over time.

The French biologist Jean-Baptiste Lamarck (1744–1829), who is credited with the first use of the word "biology," was the first scientist who proposed a theory of evolution. He used the term *transformation* rather than *evolution* to suggest that organisms change and transform as the result of "a new need that continues to make itself felt." His first reference to evolution as a process of less complex species becoming more complex appeared in 1800 in his Floreal lecture. Within next 20 years, Lamarck published three important works (*Recherches sur l'organisation des corps vivants*, 1802; *Philosophie Zoologique*, 1809; *Histoire naturelle des animaux sans vertèbres* (in seven volumes, 1815–1822) in which he developed his ideas of evolution and formulated the laws that described evolution as a process. Lamarck writes:

> **Law 1:** Life, by its own forces, continually tends to increase the volume of every body which possesses it and to enlarge the size of its parts up to a limit which it brings about itself. **Law 2:** The production of a new organ in an animal body results from the appearance of a new want or need, which continues to make itself felt, and from a new movement which this want gives birth to and maintains. **Law 3:** The

> development of the organs and their strength of action are constantly in proportion to the use of these organs. **Law 4:** All that has been acquired, impressed upon, or changed in the organization of individuals during the course of their life is preserved by generation and transmitted to the new individuals that come from those which have undergone those changes.

Lamarck used these laws to explain the two forces he saw as comprising evolution; a force driving animals from simple to complex forms, and a force adapting animals to their local environments and differentiating them from each other.

Lamarck is remembered primarily for his belief in the inheritance of acquired characteristics and the **use and disuse** model by which, according to Lamarck, organisms develop their characteristics. The theory of evolution developed by Lamarck is frequently referred to as Lamarckism or Lamarckian evolution. This theory is also often referred to as **soft inheritance**. The term was first suggested by Ernst Mayr to explain the ideas of Lamarck and Étienne Geoffroy Saint-Hilaire (1772–1844) and to contrast those ideas with the modern idea of inheritance, which Mayr referred to as **hard inheritance**. Geoffroy, a French naturalist and a colleague of Lamarck, defended Lamarck's idea of the influence of the environment on species evolution. He further developed Lamarck's idea suggesting that the environment causes a direct induction of organic change that is the transmutation of species in time.

Perhaps the first attempt at rejection of soft inheritance was made by the English surgeon William Lawrence (1783–1867) in 1819. He stated that "The offspring inherit only connate peculiarities and not any of the acquired qualities" (Lawrence and William, 1819). The inheritance of acquired characteristics was also rejected by the German biologist August Weismann (1834–1914). In the 1880s, he performed an experiment in which he cut off the tails of 22 generations of mice, thus proving that the loss of tail cannot be inherited. Furthermore, in 1893, Weismann proposed his own theory of inheritance. He discovered that the cells that produce the **germ plasm** (now known as gametes) separate from somatic cells at an early stage of organismal development. Weissman could not understand how

somatic and **gametic cells** communicated with each other, and therefore, he declared that the inheritance of acquired characteristics was impossible. He further suggested that the organism's body (the **somatoplasm**) exists for only one generation, whereas the hereditary material, which he called germ plasm, is immortal and passed from generation to generation. Although being rather naïve and futuristic, this view led to an important suggestion: Nothing that happens to somatic cells may be passed on with the germ plasm. Thus, this model underlies the modern understanding of inheritance in which germlines are main cells passing hereditary information from one generation to another. At the same time, because this model suggested that the germ plasm is a self-sufficient substance that is not influenced by the environment, it represented a unilateral understanding of evolutionary processes.

Another theory of evolution was synthesized and described by the English biologist and social philosopher Herbert Spencer. In 1857, he published his theory of evolution in his essay "Progress: Its Law and Cause." Spencer characterized the process of evolution as "evolution of complexity"; he suggested that evolution was a process in which simple organisms always evolved into more complex ones, therefore, evolution itself was progressive in nature. Currently, this view of evolution is considered to be misleading; it is generally accepted that species evolve in response to the environment in the process of natural selection that does not have directionality. The absence of the logical explanation of natural selection did not allow Spencer's theory of evolution to become more prominent. At the same time, it was Spencer who popularized the term **evolution** itself. Moreover, after reading Darwin's *The Origin of Species*, published just two years after Spencer's essay, Spencer attempted to use Darwin's theory for explanation of the role of evolution in society. Moreover, he also tried to incorporate it into his own theory of evolution, coining the now-common phrase **survival of the fittest**.

In 1859, Charles Darwin published the work *"On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life"* (Darwin, 1859) (commonly known as *The Origin of Species*) that became a foundation of evolutionary biology and a reason for plenty of scientific discussions.

Darwin's theory suggested that species in the population evolve through a process of natural selection. His book offered multiple examples of how the diversity of life on our planet arose by common descent with modification through a branching pattern of evolution. Darwin proposed that within a certain species, individuals that are less fit for their particular environment are less likely to survive and reproduce compared to those that are well-adapted and have better survival and reproductive potential. The more successful individuals leave more progeny, and thus pass their heritable traits to the next generation. As a result, a certain part of population adapts to the changed environment and eventually might become a separate species. One important thing to note here is that the environment plays a critical role in shaping species' evolution. Darwin accepted a version of the inheritance of acquired characteristics proposed earlier by Lamarck.

Later on, Darwin set forth his provisional hypothesis describing the mechanisms of heredity. In 1868, he presented this idea in his work *The Variation of Animals and Plants under Domestication* (Darwin, 1868). The theory of pangenesis suggests that each individual cell of an organism not only experiences environmental changes and responds to them but also generates molecules that accumulate in germ cells. Darwin believed that these molecules, which he called **gemmules** (Darwin, 1868; Darwin, 1971), are capable of contributing to the development of new traits and organisms. Nowadays, it is a striking fact that small non-coding RNAs such as **microRNAs** (**miRNAs**) and **small interfering RNAs** (**siRNAs**) generated by somatic cells are indeed able to travel within the organism reaching the gametes and potentially influencing the phenotypic appearance of progeny.

1-1. Ontogeny and phylogenetics

Ontogeny (ontogenesis, morphogenesis) is a branch of science describing the development of an organism from the fertilized egg to its adult form.

Phylogenetics (phylogenesis) is the study of evolutionary relatedness among various groups of organisms.

Neo-Darwinism is a comprehensive theory of evolution, frequently called the Modern Synthesis, that combines Mendelian genetics with Darwinian natural selection as a major factor in evolution and population genetics. The term Neo-Darwinism was first used by George Romanes (1848–1894) in 1895 to explain that evolution occurs solely through natural selection as it was proposed by Alfred Russel Wallace (1823–1913) and August Weismann. Neo-Darwinism suggests that evolution occurs without mechanisms involving the inheritance of acquired characteristics based upon interactions with the environment. Thus, this modernized Darwinism accepted some ideas developed by Darwin's original theory of evolution via natural selection, but at the same time it separated them from Darwin's hypothesis of **pangenesis** and the Lamarckian view of inheritance.

Historically, many scientists tried to prove or disprove Darwin's theory of pangenesis. Francis Galton (1822–1911), a cousin of Darwin, conducted many experiments that led him to refute the pangenesis theory. Initially, he accepted the theory, and, in consultation with Darwin, he tried to detect how gemmules were transported in the blood. In his very simple hypothesis, he suggested that if gemmules were transferred to gametic cells though the blood then blood transfusion between various breeds of animals would generate new traits in progeny. In a long series of experiments initiated around 1870, he transfused the blood between dissimilar breeds of rabbits and found no evidence of characteristics transmitted by blood transfusion.

Darwin challenged the validity of Galton's experiment. He wrote in 1871:

> Now, in the chapter on Pangenesis in my "Variation of Animals and Plants under Domestication," I have not said one word about the blood, or about any fluid proper to any circulating system. It is, indeed, obvious that the presence of gemmules in the blood can form no necessary part of my hypothesis; for I refer in illustration of it to the lowest animals, such as the Protozoa, which do not possess blood or any vessels; and I refer to plants in which the fluid, when present in the vessels, cannot be considered as true blood.

Until the end of the nineteenth century, Darwin's theory of pangenesis was accepted by many scientists. The work of Gregor Johann

Mendel on plant hybridization fundamentally changed scientists' understanding of the mechanism of inheritance. Although Mendel published his work in 1866, it was not until 1900 that his ideas were re-examined. Upon re-discovering the significance of Mendel's work, a new era of Mendelian genetics began in which scientists completely rejected the possibility of the transmission of information from somatic cells to gametes and thus to progeny. It was a real pushback for Lamarck's theory of evolution.

Many scientists still considered the possibility of environmentally induced heritable changes. The Russian scientist Ivan Michurin (1855–1935), one of the founders of scientific agricultural selection, also assumed that genotypes could change upon environmental pressure. He worked on hybridization of plants of similar and different origins, developing strategies for overcoming species incompatibility upon hybridization and cultivating new methods in connection with the natural course of ontogenesis (1922–1934). He was also interested in directing the process of predominance, evaluation, and selection and in working out methods of acceleration of selection processes. In the early twentieth century, he proved that the dominant traits in generation of hybrids depend on heredity, **ontogenesis,** and **phylogenesis** of the initial cell structure as well as on individual features of hybrids. Michurin was a true follower of Lamarck and Darwin, and he firmly believed that natural selection could be influenced by external factors, with man being the most influential one.

In the not-too-distant past, the ideas of Lamarck and Michurin seemed to be pseudo-scientific and impossible to believe in. But recently, a breakthrough publication describing changes in the genetic make-up of grafted plants appeared that became an eye-opener, suggesting many new possibilities for transmission of genetic material. Sandra Stegemann and Ralph Bock showed that transfer of genetic material from stock to scion is possible upon grafting of tobacco plants (Stegemann and Bock, 2009). The results of the study demonstrated that recipient plants acquired tolerance to an antibiotic in the same manner as donor plants, and they also confirmed the transfer of genetic material from a donor to a recipient. Although it is still unclear whether the acquisition of antibiotic resistance occurs via plastid transfer through plasmodesmata or via the transfer of a large portion of the plastid genome from a donor cell to a recipient cell, it

can be definitely considered as an example of changes not only in phenotypic appearance but also in the genetic make-up of a grafted plant.

The emergence of epigenetics as science was closely linked to the study of evolution and development. Nowadays, we know that chromosomes are associated with both genetic and epigenetic regulation, thus driving the developmental processes. Despite the early discovery of chromosomes by Walther Flemming (1843–1905), the founder of cytogenetics, in 1879, it took many more experiments to links chromosomes to function, phenotypes, and developmental programming. The experiments by Edmund Wilson (1856–1939), Theodor Boveri (1862–1915), Walter Sutton (1877–1916), and later on Thomas Hunt Morgan (1866–1944) provided several evidences that chromosomes were indeed involved in developmental processes, and changes in chromosomes resulted in changes in phenotype. The **Boveri-Sutton chromosome theory** suggested that Mendelian laws of inheritance could be applied to chromosomes and chromosomes might thus be units of inheritance. Morgan's work in *Drosophila* showed that the inheritance of many genes was linked to the X chromosome; among them were genes coding for eye color. This and other works enabled him to become the first scientist to receive a Nobel Prize (1933) for his work in genetics. The report by Watson and Crick (1953) describing DNA structure and proposing the mode of DNA replication further reinforced the notion that DNA is the cell's genetic material. Although the studies of chromosome morphology indicated that somatic cells contained all of the chromosomes, it was not clear why the somatic cells of different tissues had different phenotypic appearance, raising doubts whether somatic cells actually did carry all the genes and not only those that were necessary for their growth and development.

Although investigations of epigenetic regulation of an organism's development and cell fate were being actively pursued throughout the twentieth century, the actual name "epigenetics" did not emerge until 1942 when Conrad Hal Waddington (1905–1975) used it to describe how genes might interact with their surroundings to produce a phenotype. Waddington described several essential concepts, including **canalization**, **genetic assimilation**, and **epigenetic landscape**. The concept of canalization in Waddington's understanding was the capacity of the organisms of a given population to produce the same phenotype regardless of the extent of genetic and

environmental variations. He assumed that this robustness came as a result of evolution, shaping the developmental processes to perfection. Waddington's idea of genetic assimilation suggested that an organism responds to the environment in such a way that the acquired phenotype would become part of the developmental process of the organism.

To demonstrate that the phenomenon exists, Waddington induced an extreme environmental reaction in the developing embryos of the fruit fly *Drosophila*. When exposed to ether vapor, a small percentage of the *Drosophila* embryos developed a second thorax. It was obvious that bithorax embryos represent an abnormal phenotype. Waddington continued selection of bithorax mutant embryos, and after about 20 generations of selection, he obtained *Drosophila* flies that developed bithorax without being exposed to ether vapor. Waddington suggested that in this particular case, selection led to the production of the desired effect, which became canalized, and, as a result, bithorax appeared regardless of environmental conditions. Thus, Waddington's experiments demonstrated that Lamarckian ideas of inheritance of acquired characteristics could, at least in principle, be true. Finally, the epigenetic landscape, as suggested by Waddington, represents is a programmed cell fate where developmental changes would occur with increasing irreversibility, much like marbles rolling down a small-ridged slope toward the lowest elevation point. Nowadays, the term **epigenetic landscape** refers to the certain area of a chromatin in the cell with specific cytosine methylation and histone modifications involved.

During the past 50 years, the scientific community has witnessed a lot of rises and falls in an interest in epigenetics. Perhaps, the next important discovery in the area of epigenetics was Alexander Brink's report on the phenomenon of **paramutation**. In 1956, Brink described a somewhat puzzling and controversial phenomenon of the inheritance phenotype associated with the of *Red 1* (*r1*) locus in maize (Brink, 1956). It was observed that the spotted seed allele (*R-st*) was able to transform the *R-r* (purple color seeds) phenotype allele into a colorless seed phenotype in subsequent generations. As a result of the cross, all of the F_2 generation plants showed reduced anthocyanin in seeds, which was contrary to the expected segregation ratios according to the Mendelian law. The phenomenon that he

proposed to be called paramutation involved heritable transmission of epigenetically regulated expression states from one homologous sequence to another. For a more detailed description of paramutations in plants and animals, see Chapter 13, "Paramutation, Transactivation, Transvection, and Cosuppression: Silencing of Homologuous Sequences."

In her early work, Barbara McClintock (1902–1992) also suggested that the chromosomal position effect might influence on the behavior of mutable loci in maize. She assumed that the observed difference in mutability ratios of suppressor elements in maize had the mechanism similar to the earlier described phenomenon of position-effect variegation. The latter term was first brought up by Hermann Joseph Muller (1890–1967) and was meant to describe the effect of chromosomal position on gene expression. Having observed gross chromosomal rearrangements, Muller noted changes in gene expression, and the genes that were brought into the area of heterochromatin expressed poorly. McClintock noted that some controlling elements, such as *Spm,* would suppress gene expression rather than mutate a gene; she also noticed that the suppression of gene expression would take place not only at the locus where the elements had been inserted but also at the neighboring loci.

The work of David Nanney (published in 1958) showed that the cytoplasmic history of conjugating parents had an impact upon the mating-type determination of resulting progeny in *Tetrahymena* (Nanney, 1958). This phenomenon was suggested to be of epigenetic nature.

In the early 1960s, Mary Lyon and Walter Nance presented the mechanism of another epigenetically regulated process, **X-chromosome inactivation**. It was suggested that inactivation of the mammalian female X chromosome occurred before the 32-cell stage of the embryo. However, there was no clear assumption that this process was indeed of epigenetic nature. The fact that no changes were observed at the level of DNA allowed Riggs (1975) and Holliday and Pugh (1975) to propose that DNA methylation could be a mechanism of X-chromosome inactivation.

In the 1970s, Hal Weintraub's work on the expression of globin genes revealed an influence of chromosomal location on the transcriptional activity. His observations were the source from which the suggestion came that the chromatin structure might regulate gene expression.

In the early 1980s, it became clear that there was an apparent correlation between the level of cytosine methylation at GpG DNA sequences and the level of gene transcription. Moreover, the mitotic heritability of **DNA methylation patterns** was also shown. Later on, by the mid-1980s, the influence of nuclear content on the genetic/phenotypic make-up of the organism was also revealed. It was found out that not only the DNA sequence of paternal or maternal alleles had an effect on the phenotype, but the origin of a particular chromosome itself could influence the phenotype. Thus, it was suggested that besides the DNA sequence, the chromosome also carried additional information.

In the 1990s, scientists presented more discoveries in the area of epigenetics, coming from studies of various organisms including protozoa, fungi, *Drosophila*, plants, and animals. In plants, it was found that the transgene coding for chalcone synthase (*Chs*) had various degrees of suppression of expression gene expression. It was perhaps the first well-documented event of **gene silencing** (Napoli et al., 1990).

In trypanosomes, it was discovered that silencing of the group of *Variable Surface antigen Genes* (*VSG*) is maintained by the incorporation of a novel base, β-D-glucosylhydroxymethyluracil (Borst et al. 1993). Because trypanosomes do not have the mechanism of cytosine methylation, it was suggested that the insertion of the modified base would also serve as a gene-silencing mechanism. Significant progress was made in understanding the mechanisms of X inactivation. A portion of the human X chromosome was identified to function as the X chromosome inactivation center; later on, the gene *Xist* was identified that appeared to be coding for a **non-coding RNA** expressed only in an inactive X chromosome (Willard et al., 1993). The analysis of the expression of the neighboring gene *Igf2* and *H19* pair provided a further understanding of the mechanism underlying chromosomal imprinting. The genes were mutually exclusively expressed depending on the maternal or paternal origin of the chromosome; if the *Igf2* gene was expressed from the paternal chromosome, then the *H19*

gene was repressed, whereas if the *H19* gene was expressed from the maternal chromosome, the *Igf2* gene was repressed. Methylation analysis of the locus identified high frequency of occurrence of methylated CpGs. Therefore, it was proposed that methylation controlled the access to the enhancer element that functioned mutually exclusively for both genes. Indeed, in mice, it was found that a mutant impaired in the function of a 5-methyl-cytosine DNA methyltransferase lost the imprinting of the gene pair in ES cells.

The role of epigenetic regulation in control over gene expression was also demonstrated by the experiments on fungi. Gene duplication in *Neurospora crassa* often resulted in the occurrence of two events: frequent mutations and hypermethylation of both gene copies, a phenomenon known as **repeat-induced point mutation (RIP)**. Furthermore, for the first time, it was shown that cytosine methylation in *Neurospora* could occur at non-CpG sites. A similar phenomenon was observed in *Drosophila*; the duplication of the brown gene translocated near heterochromatin increased the level of repression in the active copy. Because in *Drosophila,* cytosine methylation is not used as a process of gene expression regulation, there should be a different repression mechanism. The research in this direction resulted in the development of the concept of chromosomal **boundary elements**, the areas of the chromosome that contained a 300 bp nuclease-resistant core surrounded by nuclease hypersensitive sites that were first described in *Drosophila*. It was suggested that such elements allow the separation of a chromatin domain along the chromosome, thus leading to differential areas of chromosome compaction and gene expression. In yeasts, the Sir2, Sir3, and Sir4 proteins (silent information regulator proteins) were identified that were proposed to control repressive states near heterochromatic regions. The evidence that Sir3 and Sir4 interacted with the tails of histones H3 and H4 further confirmed the importance of both these proteins and histones in the maintenance of the chromatin state. By the end of the 1990s, histone-modifying enzymes such as acetylases and deacetylases were identified, and the MeCP2 protein complex that was able to bind to methylated DNA and histone deacetylases were described.

One more important discovery made in the late 1990s was the description of the phenomenon of **RNA interference**. A series of work by Craig Mello and Andrew Fire culminated in the famous

publication in *Nature* (Fire et al., 1998) that described the ability of double-stranded RNA molecules to inactivate the expression of genes in *C. elegans*; the effect of interference was evident in both injected animals and progeny. Because just a few molecules per cell were sufficient to trigger the effect, the authors suggested the existence of an amplification component, currently known as a mechanism that involves the function of RNA-dependent RNA polymerase. The importance of this work for studying organism development, the therapy of various human diseases, as well as for the development of biotechnology and basic science was recognized with the Nobel Prize awarded to Mello and Fire in 2006.

In 1990, Robin Holliday defined epigenetics as "the study of the mechanisms of temporal and spatial control of gene activity during the development of complex organisms" (Holliday, 1990). Today, the definition of epigenetics has been changed; it is now described as the study of the mechanisms of inheritance and control of gene expression that do not involve permanent changes in the DNA sequence. Such changes occur during somatic cell division and sometimes can be transmitted transgenerationally through the germline.

The last ten years were marked by the most prominent achievements in epigenetic research. In 2000, it was discovered that the Sir2 protein of yeasts was in fact a histone deacetylase. Studies of the heterochromatin states, replication processes, the activity of the Sir3 protein in yeast, and the heterochromatin protein (HP1) in mammals showed that heterochromatin was not in a solid inert stage reversible only during replication but rather in an active equilibrium stage of protein exchange between the nuclear soluble compartment and heterochromatin itself, regardless of cell cycle status.

By the early 2000s, most of the histone modifications and the enzymes that catalyze them were discovered, and it was believed that besides histone methylation, all other modifications such as acetylation, phosphorylation, ubiquitination, and so on were reversible. Thus, various histone methylation states were regarded as a permanent epigenetic mark of chromatin status and were reversible only during replication. The results of studies by Cuthbert et al. (2004) and Henikoff et al. (2004) raised the possibility that histone methylation could be reversible, and their suggestions were met with true enthusiasm. In his works, Cuthbert demonstrated that

peptidylarginine deaminase was able to remove single methylation events at the arginine amino acid of histone H3 (Cuthbert et al., 2004). Henikoff et al. (2004) showed that H3.3, a histone H3 variant, was able to replace histone H3 in a transcription-dependent and replication-independent manner, opening the possibility for more flexible regulation of methylation after the process transcription was over.

Another breakthrough was the discovery that nuclear organization and silencing at telomeres were not necessarily completely interrelated. The experiment showed that if telomeres and the associated silencing complex were released from the periphery of the nucleus and were able to move throughout the nucleus, the silencing at telomeres was established with similar efficiency (Gasser et al., 2004). This is truly exciting—it suggests that chromatin compartmentalization and gene silencing processes are not rigid and predefined states; there indeed exists an active exchange between the nuclear pools of proteins and small RNAs that are able to establish a certain chromatin state at any given locus regardless of its nuclear location.

A curious phenomenon was reported for *Arabidopsis*; it was on the borderline of epigenetic regulation and described a non-Mendelian inheritance. An *hth* mutant is homozygous for the mutation in the *HOTHEAD* (*HTH*) gene that encodes a flavin adenine dinucleotide-containing oxidoreductase involved in the creation of the carpel during the formation of flowers. It was reported that in the progeny of the *hth* mutant, the percentage of the frequency of appearance of the *HTH* phenotype and *HTH* genomic sequence was ~15% (Lolle et al., 2005). It was first proposed that reversion was triggered by RNA synthesized by *HTH/hth* parents and stored in the progeny *hth/hth* plants. Four alternative explanations have been proposed: Two of them were in part similar to an original explanation made by Lolle et al. (2005) and dealt with template-directed gene conversion; the third one offered the process of mutation accumulation followed by selection; and the fourth one involved **chimerism**. Later on, two publications seemed to put everything in place: Peng et al. (2006) and Mercier et al. (2008) reported that the *hth* mutant showed a tendency toward outcrossing and recovered a normal genetic behavior when grown in isolation. Despite the fact that

Arabidopsis is an extreme self-pollinator (less than 0.1% of outcrossing), in the *hth* plants the frequency of outcrossing among neighboring plants was ~12%. This can be an excellent alternative explanation for the apparent genetic instability of *hothead* mutants.

Now that the genome sequences of model organisms such as *C. elegans*, *Drosophila*, *Arabidopsis*, human, mice, rice, and so on are available, more and more investigators have attempted to understand the organization of the genome and chromatin and explain the mechanisms of inheritance, maintenance of genome stability, and regulation of gene expression. What has become clear is that these mechanisms are both genetic and epigenetic in nature. As it was recently put by Daniel E. Gottschling ("Epigenetics: from phenomenon to field" in *Epigenetics*; eds. C.D. Allis, T. Jenuwein, D. Reinberg), it was time to move "above genetics"—a literal meaning of epigenetics as several important genomes have already been sequenced.

There are multiple examples of the influence of environment on the genetic and epigenetic make-up of the organism. The phenomena of stress-induced transposon activation, non-targeted mutagenesis, stress-induced communication between cells and organisms, and evidences of transgenerational changes induced by stress are just some representations of epigenetic effects of the environment on the organism.

The non-linear response to DNA damaging agents is one of the most interesting examples of an epigenetically controlled process. It has already been known that a higher dose of mutagen does not necessarily result in a higher level of damage to DNA. In fact, low doses of ionizing radiation often lead to disproportionally high levels of DNA damage. Doses of ionizing radiation that are believed to have a negligible effect on a cell often exert dramatic influence on DNA damage and cell viability.

In the past, cell-to-cell communication between neighboring cells as well as communication between cells of different tissues and organs in multicellular organisms were considered Lamarckian/Darwinian and thus improbable. There are multiple examples of physiological cell-to-cell communications in simple and complex organisms involving hormonal signaling, neurotransmission, and so

on. Moreover, it is believed that damaged tissues are able to communicate with non-damaged tissues—a phenomenon known as **bystander effect**. The phenomenon of bystander effect has also been observed between whole living organisms.

Can organisms communicate memory of stress across generations? According to Darwin, organisms evolve from the pool of individuals with spontaneous changes/mutations through the process of natural selection. The process of mutagenesis is believed to be random, and the majority of mutations are deleterious. The rare mutations that become beneficial under certain environmental conditions have a chance to be fixed in a population. Because mutagenesis does not occur frequently, the fixation of desired traits would take place very rarely. In contrast, processes of acclimation and adaptation are rapid ones that allow organisms to acquire protection against stress in a single generation after stress exposure. These processes cannot be explained by the laws of Mendelian genetics. In this book, you find multiple examples demonstrating the inheritance of stress memory in various organisms across generations.

This chapter attempted to explain what epigenetics is, how it is involved in the regulation of growth and development of the organism, how it controls interactions of the organism with the environment, and what roles epigenetics plays in the mechanisms of inheritance and evolutionary processes.

There have been many more important discoveries in the field of epigenetics, and we apologize to all those authors whose work, though relevant, is not mentioned in this chapter because of limitations of space.

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