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 the role of IL-1 in the cytokine network of the body; genetic manipulation of leishmania parasites facilitating the exploration of polyamine biosynthetic pathways as potential therapeutic targets; RNA interference-mediated cell camouflage; identification of RNA in analysis of body fluids stains for forensic purposes; cationic lipid-based nanoparticles for siRNA delivery; Interleukin-1 in osteoarthritis; fungal genetic manipulation using RNA silencing; microglial cathespin B-dependent IL-1B production; role of miRNAs in early embryonic development of drosophila melanogaster; and pHg/pSILBAy vector system for RNA silencing in mycorrhiza via agrobacterium-mediated transformation.

Chapter I – This review, based on our own data and data of other researchers, is devoted to regulatory relationships of IL-1 in the body's cytokine network in normal and pathology. The characteristics of cytokines, belong to IL-1 family (IL-1F1 – IL-1F11) are presented. The regulatory relationships between the cells, producers of IL-1, are discussed. The results of our own research on the asymmetrical expression of IL-1β and IL-1receptor type I genes in mouse brain hemispheres in relation to the intensity of the developed humoral immune response and expression of behavioral reactions are presented. The relationship between IL-1 family genes polymorphism and predisposition to some diseases, character of inflammatory process and sensitivity to cytokine and anti-cytokine therapies are characterized.

Chapter II - Parasites of the genus *Leishmania* cause a variety of devastating and often fatal diseases, ranging from cutaneous ulcerative lesions to fatal visceralizing infections that affect an estimated 12 million people worldwide. Unfortunately, vaccines are not available and the current arsenal of drugs used to treat leishmaniasis is far from ideal. Thus the need for new therapeutic targets and a better understanding of host-parasite interactions is urgent. One biochemical pathway that has been successfully exploited for the treatment of a related parasitic disease, African trypanosomiasis, is the polyamine biosynthetic pathway. In order to elucidate the polyamine biosynthetic pathway and to explore its potential as a therapeutic target in *Leishmania*, the Authors have generated and characterized gene deletion mutants and polyamine enzyme overproducer strains. These studies revealed that the polyamine pathway in *Leishmania* is significantly different from that of the mammalian host and polyamines were found to be essential for parasite proliferation. Infectivity studies in macrophages and mice

with gene deletion mutants revealed that at least two polyamine biosynthetic enzymes, ornithine decarboxylase and spermidine synthase, are necessary for *Leishmania donovani* to establish a successful infection. However, arginase gene deletion mutants of *Leishmania mexicana* are still capable of eliciting an infection, albeit at lower levels than wild type parasites. Ongoing studies address whether the disparities in infectivity are due to the loss of specific enzymes within the pathway or to differences between the two *Leishmania* species. Furthermore, the gene deletion mutants are useful tools to investigate the relative contribution of host and parasite polyamine biosynthetic enzymes in parasite infectivity. This chapter will summarize how genetic manipulations in *Leishmania* have advanced our understanding of the polyamine pathway and its role in host-parasite interactions.

Chapter III - The high variability of the major histocompatibility antigen (MHC), in humans known as the human leukocyte antigen (HLA), loci remains a major obstacle to allogeneic transplantation. In this chapter the Authors describe the use of RNA interference (RNAi) technology to reduce the immunogenicity of cell-based therapeutics. This approach does not only overcome the drawbacks of HLA protein variations, but also prevents the presentation of minor histcompatibility antigens derived by the proteasomal cleavage of other polymorphic molecules. The cell surface expression of HLA molecules was silenced by the delivery of short hairpin RNAs (shRNA) targeting HLA class I or HLA class II transcripts. Lentiviral vector systems encoding for the shRNA sequences demonstrated to efficiently contribute for a permanent knockdown of the target expression. The expression of HLA proteins was suppressed in several cell lines and primary cells including B-lymphoblastoid cells, monocytes, fibroblasts or CD34+ progenitor cells. Furthermore, the expression of HLA proteins was stably silenced in tissue in its original 3D-structure. *In vitro*, HLA class I silencing showed to protect the target cells against antibody-mediated cytotoxicity. Furthermore, this strategy prevented the recognition of HLA class I or class II silenced cells by allogeneic antigen-specific CD8+ or CD4+ T-cells and abrogated the capacity to initiate a *de novo* immune response. Levels of cytotoxic T-cell lysis or pro-inflammatory cytokine T-cell secretion such as interferon-γ, TNF-α, IL-1β, IL-6 or IL-8 were significantly lower when HLA silenced cells were used as targets in comparison to non-modified cell targets. Also using an allogeneic transplantation rat model, RNAi-mediated MHC class I silencing showed to significantly contribute to the graft survival. The shRNA-mediated HLA silencing effect of CD34+ progenitor cells showed to be maintained during differentiation. In fact, the adult cell types differentiated from HLA-silenced CD34+ cells showed to lack cell surface HLA expression, but their functional activity was comparable to those differentiated from non-engineered progenitor cells. This data suggests that shRNA-mediated HLA knockdown confers an efficient camouflage to cells with an allogeneic origin via preventing their recognition by the recipient's immune system. RNAi-mediated HLA silencing has the potential to revolutionize the field of transplantation by promoting the survival of allogeneic grafts with very low or no immunosuppressive regimens. RNAi technology may pave the development of new therapeutic concepts in the field of regenerative medicine.

Chapter IV - Body fluid stains recovered at crime scenes are important types of evidence to forensic investigators. The first step of identifying a particular body fluid is essential since the nature of the fluid is itself very important to insure the correct handling of the sample (e.g. in mixtures from different sources). However, the destructive nature of the conventional screening test must be considered when only a large amount of material is available. The ability to characterize an unknown stain at the scene of the crime without having to wait for

results from a laboratory is another very critical step in the development of forensic body fluid analysis.

In the last years, mRNA and microRNA (miRNA) analysis has demonstrated to be a promising method for the identification of body fluids resulted in a trend to bypass conventional body fluids identification methods for the advantage that RNA can be isolated simultaneously with DNA, therefore avoiding sample loss.

First publications showed that RNA can be isolated in suitable quality and quantity from menstrual blood, blood, saliva and vaginal secretions. Afterwards, several authors published a number of articles regarding the applications of RNA to further stains as seminal fluid and epithelial cells. These works address the challenge of isolating RNA and DNA in suitable quality and quantity from limited amounts of stains as well as the validation of methods to analyse certain transcripts, e.g. by end point PCR or real time PCR and suitable sets of stable RNA markers.

mRNA and miRNA present different advantages and limitations in their application of body fluids stains analysis. This chapter aims to give an overview of the possible advantages of RNA application and to shed further light into the limitations of this method.

Chapter V - Since the RNA interference discovery, siRNAs have emerged as an undisputed laboratory tool to knockdown a gene target. However, even though RNAi is a highly specific technique and has the potential to selectively silence the expression of any gene, the systemic delivery of RNAi molecules remains a challenge.

Recently, the Authors developed a clinically acceptable efficient formulation of siRNA. This tri-component delivery system consisted of (i) the cationic lipid 2-{3-[Bis-(3-amino-propyl)-amino]-propylamino}-N-ditetradecyl carbamoyl methyl-acetamide, which is termed DMAPAP, (ii) the neutral lipid 1,2-dioleoyl-sn-glycero-3-phospho-ethanolamine or DOPE and (iii) an anionic polymer that served as an enhancer of lipoplexes efficiency. Several anionic polymers are able to enhance the gene silencing effect of the lipoplexes into which they are incorporated. This Chapter characterized the physico-chemical properties of these siRNA lipoplexes as well as their gene silencing efficiency, cytotoxicity and in vivo localization upon i.v. administration to mice. Also shown is that these lipoplexes are suitable to be used as a systemic siRNA vector.

Chapter VI - Osteoarthritis (OA) is the most common form of joint disease. It is characterized by the progressive destruction of articular cartilage and subchondral bone accompanied by low-grade inflammation that together result in pain and deformity. During the progression of OA, there are several cellular changes in joint, including an increase of interleukin-1 levels in synovial fluid. Although the etiology and pathogenesis underlying this disease are poorly understood, *in vitro* experiments, as well as animal studies, provide support for a key-role of IL-1 in the development of OA. This pro-inflammatory cytokine controls, in particular, the degeneration of articular cartilage matrix, by stimulating the expression of metalloproteinases and aggrecanases. It also counteracts the majority of anabolic effects of Transforming growth factor-beta (TGF-beta) and reduces the production of cartilage-specific macromolecules, including type II collagen. This chapter focuses on IL-1 functions in cartilage and discusses the experimental trials of the neutralization of this mediator as an OA therapy.

Chapter VII - RNA silencing is a homology-dependent negative gene regulation mechanism widely conserved in eukaryotes. Among lower eukaryotes, filamentous fungi were pioneering in the RNA silencing studies. Since it was initially described as "quelling" in

the filamentous fungus *Neurospora crassa*, a large number of players, effectors, pathways and functions have been identified and characterized in the complex mechanism known as RNA silencing. The exhaustive research undertaken in different fungal study models has allowed the accumulation of an important amount of knowledge, and yet new and interesting aspects of RNA silencing are being discovered. Besides that, research on fungal RNA silencing has also generated an extensive list of genetic tools designed to explore and manipulate gene function in fungi. This chapter summarizes the most important discoveries related with RNA silencing in fungi and specially reviews the different strategies that have been developed to manipulate gene expression in the fungal kingdom.

Chapter VIII - Interleukin-1β (IL-1β) and IL-18 are potent proinflammatory cytokines that play key roles in the pathogenesis of the brain aging and diverse range of neurological diseases including Alzheimer's disease, Parkinson's disease, stroke and persistent pain. Activated microglia are the main cellular source of IL-1β and IL-18 in the brain. Two signals mediated by Toll-like receptors (TLRs) and Nod-like receptors (NLRs) are required for the production and secretion of IL-1β and IL-18. TLRs leads to the induction of pro-IL-1β, pro-IL-18 and NLRP3, the best characterized inflammasome. The leakage of cathepsin B (CatB) from lysosomes has suggested to trigger the activation of the NLRP3 inflammasome in microglia/macrophages after phagocytosis of various molecules including β-amyloid peptide. After activation, the NLRP3 inflammasome can mediate pro-caspase-1 activation to promote the processing and secretion of IL-1β and IL-18. However, the precise role of leaked CatB in the activation of NLRP3 inflammasome remains to be determined. Furthermore, there is still evidence suggesting that CatB is associated with the maturation of pro-IL-1β and pro-IL-18 in the endosomal/lysosomal system, because CatB can effectively cleave pro-caspase-1 in a cell-free system only at acidic pH. Recently, we have further provided evidence that CatB can directly activate pro-caspase-1 in the phagolysosome of microglia. Here, the chapter reviews the current understanding the mechanism by which the roles of CatB in the processing and secretion of IL-1β and IL-18. Also discussed a possible involvement of CatB in the pathogenesis of inflammatory pain and brain aging.

Chapter IX - MicroRNAs (miRNAs) are small (≈22 nucleotides) noncoding RNA molecules and are thought to play an important role in regulating gene expression at various biological stages. Although the biological functions of most miRNAs are unknown, miRNAs are thought to regulate the gene expressions in embryo development. Since cis-regulatory elements regulate the gene expressions in the early embryonic development of *Drosophila melanogaster*, this article analyzes the relations between the nucleotide sequences of *D. melanogaster* miRNAs and cis-regulatory elements *bcd, hb, eve, kr,* and *gt*. Finding from consensus sequences that individual miRNAs are closely related to the 5' upstream regions of these genes, the article proposes that miRNA mediates inquiries and responses in regulatory interactions between genes. This article also analyzes the relations between the frequency probabilities of miRNA consensus sequences and random sequences in the 5' upstream regions of the target genes and shows that the probabilities of consistencies between individual miRNAs and 5' upstream regions are greater than those expected for same-length sequences with random nucleotide frequencies. These results thus imply that some miRNAs are closely related to the 5' upstream regions of certain genes and play important roles in the regulation of gene expression during the embryonic development of *D. melanogaster*.

Chapter X - Mycorrhizas, fungal-root associations, are the most prevalent mutualistic symbiotic relationships on earth. In nature the plant nutrient uptake from the soil takes place via the extraradical mycelia of the symbiotic fungi as an exchange for photosyntates. While most herbaceous plants and tropical trees form endomycorrhiza-type interactions, trees of boreal and temperate ecosystems are typically ectomycorrhizal (ECM). These species include the majority of ecologically and economically important trees and the fungal symbionts are mainly homobasidiomycetes. The symbiotic phase in the life cycle of ECM homobasidiomycetes is the dikaryon. Therefore, studies on symbiosis regulated gene functions in these organisms require the inactivation of both gene copies. RNA silencing is a sequence homology-dependent degradation of target RNAs in the Eukaryota domain. In different such organisms, like fungi, the RNA silencing pathway can be artificially triggered to target and degrade gene transcripts of interest, resulting in gene knock-down. Most importantly, RNA silencing can act at the cytosolic level affecting mRNAs originating from several gene copies and different nuclei thus offering an efficient means of altering gene expression in dikaryotic, diploid and polyploid organisms. The pHg/pSILBAγ silencing vector was constructed for RNA silencing triggering in homobasidiomycetes using the ECM fungus *Laccaria bicolor* as a model. This cloning vector harbors the *Agaricus bisporus gpdII*-promoter, two multiple cloning sites separated by a *L. bicolor* intron and the *Aspergillus nidulans trp*C terminator. The pSILBAγ plasmid allows a two-step PCR-cloning of hairpin sequences for expression in homobasidiomycetes. Further cloning into pHg, a pCAMBIA1300-based binary vector carrying a hygromycin resistance marker for fungal selection, makes the pHg/pSILBAγ system compatible with *Agrobacterium*-mediated transformation. The pHg/pSILBAγ vector usually produces single integrations of T-DNAs in the fungal genome. Moreover, the integration sites can be determined by plasmid rescue in *E. coli* as pHg/pSILBAγ carries an ampicilline selection marker and an ORI from pBluescript in its T-DNA. Besides the optimized use in *L. bicolor* for RNA silencing and overexpression, this vector system is compatible with other homobasidiomycetes and different transformation techniques.