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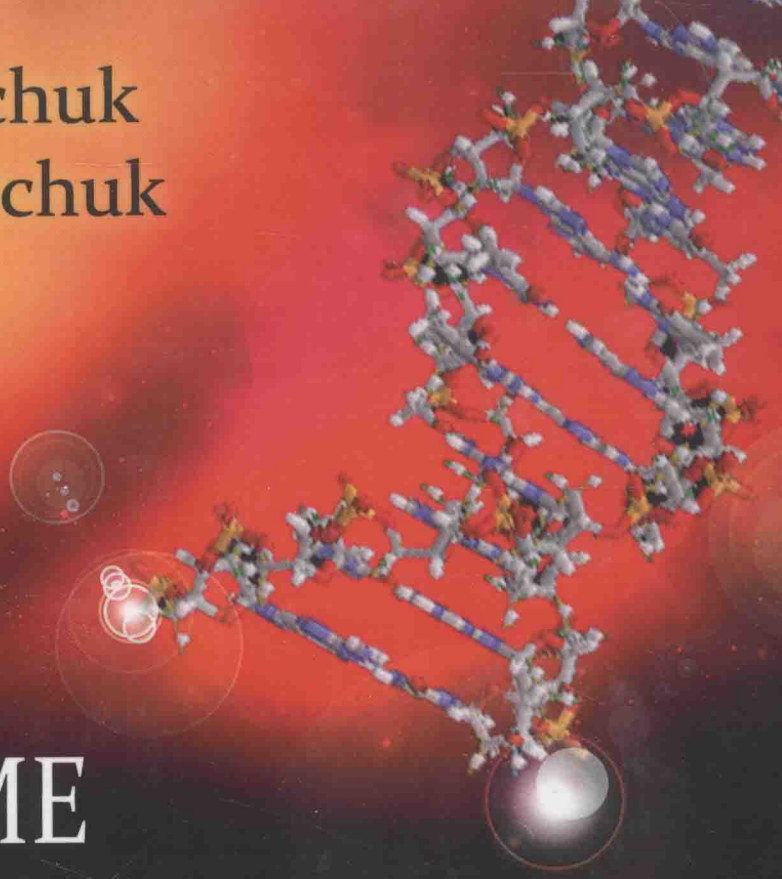

Genome Instability and Transgenerational Effects

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GENOME INSTABILITY AND TRANSGENERATIONAL EFFECTS

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PREFACE

Chapter 1 - Genome stability of every species depends on complex interaction of predefined and environmentally induced genetic and epigenetic states. Predefined states consist of chromatin structure and cell metabolic processes such as DNA repair, radical scavenging and cell signalling, whereas induced states depend on interactions with the environment. Organisms are able to respond to a changing environment by various alterations in their somatic cells as well as in their germline and progeny. This book, will describe various phenomena associated with the maintenance of genome stability. These include genetic and epigenetic responses to various stresses in exposed cells and organisms, bystander and, bystander-like effects, transgenerational changes in genome stability and stress tolerance in bacteria, plants and animals.

Chapter 2 - Maintenance of genome stability is a key to a disease-free survival of mammalian organisms. Mammalian cells are affected by a wide variety of endogenous and exogenous DNA damaging agents. To effectively deal with DNA damage, mammalian cells harbor a versatile network of repair, cell cycle control and genome maintenance mechanisms, since failure to eradicate DNA damage may lead to genome instability.

Yet, the correct function of these and other cellular mechanisms heavily depends upon gene expression and organization as well as on the accessibility of DNA for DNA-protein interactions. These domains are governed by epigenetic processes—meiotically heritable and mitotically stable alterations in gene expression that include DNA methylation, histone modification, and RNA-associated silencing. This chapter will introduce some of the key strategies mammalian cells have evolved to maintain the integrity of their heritable information and will describe some interconnected genetic and epigenetic tactics that frequently work in concert to achieve a common goal of maximum stability of the genome.

Chapter 3 - Maintaining genome stability is one of the most critical tasks for each and every living organism. During their life span, organisms are constantly exposed to a variety of internal and external stimuli, namely stresses, which can alter genome stability and lead to heritable changes. Over the past several years, a number of naturally occurring stresses were revealed to affect genome stability. They include: light spectrum and the day length, various types of ultraviolet and ionizing radiation, chemical mutagens and herbicides, temperature, salt, water stress and pathogen attacks. In fact, many of those stresses can change genome stability at both genetic and epigenetic levels, inducing rearrangements and altering existing DNA methylation patterns. Control and/or prevention of these changes is an issue of vital importance as they exert their influence on organisms experiencing stress, and they can be

transmitted to progeny altering genome stability for several generations. The epigenetic regulation, including DNA methylation, histone acetylation/methylation and chromatin remodeling, play a key role not only in controlling gene expression but also in preventing undesirable rearrangements and transposon activation. The presence of high amounts of heterochromatic DNA containing various repetitive elements, pseudo genes, and virus-derived sequences that often carry strong promoters and/or enhancers emphasizes the importance of epigenetic mechanisms in controlling genome stability. The system of epigenetic DNA modifications relies on a complex network of various protein factors, including numerous DNA and histone methyltransferases, histone deacetylases, methyl-CpG-binding domain proteins etc., many of which are guided via multifaceted systems of small RNA molecules. Overall, these systems permit sequence-specific changes in DNA epigenetic modifications, which allow adjusting transcriptional profiles of cells to new conditions and also modifying genome stability, if needed. This chapter is focused on various aspects and mechanisms involved in the maintenance of genome stability. It also discusses how changing the stability of a given locus may contribute to genome evolution and the ability to adapt to stress.

Chapter 4 - Oxidative damage to DNA results in lesions such as thymine glycol, 5-hydroxycytosine, formamidopyrimidine and 8-oxoguanine that have very different structures. Base excision repair is the main biological process for repairing DNA damage caused by oxidative stress. During the first step of base excision repair, an oxidative DNA lesion is recognized and excised by a DNA glycosylase. The Fpg/Nei family of DNA glycosylases is one of two families, each containing members that have distinct substrate specificities which collectively excise the oxidative lesions listed above. Events in the Fpg/Nei phylogeny include acquisition of novel substrate specificity, horizontal transfer, changes in a major structural motif (a zinc finger), and expansion within a bacterial clade. Importantly, the function of one clade, Fpg2, remains unknown.

Chapter 5 - Stress-induced mutagenesis in bacteria has been puzzling researchers for decades. Bacteria regularly encounter a variety of chemical, physical and biological stressors. Some bacteria manage to survive and proliferate, very often giving rise to cell populations with new phenotypes. Numerous attempts have been made to study mutagenic factors and their influence on microbial physiology, genome stability and evolution. These attempts have raised more questions than answers. A classical example is the fact that until now there is no unambiguous theory regarding mechanisms of adaptive mutation in a Lac$^-$ strain of *Escherichia coli*. This chapter will attempt to outline the influence of main mutagenic factors on bacterial physiology and the bacteria's ability to adapt to these factors.

Chapter 6 - Bacteria and archaea lacking sexual reproduction need to use a variety of mechanisms for gene exchange/gene acquisition. These mechanisms of horizontal gene transfer (HGT) in bacteria and archaea include phage transduction, transformation, and conjugation. At the same time, bacteria require protection against their ancient enemies, phages. One of the most interesting mechanisms of protection is based on the function of regulatory RNAs encoded by clustered regularly interspaced short palindromic repeat (CRISPR) loci to confer sequence-directed immunity against phages. According to recent publications, this is an active protection mechanism by which prokaryotes integrate short fragments of phage/viral nucleic acids into clusters of CRISPRs. As such, this process represents an ancient adaptive mechanism of protection against pathogens.

Chapter 7 - In contrast with a wide definition of 'epigenetic variation', including all changes in gene expression that do not result from the alteration of gene structure, a more restricted class has been defined, initially in plants, under the name 'paramutation'. It corresponds to epigenetic modifications distinct from regulatory interactions of cell differentiation pathways, which are meiotically stable and sexually transmitted by non-Mendelian ratios. This class of epigenetic changes appeared for some time restricted to the plant world, but examples of epigenetic inheritance progressively accumulate in organisms ranging from mice to humans.

Occurrence and possible mechanisms of paramutation in the mouse were first established with a tail color alteration in the paradigmatic case of a mutant phenotype maintained and hereditarily transmitted by genotypically wild-type homozygotes. Studies in the mouse point to a new role of RNA as an inducer and hereditary determinant of epigenetic variation. Given the known presence of a wide range of RNAs in human spermatozoa as well as a number of unexplained cases of familial disease predisposition and transgenerational maintenance, one may consider a role of RNA-mediated inheritance in mammals. A possible involvement in evolutionary processes makes for interesting speculations.

Chapter 8 - Ionizing radiation, in addition to being an important treatment modality, is a potent tumor causing agent. The resultant risk of secondary radiation treatment-related cancers is a growing clinical problem. Some studies link secondary radiation-induced cancers to an enigmatic phenomenon of bystander effects whereby exposed cells signal damage and distress to their naïve neighbors resulting in genome instability. There is also well-documented evidence that radiation exposure leads to transgenerational genome instability in the offspring of exposed parents. The exact molecular mechanisms of these indirect/non-targeted radiation effects have yet to be defined; however, recent evidence suggests that they may be epigenetic in nature.

Epigenetic phenomena seem to be mediators of indirect radiation effects, including radiation-induced genome instability, bystander and transgenerational effects. DNA methylation and histone modification changes directly impact chromatin packaging and therefore influence gene expression and susceptibility of DNA to rearrangements. Short RNAs (such as microRNAs and piRNAs) may potentially be acting as key mediators of these non-targeted effects due to their small size, relative stability, their roles in maintenance of gene expression, stability of transposable elements, and the genome as a whole.

This chapter will summarize the current knowledge of the existence of non-targeted radiation-induced bystander and transgenerational effects and the roles of epigenetic changes in their initiation and maintenance.

Chapter 9 - The study of germline mutations is extremely difficult because rates of mutation are extremely low for gene sequence DNA. An alternative approach uses highly variable non-coding regions of the genome that possess rates of mutations that are orders of magnitude greater than protein-coding sequences. Mutations result from gains and losses of repeat units that arise during replication, recombination and repair. Germline mutation frequencies range from 1 – 20% and facilitate the measurement of induced mutations in small sample sizes. Mutations can be detected in pedigrees using Southern blotting, or by PCR analysis of sperm DNA. Expanded simple tandem repeat loci (ESTR) and minisatellites have been shown to undergo elevated rates of mutation following exposure to radiation and chemicals. As such, the study of repeat mutations in populations exposed to toxicants in the environment showed that induced germline mutations may result following exposure to

ambient levels of contaminants. This chapter reviews the current state of knowledge in this field of study and provide an overview of repeat types and structure, the mechanisms of repeat mutations, the methods used to study mutations and discuss some experiments applying repeat analysis to study germline mutations.

Chapter 10 - This chapter will review the data for somatic and germ line transgenerational effects in fish. The emphasis will be on radiation studies with reference, where appropriate, to chemical or mixed exposure studies. The terms will be defined as used in most of the literature with discussion of confusing elements. There are two main sources of data for fish transgenerational studies. These are the use of fish in the laboratory as models for human studies and the study of impacts of radiological accidents or planned exposures on fish as critical elements in the aquatic ecosystem. Both data sets will be reviewed. Comparative studies of mammalian and fish radiation responses are rare but important for both mechanistic understanding and environmental risk assessment. Common assumptions are that fish are "radioresistent". The basis for this will be reviewed and discussed in detail. Work from the authors' laboratory concerning bystander signaling and associated induction of genomic instability will also be reviewed. Finally, reasons for these studies will be discussed in terms of relevance for evolutionary biology and environmental protection of seeking a mechanistic understanding of vertebrate responses to low doses of environmental stressors.

Chapter 11 - Transgenic animal models that carry mutation target genes have proven invaluable in addressing the need for improved approaches to study mutations induced in somatic cells. Recently, a small laboratory fish model, the λ transgenic medaka, was introduced as a new model for germ cell-mediated mutagenesis. Attributes of medaka as an animal model and the *cII* transgene as a mutation target gene combine to provide numerous practical and scientific benefits for such investigations. In this chapter, features of using the λ transgenic medaka and the *cII* mutation assay, and results from this recent investigation using the germ cell mutagen ethylnitrosourea (ENU) are reviewed with a focus on comparisons with investigations using mouse models. The ability to characterize both the frequencies and specific types of *cII* mutations carried by individual mutant offspring proved invaluable in distinguishing non-mutant, whole body and mosaic mutant offspring. The frequencies of mutant offspring derived from ENU-treated spermatogonial stem cells of fish were remarkably similar to those reported for transgenes and endogenous genes of mice, thereby supporting the use of medaka as a comparative animal model for germline mutagenesis. The prevalence of mosaic mutant offspring and the distinctive spectra of mutations they carried revealed that, in addition to the role germ cells play as direct transmitters of mutations, germ cells carrying persistent DNA damage act as mediators of indirect mutagenesis in cells of early stage embryos. Emerging evidence suggests that error-prone DNA repair processes and likely, epigenetic processes acting independently or together contribute to a phase of hypermutagenesis manifested as genomic instability in offspring of mutagen-exposed germ cells. Whereas the processes of mutagenesis mediated by germ cells remain incompletely characterized, a model for the mechanism(s) will incorporate the interplay between persistent DNA damage, delayed and untargeted mutations, and constraints of rapidly dividing cells of early stage embryos in responding to damage contributed by germ cells.

Chapter 12 - The field of transgenerational genomic instability in mammals, including humans, is a confusing area of contradictory results, generalizations based on limited data sets

and confusion about terminology. This chapter will focus on radiation-induced effects on genome stability, with some discussion of the literature on chemical-induced effects where relevant. The old and recent radiation literature will be summarized, and controversial issues will be highlighted. The relevance of non-targeted effects, such as bystander signaling and stress-induced instability, will also be discussed. An emphasis will be placed on the recent data from the authors' laboratory concerning medium- and blood-borne signals, which appear to regulate behaviors of lower hierarchical levels to achieve coordination of tissue, organism and ecosystem response at higher levels of organization. The hypothesis is put forward that such signal-mediated coordination represents a natural response to stress such as radiation exposure, which enables an appropriate reaction to ensure an optimal outcome for irradiated organisms. Data sources will be reviewed, and limitations of these studies will be discussed. Most of the discussion will relate to experimental studies in (mainly) rodents, but available data on wild mammals (e.g., post Chernobyl) will also be discussed.

Chapter 13 - Phenotypic diversity is shaped by both genetic and epigenetic mechanisms that program tissue specific patterns of gene expression. Cells, including neurons, undergo massive epigenetic reprogramming during development through modifications to chromatin structure and by covalent modifications of DNA through methylation. There is evidence that these changes are sensitive to environmental influences, leading to sustained differences in phenotype. As in humans, variations in maternal behavior of rat mothers have long-term consequences for the behavior of their offspring. For example, in rats, species-typical differences in maternal behavior have long-term effects on the Hypothalamic-Pituitary-Adrenal (HPA) axis, cognitive and emotional function of offspring. Thus, the offspring of mothers that engage in high amounts of licking and grooming and arched-back nursing exhibit less stress reactivity than do the offspring of mothers that exhibit low licking and grooming and arched-back nursing. These differences induce long-term changes in gene expression, including those in the glucocorticoid receptor, which is associated with altered histone acetylation, DNA methylation, and NGFI-A transcription factor binding. Thus, maternal care plays a critical role as a mediator of the relationship between early-life events and health in adulthood. Interestingly, these effects can be reversed by early post-natal cross fostering, and by pharmacological manipulations in adulthood, including Trichostatin A and l-methionine, that influence the epigenetic status of critical loci in the brain. Recent evidence suggests analogous mechanisms may be at work in humans. These findings provide evidence for a stable yet dynamic epigenome capable of regulating phenotypic plasticity through behavioral programming.

Chapter 14- Classically, the development of cancer in humans has been viewed as a progressive multistep process of transformation of normal cells into malignant cells driven by genetic alterations. However, a wealth of data in the past decade indicating the importance of epigenetic mechanisms has largely changed the view on cancer as being a solely genetic disease.

Currently, cancer is recognized as a disease provoked by both genetic and epigenetic alterations, and both of these components cooperate and complement each other at every stage of cancer development. It is widely believed that the major causes of human cancer are due to environmental exposure to natural and man-made chemical and physical agents.

However, during recent years, an interest to the causative role of personal lifestyle factors and nutrition in the origin of cancer has increased significantly. This is driven by growing evidence that lifestyle may substantially compromise the stability of the genome by inducing

genetic and epigenetic alterations that predispose cells to tumorigenesis. Therefore, the elucidation of the effects of personal lifestyle factors on epigenetic processes during carcinogenesis is of great importance, as these epigenetic alterations may not only have been potential early-detection and prognostic markers but also targets for cancer prevention.

Chapter 15 - Epigenetic regulation of genes serves as a flexible way for variation in expression without alterations in existing genetic sequences of genes. Paramutation is one such manifestation of epigenetic regulation. Paramutation leads to heritable alterations in the expression status of one allele in accordance with the second paramutagenic allele. Such examples exist in a wide range of organisms including plants, mammals and fungi. Causes and mechanisms responsible for paramutation have long been sought for. A siRNA-based model is emerging as the most promising candidate for paramutations. Yet, exact details of paramutations are inconclusive. Here, two well-studied examples of paramutations in plants are discussed. Also, the most evident mechanisms of paramutation are discussed in relation to the two examples.

Chapter 16 - Plants are sedentary organisms that respond to stress with reprogramming of patterns of gene expression, thus allowing fast acclimation and adaptation in response to specific environmental conditions. Mechanisms underlying the ability of plants to respond to stress so rapidly and efficiently could be broadly classified into the mechanisms associated with physiological changes in exposed plants and accumulation of newly synthesized metabolites in formed seeds and into those that can be associated with epigenetic mechanisms of reprogramming of gene expression. The latter ones are the mechanisms of inheritance that do not involve permanent changes in DNA sequence. However, epigenetic modifications may also trigger permanent changes in the genome.

This chapter describes what changes occur in progeny of plants exposed to stress and will discuss epigenetic mechanisms regulating a transgenerational response to stress, describe what is known from current literature reports, and present several sets of data accumulated in our lab that show various examples of heritable changes in response to stress.

Cgapter 17 - Transposable elements (TEs) are ubiquitous mobile DNA sequences that have the ability to insert at different positions in the genome and sometimes to amplify at high levels. The activity of TEs can deeply modify host genome structure, it also creates genetic variation and affects gene expression and function. In plants, TEs represent a major genome component, and their activity is often associated with stress conditions. The expression of LTR-retrotransposons in particular is tightly linked to plant stress response pathways. Several evidences of heritable genome restructuring associated with the plant TE activity have been reported in response to environmental challenges and genome shocks such as interspecific crosses. More importantly, TEs may affect gene functions in multiple ways, from contributing to proteic sequences or even metamorphosing into new genes to relocating genes and genic sequence to new positions. They also provide new or alternative expression patterns and act as mediators of epigenetic regulations. Retrotransposon LTRs, in particular, may act as promoter capsules modulating neighbouring gene expression in response to their own transcriptional and epigenetic responses to specific stress stimuli. An emerging paradigm thus considers TEs as sensors of specific challenges that are able to both restructure genomes and modulate gene expression and function in response to these challenges, thus playing a significant role in the generation of phenotypic plasticity.

Chapter 18 - Bystander effects can be defined as unexposed 'units' that exhibit molecular symptoms of stress exposure when adjacent or nearby 'units' are subject to stress. Here a unit

may be referred to as a cell, organ, or even another organism. Plants have a variety of bystander-like effects, most of which involve local pathogen infection and pest infestation that result in a systemic resistance response to current and future attacks. These bystander effects are critical for plants to survive in their environment. The following chapter will outline some of the known inter- and intraplant signalling events that occur in response to pathogen or pest detection. As such, it will involve an overview of the plant immune system as it is understoon today, and how a plant signals resistance to the rest of the plant, or in some cases to other plants. By no means does the chapter mean to cover all of what is known in each of these areas, only the most accepted paradigms and the ideas behind how they function. This chapter may be utilized by undergraduate and graduate students who want a taste of the plant signalling field or for those with little experience in plant signalling biology. More experienced molecular botanists can use this chapter as a 'jumping off' point, as the chapter directs readers to numerous current reviews in each of the discussed areas.

Chapter 19 - Since the time man has domesticated plants for food, this chapter seeks to expand the range of benefits they can provide. Starting in the late 19^{th} century, we began to apply the principles of modern science in order to achieve these aims faster, more consistently and on a wider scale. In modern times, the goals included higher, more sustainable yields in the face of changing environments. Recently increasing emphasis is on improving aesthetic and sensory experience and addressing health concerns. The complexity of the goals is clear.

In this review, attempts are made to make the case that geneticists are ready to exploit new possibilities arising from a better appreciation of the complexity of inheritance and its interactions with the environment. In doing so, one should seek to preserve and consolidate the undeniable extensive gains made by what might be now regarded as classical or conventional plant breeding approaches. Standing on such a solid foundation,will show that by recognizing and more fully respecting the complex, adaptive and newly-discovered, self-modifying nature of biological information, will have developed novel, complementary approaches to current plant breeding practice. With the benefit of this new perspective, one may better see the way to reach our goals faster, with less costly screening, and for a wider range of environments.

CONTENTS

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Chapter 1

GENETIC AND EPIGENETIC REGULATION OF TRANSGENERATIONAL CHANGES IN GENOME STABILITY: AN OVERVIEW

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Genome stability of every species depends on complex interaction of predefined and environmentally induced genetic and epigenetic states. Predefined states consist of chromatin structure and cell metabolic processes such as DNA repair, radical scavenging and cell signalling, whereas induced states depend on interactions with the environment. Organisms are able to respond to a changing environment by various alterations in their somatic cells as well as in their germline and progeny. In this book, we will describe various phenomena associated with the maintenance of genome stability. These include genetic and epigenetic responses to various stresses in exposed cells and organisms, bystander and bystander-like effects, transgenerational changes in genome stability and stress tolerance in bacteria, plants and animals.

The history of studies of the environment's influence on the phenotypic appearance of organisms and inheritance goes all the way back to Jean-Baptiste Lamarck and Charles Darwin. Their ideas laid an important foundation for the development of the field of research that we are going to introduce you to. With this in mind, we would like to briefly recall how our understanding of interactions between the genetic make-up of organisms and their environment have been formed and developed over time.

The theory of pangenesis suggests that every cell in every organism not only experiences environmental changes and responds to them but also generates molecules capable of contributing to the development of new traits and new organisms. Darwin called these molecules "gemmules" [1]. Darwin's ideas and views were strongly influenced by the work of another great scientist of 19th century, the French biologist Jean-Baptiste Lamarck.

Lamarckism (often referred to as Lamarckian evolution) is a widely accepted idea that an organism can pass on characteristics that it acquired during its lifetime to its offspring. This

theory which is often referred to as a "soft inheritance" is named for its founder Jean-Baptiste Lamarck, who was the first to suggest that acquired characteristics can be passed to progeny. Specifically, he proposed that individual adaptational efforts during the lifetime of the organisms were the main mechanism driving species to adaptation to a changing environment.

Neo-Darwinism is another theory of evolution that represents the "modern synthesis" that combines Darwin's theory of natural selection as a major factor of evolution and population genetics. The term Neo-Darwinism was first used by George Romanes in 1895 and referred to the idea that evolution occurs solely through natural selection, as proposed by Alfred Russel Wallace and August Weismann. Neo-Darwinism suggests that evolution occurs without mechanisms involving the inheritance of acquired characteristics resulting from their use or disuse.

Thus, this 'modernized' Darwinism accepted some ideas that were 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 as a Lamarckian source of variation involving blending inheritance.

Complete rejection of Lamarckian theory of inheritance was based on the germ plasm theory proposed by Weissmann in 1893. He discovered that the cells that produce the germ plasm (gametes) separate from the somatic cells at an early stage of organismal development. Weissman could not understand how somatic and gametic cells would communicate with each other and declared that the inheritance of acquired characteristics was therefore impossible.

Overall, many scientists further tried to prove or disprove Darwin's hypothetical theory of Pangenesis. Francis Galton conducted many experiments that led him to refute this theory. Initially he believed in the pangenesis doctrine and theory, and in consultation with Darwin, he attempted to detect how gemmules were transported in the blood. His hypothesis was very simple. He hypothesized that if gemmules were transferred to gametic cells though the blood, then blood transfusion between various breeds of animals would allow obtaining new traits in offspring. 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 in the transfused blood.

Darwin challenged the validity of Galton's experiment. In his article published in 1871, he wrote: "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 19th century, the scientific world still believed in Darwin's theory of pangenesis. The work of Gregor Johann Mendel on plant hybridization completely changed the scientific approach to understanding the mechanism of inheritance. Although Mendel published his work in 1866, it was not until 1900 that the importance of his work has been re-analyzed. Once Mendel's work was re-discovered, a new era of 'Mendelian' genetics began which rejected the possibility of transmission of information from somatic cells to gametes and thus to the progeny.

It should be noted that many scientists still considered the possibility of environment-induced heritable changes. The Russian scientist, Ivan Michurin was one of the founders of scientific agricultural selection. He worked on hybridization of plants of similar and different origins and on development of new cultivating methods in connection with the natural course of ontogenesis. He was also interested in directing the process of predominance, evaluation and selection and in working out methods of acceleration of selection process with the help of physical and chemical factors. In early 20th century, he proved that 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. Like Lamark and Darwin, Michurin in his works assumed a possibility of changing genotype under external influence.

The fact that phenotypical characteristics can be inherited during hybridization/grafting experiments using different plant cultivars has been reported by many famous plant breeders. Over the past decades independent scientists have repeatedly shown that graft-induced phenotype variations are stable and heritable [2].

In 1928, Trofim Lysenko, one of the proponents of Michurin's ideas of hybridization and grafting, claimed to have developed an agricultural technique of vernalization which used the combination of humidity and low temperatures to make wheat grow in spring. He suggested that using this technique would increase crop yields by several times, which turned out to be impossible. Lysenko attempted to further develop the Lamarckian idea of inheritance of acquired characteristics in an organism. At the same time, he had some fairly strange ideas, suggesting for example, that the state of being leafless as a result of having been plucked could be inherited by the organism's descendants.

Some time ago, the ideas of Lamark and Michurin seemed to be pseudo-scientific and impossible to believe in, did not they? Recently, a breakthrough publication describing changes in genetic make-up of grafted plants was an eye-opener that brought many new possibilities for transmission of genetic material. Sandra Stegemann and Ralph Bock reported transfer of genetic material from stock to scion upon grafting of tobacco plants [3]. The study showed that recipient plants acquired tolerance to an antibiotic in the same manner as donor plants, and it also confirmed the existence of transfer of genetic material from a donor to a recipient. Although it is still unclear whether it occurs via plastid transfer through plasmodesmata or via transfer of a large portion of the plastid genome from a donor cell to a recipient cell, it can definitely be considered an example of changes not only in phenotypic appearance but also in a genetic make-up of a grafted plant.

Ideas of epigenetic regulation of organism development and cell fate have been developing very actively throughout the entire 20th century. The actual name 'epigenetics' did not, however, emerge until 1942 when Conrad Hal Waddington used it to describe how genes might interact with their surroundings to produce a phenotype. During the past 50 years, interest in epigenetics has fallen and risen many times.

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" [4]. The current definition of epigenetics is not so broad; it refers to heritable traits that do not involve changes in DNA sequences. These changes occur during somatic cell divisions and sometimes can be transmitted transgenerationally through the germline.

The field of epigenetics has again exploded onto the scientific scene in the recent years. Numerous articles have been published in top-ranking journals. Nowadays, epigenetics is perhaps one of the most popular fields of studies. Genome sequences of model organisms

such as C. elegans, Drosophilla, Arabidopsis, human, mice, rice, etc. having become available, more and more studies appeared that attempted to understand genome and chromatin organization and explain mechanisms of inheritance, maintenance of genome stability, and regulation of gene expression. What has become clear is that these mechanisms are of genetic and epigenetic nature.

In this book, we will discuss various genetic and epigenetic mechanisms of genome stability regulation in animals and plants. We will also describe the influence of environment on genome stability and epigenetic changes; evidence of inheritance of such changes will also be presented. We will also introduce concepts of non-targeted events, bystander and bystander-like effects, paramutations, and other epigenetics-related phenomena.

Several introductory chapters will describe normal DNA repair and genome maintenance processes in bacteria, plants and animals. Specifically, the chapters by Jody Filkowski and Olga Kovalchuk will introduce the reader to cellular mechanisms of genome stability regulation, including DNA repair and epigenetic regulation of chromatin structure and gene expression in animals. Alex Boyko and Igor Kovalchuk will cover these processes in plants. The chapter by Susan Wallace and colleagues will give a specific example of evolution of DNA repair enzymes - glycosylases in various organisms and underscore their role in maintaining genome stability and species evolution.

The influence of stress on genome stability and genome plasticity will be demonstrated by Grandbastien on the examples of transposon activation in plants.

Several chapters will discuss targeted and non-targeted mutagenesis, stress-induced communication between cells and organisms, and even evidences of transgenerational changes induced by stress.

The chapter by Andrey Golubov will introduce the reader to stress-induced mutagenesis in bacteria and will attempt to analyze whether such events are random or directed. The chapter by Igor Kovalchuk and Andrey Golubov will describe a specific case of sequence-specific DNA degradation used by bacteria for the protection against bacteriophages and will discuss some recent examples of how bacteria can rapidly acquire tolerance to phages.

One of the most interesting examples of an epigenetically controlled process - the non-linear response to DNA damaging agents – will be described by Carol Yauk and John Stead. 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 result in 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. The chapter focuses on the stability of tandemly repeated sequences and analyzes the phenomenon of non-targeted instability of these repeats.

Cell-to-cell communication between neighbouring cells and communication between cells of different tissues and organs of multicellular organisms is well-known and occurs regularly. These communications involve hormonal signalling, neurotransmission, etc. It is believed that damaged tissues are able to communicate with non-damaged tissues - a phenomenon known as bystander effect. The chapter by Jody Filkowski, Yaroslav Ilnytsky and Olga Kovalchuk will present several examples of such bystander effects triggered by ionizing radiation in vivo in an animal model. The chapter by Carmel Mothersill and Colin Seymour will introduce the reader to the phenomenon of transgenerational genome instability in somatic and germ cells in mice.

Organisms are also able to communicate danger to each other through water and air. These communications are often chemical in nature, although in many cases an exact nature of signals is not known. Another chapter by Carmel Mothersill and Colin Seymour will introduce the reader to bystander effect between irradiated and non-irradiated fish.

In plants, mechanisms of intra- and inter-plant communication are often used for pathogen response. Molecules involved in this process include small peptides, volatile organic compounds (methyl salicylate, methyl jasmonate) and even small non-coding RNAs. The chapter by Franz Zemp will introduce the reader to various bystander-like phenomena used by plants to communicate danger between treated and non-treated tissues.

Can organisms communicate memory of stress across generations? According to Darwin, organisms evolve through the process of natural selection from a pool of individuals with spontaneous changes/mutations. 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. Since mutagenesis is a rare process, the fixation of a desired trait would be extremely rare. In contrast, processes of acclimation and adaptation are rapid ones that allow organisms to acquire protection against stress in a single generation after exposure. These processes cannot be explained by the laws of Mendelian genetics.

Several chapters that include works by Igor Kovalchuk, Richard Winn, McGowan, Meaney and Szcyf, Olga Kovalchuk, Carmel Mothersill, Andere Comeau present the data demonstrating the inheritance of stress memory across generations in various organisms.

The chapter by Igor Pogribny shows how the epigenetic machinery of the cell is influenced by a diet and life style.

Chapters by Palak Kathiria and Minoo Rassoulzadegan will describe paramutation in plants and animals, the phenonon that is based on allelic interactions and changes in the phenotype influenced by the environment.

In sum, the current book covers a variety of topics associated with direct and indirect damage to DNA, genetic and epigenetic control of genome stability, local and systemic responses to mutagens, phenomena of non-targeted effects including bystander effect and transgenerational genome instability.

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