Preface

"Advances in Genetics Research" presents original research results on the leading edge of genetics discovery. Each article has been carefully selected in an attempt to present substantial research results across a broad spectrum. In this continuing series compilation, the authors present and discuss varied topical data such as cytogenic mapping of animal genomes; somatic cell counts in cow's milk and implications for dairy cow breeding; mutations and the carcinogenic process; genetic mapping projects and functional genomics.

Chapter 1 - Decades before the recent advances in molecular biology and the knowledge of the complete nucleotide sequence of several genomes, cytogenetic analysis provided the first information concerning the genome organization. Since the beginning of cytogenetics, great effort has been applied for understanding the chromosome evolution in a wide range of taxonomic groups. The exploration of molecular biology techniques in the cytogenetic area represents a powerful tool for advancement in the construction of physical chromosome maps of the genomes. The most important contribution of cytogenetics is related to the physical anchorage of genetic linkage maps in the chromosomes through the hybridization of DNA markers onto chromosomes. Several technologies, such as polymerase chain reaction (PCR), enzymatic restriction, flow sorting, chromosome microdissection and BAC library construction, associated with distinct labeling methods and fluorescent detection systems have allowed for the generation of a range of useful DNA probes applied in chromosome physical mapping. Concerning the probes used for molecular cytogenetics, the repetitive DNA is amongst the most explored nucleotide sequences. The recent development of bacterial artificial chromosomes (BACs) as vectors for carrying large genome fragments has allowed for the utilization of BACs as probes for the purpose of chromosome mapping. BACs have narrowed the gap between cytogenetic and molecular genetics and have become important tools for visualizing the organization of genomes and chromosome mapping. Furthermore, the use of chromosome probes has permitted the development of chromosome painting technologies, allowing an understanding of particular chromosomal areas, whole chromosomes or even whole karyotypes. Moreover, chromosomal analysis using these specific probes has contributed to the knowledge of supernumerary chromosomes, sex chromosomes, species evolution, and the identification of chromosomal rearrangements. Finally, the synergy between chromosomal and molecular biology analysis makes cytogenetics a powerful area in the integration of knowledge in genetics, genomics, taxonomy and evolution.

Chapter 2 - This chapter is a review of studies on SCC in cow's milk and its practical implications for dairy cows breeding, in particular for improving cows' resistance to mastitis. First, the morphology of somatic cells in milk is discussed. Then, an outline is given of various methods for SCC determination and mastitis diagnosis, including their advantages and disadvantages. Furthermore, the factors that affect SCC and mastitis are presented. These have been divided into quarter-, cow- and herd-level environmental factors and genetic factors. The most important environmental factors include mastitis-causing pathogens, other diseases, teat injuries, udder conformation, cow's condition, temperature and housing hygiene. These factors, directly or in combination with genetic factors, lead to udder inflammation account for 90–95% of all the stimuli that affect the udder. Genetic parameters related to SCC such as heritability, repeatability, and genetic and phenotypic correlations have been also discussed.

Despite the considerable advances in technology, the problem of *mastitis* persists and the costs related to the incidence of this disease constitute an increasingly high portion of the total expenses incurred on animal keeping. It is therefore necessary to focus on the genetic aspects of the disease. In many countries, dairy cattle breeding programs have given priority to immunity to mastitis, even at the cost of slowing down or halting the progress of research on improving milk utility traits.

Chapter 3 - Mutation is a possible consequence of DNA damage and it is the most prominent event of the carcinogenic process. Spontaneous mutations originate from endogenous metabolic processes, such as inaccurate DNA replication or repair, whereas induced mutations arise from exposure to exogenous agents. Once DNA damage occurs, a complex network of repair functions is triggered to protect cells against the deleterious consequences of mutations and in order to maintain genomic stability. Among the several DNA repair pathways available in eukaryotic cells, base excision repair (BER) is required to repair non-bulky DNA adducts such as oxidized bases. Poly (ADP-ribose) polymerase-1 (PARP1) is a ubiquitous protein in mammalian cells, with a relevant role in BER and participating also in other complex biochemical processes including double strand break repair, chromatin remodelling and cell death. Hence, defective PARP1 activity would be expected to influence the number or type of gene and/or chromosome mutations resultant from exposure to genotoxic agents and, eventually, to be implicated in cancer. Recently, we generated a *Parp1*$^{-/-}$ *lacZ* transgenic mouse model to investigate the effect of PARP1 deficiency *in vivo* on the mutagenic and clastogenic responses to carcinogens. In this chapter, we present data showing the occurrence of a higher spontaneous genomic instability and a modified mutagenic response to genotoxic insults in *Parp1*-deficient mice. Particularly, the higher level of deletion mutations in the germ line of those mice might constitute an added risk for hereditary diseases. Inversely, it might provide genetic diversity, representing an advantage for survival. Interestingly, although we found that PARP1 inactivation causes some genomic instability *in vivo*, cancer propensity was not observed either in this mouse line or in two other different *Parp1*-deficient mouse models. Even though, defects in DNA damage responses that are not directly responsible for a disease can be important and exploited to be used for the benefit of health. This has been the case of molecules acting as PARP inhibitors, which have been developed to enhance the effectiveness of anticancer drugs or even to function as chemotherapeutic agents *per se*. The rational for their therapeutic application is based, precisely, on the increase of mutations in tumor cells upon PARP1 abrogation, triggering cell death. However, considering the present knowledge about the impact of

PARP1 deficiency in mutation accumulation and the uncertainties about its association with somatic or hereditary diseases, the cost/benefit of the clinical use of PARP inhibitors in humans should be carefully evaluated.

Chapter 4 - With the completion of the Human Genome Project the physical location of all the human genes have been identified, but the function of many has yet to be studied or described in detail. Whilst there are many ways to identify the function of a gene, one of the most powerful methodologies is to link a gene to a disease phenotype. This can be done with large population-based association studies for common polygenic conditions with low penetrance and strong environmental factors, or with smaller pedigree-based mapping projects that are better suited to rare monogenic diseases for which the pathogenic mutations show 100% penetrance. For the latter, a candidate-positional approach has superseded a purely positional cloning strategy for the identification of human disease genes. However, the essential prerequisite for candidate-positional gene identification is unequivocal mapping of the disease locus. Autozygosity mapping (originally homozygosity mapping) has led to the identification of a plethora of disease genes for recessive conditions. The "On-line Mendelian Inheritance in Man" database, as of October 2009, lists about 2700 autosomal mendelian phenotypes with a known molecular basis, of which over 1100 are recessive genes identified by autozygosity mapping. However, over two decades on from the first positional identification of a human disease-causing gene, it is notable that an estimated 2300 autosomal recessive conditions, or 37% out of a total of about 6200 annotated mendelian phenotypes, still do not have any identified molecular defect. Autozygosity mapping therefore remains a very active area of medical research, with a succession of major insights into disease pathogenesis resulting from the greater power of modern genetic technologies. Furthermore, the insights obtained from rare recessive disorders often elucidate the pathogenesis of more common complex diseases, including cancers, hypertension, neurodegenerative conditions and diabetes (Peltonen et al., 2006).

Mapping projects generally consist of three main stages: the identification of suitable pedigrees, the detection of regions with linkage to the disease phenotype, and finally the detection of the disease mutation. This chapter will focus solely on the identification of suitable pedigrees and then the detection of linkage to the disease locus. Pedigree-based mapping can be applied to both recessive and dominant diseases, as long as the condition shows 100% penetrance. Since the strategies differ significantly between the two approaches, it is worth expending effort to ensure that the inheritance patterns follow the expected model. A common cause of error often arises when both homozygous and heterozygous individuals present with the same phenotype, although heterozygous carriers may have a milder or attenuated form of the condition which could be missed on a cursory clinical examination. For a condition modelled as dominant this could lead to mildly affected (heterozygous) individuals to be scored as unaffected, whereas, if a recessive model is used, it could result in obligate carriers being analysed as fully affected individuals. In both instances this would confound linkage analysis. A similar error may arise if the affected individuals are not easily distinguished from individuals with a similar phenotype but who are affected with a different condition (genetic heterogeneity) or even an environmental mechanism (a phenocopy). For example, certain forms of ataxia show a strong genetic component, but also have environmental causes. If a distantly related ataxic individual is included in the analysis of a family with multiple affected sibs it is possible that the former cause of ataxia is due to an environmental mechanism and not a common genetic lesion.

Chapter 5 - In the light of biological achievements, in particular, the discovery of the somatic cell hybridization process, the karyogamic theory of the tumor genesis acquirs more real contours. This theory maybe considered as general (common or integrate) theory of carcinogenesis, which includes the principal aspects of the most popular and acceptable theories and hypotheses for today. Influence of physical, chemical and biological carcinogenic agents on somatic cells probably are adequate. After their influence, cells' fusion originates as a result of cytoplasmic membranes perforations. Malignancy should be ascribed to one of the rather widespread processes taking place in the organism daily and being genetically inherited in a normal somatic cell. Any combinations of cancer cells with other differentiated and nondifferentiated normal somatic cells are possible. Cellular subpopulations are constantly formed in tumors without any obvious regularity and in any other tumor coexist phenotypically and genotypically different cells. This is the reason of different histogenesis, and heterogeneity of tumors. True tumorous cells (synkaryons) are probably formed very rarely. From numerous heterokaryons, homokaryons and hybrid cells after the influence of carcinogenic agents and promoters only few precancerous cells can acquire the potency of unlimited proliferation. There are indirect data and experimental works confirming hybrid essence of cancer cell. Proceeding from the cancer cell's hybrid nature, we try to interpretate the possible mechanism of carcinogenesis in case of some pathologies.

Chapter 6 - Alkylators, originally used for chemical warfare during World War I, were the first chemical therapeutics to be developed for cancer treatment. Currently there are five classes of alkylating agents used in the clinic: nitrogen mustards, ethyleneimines, alkyl sulfonates, nitrosoureas, and triazenes. These chemicals are additionally divided into 2 categories; monofunctional agents that modify DNA by single alkyl group modification and bifunctional agents containing two reactive alkyl groups that can crosslink DNA. This chapter will focus on the triazenes Temozolomide (TMZ), a chemotherapeutic monofunctional alkylator utilized for treatment of glioma, and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), a direct monofunctional alkylator employed as a tool in preclinical investigations. Cell death from these chemicals is associated with the accumulation of DNA alkylation lesions that do not manifest cytotoxicity until after chromosomal replication. The cellular pathways culminating in death, or in the escape from death, are not well understood. The DNA repair processes that protect cells from cytotoxic effects of TMZ and MNNG have been identified; the base excision repair (BER) pathway, the methylguanine methyltransferase (MGMT) enzyme, and homologous recombination (HR). Conversely, DNA mismatch repair is required to elicit the cytotoxic DNA damage response triggered by unrepaired O^6methylGuanine lesions. The efficacy of specific agents and methodologies to inhibit BER, MGMT or HR repair, or to enhance pre-existing repair deficiencies are reviewed in this chapter.

Chapter 7 - Ionizing radiation has been proved a major stress that can induce carcinogenesis. Nuclear DNA is the main target of ionizing radiation, exposure of which is followed by many types of DNA damages. DNA double-strand breaks (DSBs) induced by ionizing radiation are considered the most relevant lesion for mutations and carcinogenesis, and unrepaired or misrepaired DSBs are a serious threat to genomic integrity. The increased mutation induced by radiation has been proved to be tightly associated with carcinogenesis. Radiation-induced bystander effect (RIBE), which was found in the 1990s, challenged the conventional dogma that no effects were expected in the cell population that had not been exposed to radiation. With the RIBE, the irradiated cells could secret some signal factor(s) to

affect the nearby non-irradiated cells or cells that had received the transferred conditioned medium, and then to induce DSBs, mutation and cell death etc. in the non-irradiated cells. As such, RIBE "enlarges" the area or the target of bio-effect of radiation from the directly irradiated cells to non-irradiated cells surrounding or even away from the irradiated cells, with the effect particularly significant in the low radiation-dose regime. The existence of RIBE led to a non-linear relationship between the cancer risk and the radiation dose (in the low-dose regime). Studies on the mechanism underlying RIBE have also enlightened us on directions to radiation protection against low-dose environmental radiation as well as during radiotherapy.

Chapter 8 - Somatic cells can be reprogrammed and serve as genetic material to generate: 1) a new individual (by somatic cell nuclear transfer (SCNT)), 2) embryonic stem cells (ESCs) (by SCNT or induced pluripotent stem cells (iPS)), or 3) a cell type of another kind than the original somatic cell. Production of ESCs from the patient's own somatic cells is an attractive supply in medicine, in particular as individual human model system for drug-screening, -design or toxicology testing, but could also be used in regenerative medicine. ESCs containing patient-specific genome can multiply in tissue culture and upon external signals be differentiated into any cell type of interest. In this way, patient-specific insulin producing cells can be generated from somatic cells like skin cells and transplanted into patients suffering from Diabetes or patient-specific dopamine producing cells can be grafted into individuals suffering from Parkinson's, thereby avoiding immune-suppression. This article provides some background, molecular mechanisms and breakthroughs underlying reprogramming of somatic cells and discusses some of the obstacles to overcome.

Chapter 9 - Functional genomics studies play very important roles in gene discoveries and genetic improvements in soybeans (*Glycine max*). Reverse genetics plays a unique role in soybean functional genomics, which would be impossible to achieve through either forward genetics or other conventional genetic approaches. To date, soybean genomics research has already entered post-genome era, as the soybean genome sequencing comes to a completion, and functions of its sequences at a whole genome level remain to be verified. This task requires high-throughput and simple means of functional analysis of soybean genes. There are several reverse genetics tools currently available to aid in soybean functional genomics study. These mainly include TILLING (Targeting Induced Local Lesions IN Genomes), fast neutron mutagenesis, *Ac/Ds* (*Activator/Dissociation*) transposon tagging, *Tnt1* retrotransposon mutagenesis, and *mPing*, etc. Nonetheless, each of these approaches has its advantages and disadvantages. Additionally, because soybean possesses a complex genome, with a large percentage of genes having gene family members, it is a challenge to verify the functions of many soybean genes through any of these approaches. Therefore, RNAi (RNA interference), gene trap mutagenesis system, and complementation with BIBAC (Binary Bacterial Artificial Chromosome) libraries could provide better means for verifying functions of many genes. In any case, a highly-efficient soybean transformation system that is also capable of generating high quality events is yet to be developed for utilizing the above reverse genetics approaches. However, development of such high-throughput transformation system in soybean has been proven to be very challenging. The large size and long life cycle of a soybean plant present additional difficulties in the growing, maintaining, and functional screening of a large number of transgenic soybean plants, owing to the constraints in growth chambers, seed storage, greenhouses or field plots. These challenges must be resolved, otherwise they will adversely

impact soybean functional genomics studies. This review will also discuss possible solutions for some of these critical issues.

Chapter 10 - Tissue culture of plant cells requires stable conditions for its continuous growth therefore alterations to such conditions can lead to the expression of different compounds resulting in physiological changes and growth reduction. Coffea canephora cell lines are prone to cell death upon alterations in their growing conditions. Here we determine that cell communication ligated with auxin deprivation induces cell death and expression of phenolic compounds as well as DNA fragmentation. These conditions also reduce the amount to the transcription factor E2F which has been ligated to several cell cycle steps as well as in apoptosis.

Chapter 11 - One of the best examples of modern analogues of early microbial life are living stromatolites. Stromatolites are biosedimentary structures produced by the sediment-trapping, binding and/or precipitation activity of resident microbial communities. The presence of stromatolites dates back to some 3.5 – 3.8 billion years in earth's history, a time period that witnessed the appearance of the first forms of life. Modern stromatolites provide scientists with a window to the past: they provide an unprecedented opportunity to study some of the Earth's earliest ecosystems and the oldest convincing evidence of life on Earth. From an astrobiological point of view, the biological and geological information stromatolites contain also offer the tantalizing prospect of allowing a more rational search for both past and present life on other planets. Recent studies have begun to reveal the true and astounding complexity of these microbial ecosystems, a process that accelerated greatly with the development and application of current techniques of functional genomics and proteomics. This short review will discuss some of the major advances in understanding the biological complexity of these systems, underpinned by employing cutting-edge techniques in functional genomics. The area of functional genomics and metagenomics are likely to lead the way in future research that will provide us with an unprecedented understanding of early life.

Chapter 12 - An active nonautonomous DNA transposon, *nDart1-0*, belonging to the *hAT* superfamily, was identified as a causative element for a variegated pale-yellow leaf mutant derived from F2 of a remote cross between *indica* and *japonica* varieties. Active transposition of *nDart1-0* was promoted by an active autonomous element, *aDart1-27*, on chromosome 6. By using the endogenous *nDart1/aDart1-27* system in rice, a large-scale *nDart*-inserted mutant population could be easily generated under normal field conditions, and the resulting tagging lines were free of somaclonal variation. Thus, most of the mutants in the *nDart*-tagging population are assumed to be caused by insertions of *nDart1* transposons. Indeed, we have identified several tagged genes for dwarfs, chlorophyll aberrations, and other traits through *nDart1-0*-specific iPCR or transposon display methods. In particular, our finding of gain-of-function mutants for increasing grain number could be useful for a breeding material in the *nDart*-tagging population. The *nDart1* transposons tend to insert into the promoter, 5' UTR region, or exon of a gene, which suggests that the *nDart1/aDart1-27* system is a powerful tool for rice functional genomics. Furthermore, we are developing several *indica* lines bearing the active *nDart1/aDart1-27* system. These lines would effectively contribute to gene functional analysis and breeding for the *indica* rice varieties.