Preface

The term "Gene" refers to a segment of DNA present inside the genome that codes for a protein. Depending on the organism, a particular genome may encode thousands of diverse types of genes responsible for various functions. These genes are differentially expressed and tightly regulated as needed at different stages of cellular and physiological functions including cell cycle progression, differentiation, and development. Gene expression may be influenced by various environmental factors and stimuli such as temperature, nutrients, hormones, stress etc. Though the fundamental mechanism of transcription (production of mRNA from the protein coding genes) is very similar in different organism, the mechanism of gene regulation and the machineries involved are distinct for different organism. In the post genomic and epigenomic era, it is increasingly being recognized that gene expression is not only controlled by gene sequences but also various other non-genomic factors that include protein and DNA modifications and non-coding RNAs. Various aspects of gene expression in prokaryotes and eukaryotes, the mechanism of transcription and gene regulation and influences of various environmental factors are highlighted in different chapters of this book.

Chapter I - Transcription is the process in which a complementary copy of RNA is produced from a sequence of DNA. RNA polymerase (RNAP) is the enzyme responsible for RNA synthesis. Transcription is the first step leading to gene expression and is the step at which most of gene regulation occurs.

Chapter II - One of the most fascinating and intriguing aspects about living organisms is their diversity. Even in bacterial populations composed of sister cells with identical genotypes there is clear evidence for wide phenotypic diversity. For example, this diversity is visible in distinct division rates, genes expression levels, cells size, among many other traits. Fluctuations in environmental conditions tend to enhance the degree of diversity as the responses of the cells to the changes will be diverse as well. Several sources of this diversity have been identified. In bacteria and other prokaryotic organisms, one of the sources that has been studied in greater detail, as it is likely to be one of the key sources, is the process of gene expression. The expression of each gene, the process by which RNA and proteins are produced, generally involves a very small number of molecules in the cell, and can occur rather infrequently. Recent observations of the number of RNA or of protein molecules expressed by individual genes at the single cell level in monoclonal bacterial populations revealed wide cell-to-cell diversity. The diversity is a consequence of the stochastic (i.e. 'noisy') nature of gene expression. Consequently, during a given time interval, the same gene

in two sister cells under the same environmental conditions, by chance, produces different numbers of RNA molecules and/or proteins. As proteins participate in virtually all processes taking place in cells, differences in protein numbers tends to result in distinct 'macroscopic' phenotypic traits such as cell size, lifetime, etc. The cell-to-cell diversity in the numbers of RNA and protein molecules is further affected by the process of degradation of these molecules, as this process is also stochastic in nature.

Nevertheless, as noted, there are other sources for the observed cell-to-cell diversity in RNA and protein numbers in bacterial (and other) cell populations. Recent evidence suggests that one important source is the process of cell division. During the division of a cell, even if this process is morphologically symmetric, the partitioning of RNA and proteins, among other molecules, is not likely to be perfectly symmetric. Further, there is also evidence that is some cases, the partitioning is purposively biased. Deviations from a perfectly symmetric partitioning are likely to be the first source of diversity between two daughter cells. Due to the existence of these and other sources of cell to cell diversity in RNA and protein numbers following their production, to assess the degree of noise in gene expression the authors must observe the kinetics of production of individual RNA and protein molecules, one at a time, in live cells. For that, they need to visualize these molecules, as soon as each is produced, and study the intervals between production events. Also, to study the contribution to cell to cell diversity of the partitioning of these molecules when cells divide, the authors need to observe the partitioning dynamics in individual division events. Recent techniques made these goals possible. Here, the authors describe studies that made use of these techniques to achieve this goal, as well as the conclusions derived from them.

Chapter III - Factor 420 (F_{420} Cofactor, (7,8-didemethyl-8-hydroxy-5-deazariboflavin)) is an NAD(P) analog whose structure is reminiscent of 5-deazaflavin that is found in *Streptomyces* mycobacteria, Nocardia, methanogenic archaea, sulfate-reducing archaea, and haloarchaea. Because of its high abundance in cells, F_{420} Cofactor is believed to be the major electron transfer entity in methanogenic bacteria by transferring electrons from hydrogen atoms to the consecutive intermediates of methane biosynthesis, thereby mediating the bulk of electron flow. Like NAD(P), F_{420} mediates exclusively two-electron transfer reactions and has a low redox potential (-340 to -350 mV) which is required for specific energy producing reactions such as the conversion of carbon dioxide to methane. Here the step-by-step biosynthesis of the F_{420} Cofactor including involved enzymes, their mechanisms, biological significance and medical relevance are described. The phylogenetic profiling of the enzymes dependent upon this cofactors will also be discussed.

Chapter IV - Regulation of gene expression through mRNA stability is conventional cellular mechanism in post-transcriptional mRNA regulatory network. Systemic or cell specific mRNA stability modulates the steady state level of different mRNAs in transcriptome by altering mRNA half life to finally regulate connectivity and network cross-talks in cellular homeostasis. Structural processing of various mRNAs like co-transcriptional 5'-end capping, deadenylation, formation of splice variants and alternative cleavage affects mRNA stability and gene expression dynamics. Intrinsic codes in mRNA like AU Rich Elements, GU Rich Elements, G-Quadruplex and coding region determinants represent other controlling elements for formation of specialized secondary and tertiary mRNA domains that act as functional sites for mRNA sensors, effectors and remodeling machineries to maintain regulated mRNA life cycle, in terms of mRNA stability, mRNA accumulation and translation. Signal specific changes in mRNA stability are mediated by cooperative and coordinated activity of mainly

RNA binding proteins like HuR, AUF1, nucleolin and non-coding RNAs. Differential mRNA stability plays major role in cell proliferation, differentiation and programmed cell death and its deregulations have significant implications in cancer development and manifestations in metastasis associated microenvironment modulations. This review highlights the molecular aspects of mRNA stability in the light of cancer development and potential therapeutic targets.

Chapter V - In nature, plants are under regular stress from various biotic agents such as herbivores, pathogens and even conspecifics competition. Complicated fitness-enhancing responses are initiated that subsequently are transduced by a MAPK cascade and a complex network of phytohormones (such as Salicylic Acid (SA), Jasmonic Acid (JA) and Ethylene). These signals spread throughout the plant via the vasculature to reconfigure its transcriptome, proteome, and metabolome. Such reconfigurations ultimately form the basis of the direct and indirect defenses and tolerance responses that enable plants to survive in stressful environments. Although transcription factors mediate changes in levels of phytohormones, the speed and magnitude of responses suggest other regulators such as small RNAs (smRNAs) are involved. smRNAs have appeared as an important layer of regulation of gene expression in several developmental and ecological processes. Here, the authors review the recent findings that illustrate how the smRNA pathways in plants regulate phytohormone signaling and crosstalk during adaptation to stressful environments. They further probe the conceptual possibilities of smRNAs acting as direct defenses as well as parallels between the prokaryotic and eukaryotic smRNA pathways.

Chapter VI - Over a century ago, Carl Rabl (1885) and Theodor Boveri (1909) proposed that chromosomes assume a distinct sub-volume in the nucleus, referred to as the chromosome territory. Chromosomes assume a non-random position in the interphase nucleus with gene rich chromosomes predominantly localized towards the nuclear center while gene poor chromosomes are closer to the nuclear periphery. Such an arrangement is also conserved in higher eukaryotes suggesting a strong functional significance for non-random chromosome positioning. Gene loci on chromosomes also show a non-random position in the nucleus, where in actively transcribing gene loci are "looped-out" of their respective chromosome territories to access transcription factories. The molecular mechanisms that regulate non-random positions of chromosome territories (~10^8 bp) or of individual gene loci (~10^3 bp) in the interphase nucleus are unclear. Diseases of the genome such as cancers are characterized by genome instability and aneuploidy. Studies of chromosome positions in either diploid or aneuploid cancer cells using 3-dimensional fluorescence *in situ* hybridization (3D-FISH) followed by confocal imaging and 3D reconstructions of labeled chromosomes, show that human chromosome 18 (peripheral) and 19 (central) largely assume conserved nuclear locations. However, the extent of aneuploidy could disturb conserved chromosome locations that may in turn affect transcription. In cancer cells, individual gene loci are amplified, which along with whole chromosomal aneuploidies severely destabilizes transcription in cancer cells. However, cancer cells may achieve further transcriptional deregulation by allowing extra chromosomes and amplified genes to assume diverse nuclear locations that additionally imbalance transcription. Taken together, the authors speculate that cancer cells acquire a hyperdynamic genome organization, where in amplified gene loci and transcription factories populate the entire nucleus that further facilitate a strikingly elevated level of transcriptional deregulation – a hallmark of cancer cells. A careful study of genomic imbalances in cancer in the context of nuclear architecture therefore assumes paramount significance.

Chapter VII - The class Insecta has more than 1 million different cataloged species living in almost all habitats throughout the world, and they encompass many different lifestyles. Understanding how these animals evolved in terms of their physiology and biochemistry is reason enough to perform research in the basic science field. However, hematophagous insects spread vector-borne diseases, such as malaria, dengue fever and Chagas' disease through human populations living in tropical regions. In addition, the herbivorous species can damage plantation fields, leading to both reduced production and economic losses. Moreover, insects can be easily raised in laboratory colonies, and some can be readily genetically manipulated. Finally, some similarities between insect and mammalian physiology make these invertebrates excellent models to study human problems; for example, lipid metabolism deregulation, which can be responsible for the development of diseases such as obesity, arteriosclerosis and diabetes, one of the major health problems concerning the Western population. For all the above reasons, research groups around the world are working with insect models to generate data that can be used to control disease vectors and agricultural pests and may even help to understand human metabolism. Here, the authors will review the findings achieved in insect lipid metabolism, particularly over the last 10 years, and they will focus on how genes involved in these pathways are regulated. We will discuss how different environmental signals or metabolites and hormones produced by insects can control the activity of transcription factors to change gene expression profiles. The gaps found in the authors' current knowledge and possible future directions in this research field are also discussed.

Chapter VIII - There is today an expressed need for approved biomarkers that both can aid the diagnosis of Alzheimer's and Parkinson's diseases and guide the de The authors velopment of new treatments for these diseases. There are today two tests approved in Europe to aid the diagnosis of Alzheimer's disease at the dementia stage and both are based on the expression of several genes in blood that together generates a model that can predict the disease. At the same time no biomarker based on proteins or metabolites in blood has yet been approved. This chapter will review the current state of development of novel biomarkers for Alzheimer's and Parkinson's diseases based on the expression of genes in blood. The chapter will also try to explain why using gene expression likely will be a viable approach for the development of novel blood based biomarkers for neurodegenerative diseases.

Chapter IX - The human transcriptome is exceptionally complex. Over 95% of human DNA is transcribed into RNA; the transcriptome containing not only the messenger RNAs (mRNAs) but also a range of large and small non-coding regulatory RNAs and components of the translational machinery. The transcriptome is therefore at the heart of the gene-environment interaction, and this complexity, together with the diversity brought about by alternative mRNA processing gives the human transcriptome unprecedented plasticity and adaptability. Many human diseases are associated with effects at the level of the mRNA transcript, either as part of the causal pathway, or as a response to disease. Analysis of the transcriptome thus has enormous potential for the development of potential biomarkers of disease, and to increase our understanding of the disease processes themselves. In the last few decades, technological advances have made profiling of the entire transcriptome an affordable and manageable prospect. These advances have made high-throughput analysis of the output of the human genome accessible, allowing transcriptional alterations with disease phenotypes to be determined in large human populations. In this review, the authors will describe some of the recent advances in our understanding of fundamental human processes such as human

ageing, and common chronic disorders that have been achieved by whole genome transcriptional profiling. The authors will outline some of the methodologies that have been employed to answer these important questions, as well as present some examples from the authors' own laboratory where they have used these approaches to investigate mechanisms ofageing and in specific age-related disorders such as frailty and cognitive decline. Gene expression profiling has an important role to play in inferring mechanisms underlying certain diseases, and technological advances will render it an increasingly powerful tool in the future.

Chapter X - The increasing incidence of various autoimmune diseases represents a serious medical, economical and social burden on society. They affect approximately 7% of the population and are often associated with the deterioration of a patient´s quality of life and shortening of lifetime expectancy. Type 1 diabetes (DM1) is an autoimmune disease whereby self-reactive T lymphocytes selectively attack insulin-producing pancreatic beta-cells. Since a number of important DM1-related autoantigens and genes have been already characterized, this disease represents a general model to study the mechanism of initiation and progression of autoimmune diseases. Advances in this field have lead to the introduction of several immunointervention strategies, the efficiency of which, unfortunately, lags somewhat behind expectations. There are two main problems: (i) pancreatic beta-cells display a poor proliferative capacity, and (ii) clinical signs of DM1 appear only after > 80% of beta-cells are irreversibly destroyed leading to a lifelong self-administration of insulin. Thus, it seems that the most effective resolution to this problem would be to recognize those who are at high risk or at the preclinical stage of diabetes when a sufficient mass of beta-cells is still present. Data generated in the authors' and other laboratories show that gene expression profiling could significantly improve such screening. This short review summarizes the current advances in using the gene expression microarray technique to characterize the expression features of genetic landscape that predispose an individual to DM1. This notion especially relates to the search for new autoimmune disease-related biomarkers and how this knowledge could be used in a daily clinical practise.

Chapter XI - At present, epigenetic mechanisms comprise DNA methylation and post-translational histone modifications. In this chapter, the methods currently being used for assessing DNA methylation and post-translational histone modifications status in cells are described. Metal and metal compounds are widespread occupational and environmental pollutants. Chronic exposure to these metals has been associated with various health outcomes, including hypertension, cardiovascular disease, neurologic deficits, as well as increased risks of numerous cancers. Mounting experimental evidence suggests that an important feature of metal induced alterations in gene expression involve epigenetic-mechanisms. In this chapter the mechanisms of carcinogenicity of nickel, arsenic, and chromium metal compounds are discussed. Emphasis is placed on the importance of having a thorough understanding of the current methodology used to study epigenetic mechanisms in order to comprehend the complete picture.

Chapter XII - Chromium is an industrially relevant transition metal. Chromium like other transition metals exhibit variable valency. However, the most relevant oxidation states of chromium are +3 and +6. Chromium in both these oxidation states finds wide industrial application. To name a few, chromium is used in making of alloys. Chromium(VI) finds application in electroplating industry. Chromium(III) is widely used in leather making. Any industry which makes use of chromium in its different oxidation states also discharges large amount of it as solid waste as well as liquid waste. Nowadays there is a lot of concern about

the indiscriminate discharge of chromium bearing waste because of the fear of their toxicity. Chromium(VI) is known to be a carcinogen and has been known to bring about cell death. Chromium(III) on the other hand is an essential trace element with recommended daily intake of 50-200 micrograms. Chromium(III) is part of glucose tolerance factor and known to be essential for glucose and lipid metabolism. One of the synthetic complexes of chromium(III) namely, trispicolinatochromium(III) is currently being used as a drug for diabatic patients. This particular chromium(III) complex is also being used as a nutritional suppliment. However, some recent experiments suggest that chromium(III) complexes may be toxic. To understand the biotoxicity of chromium(III) complexes, interaction of few chromium(III) complexes with nucleoside, nucleotides, DNA, protein and cell lines have been investigated using various spectroscopic and physical techniques. It has been shown through mass spectral experiments that chromium(III) complexes bind to the nucleic acid bases. Binding of chromium(III) complexes to nucleic acid is largely governed by the ligands associated with the metal ion. In the case of complexes with ligands having planar aromatic fused ring system, intercalative binding to DNA has been observed. In other cases favoured mode of binding is groove binding. Many of these Cr(III) complexes brought about cleavage of supercoiled DNA to the nicked or linear form in the presence of peroxide. EPR spin trap experiments show that these cleavages are due to the formation of hydroxy radical in the presence of Cr(III) complex and peroxide. Chromium(III) complexes with very high excited state redox potential has benn found to oxidatively cleave DNA in the presence of light.

Interaction of chromium(III) complexes with protein also has been shown to be governed to a large extent by the coordinated ligand. A complex like tris (ethylenediammine) chromim(III) has been found to bring about protein aggregation; whereas complexes which interact coordinatively with the protein did not bring about any protein aggregation. The tricationic ethylenediamine complex of chromium has also been found to bring about confirmational changes in the protein unlike the other complexes. The Cr(III) complexes which bind coordinativelt to proteins have been found to bring about oxidative damage to proteins in the presence of peroxide. These experiments clearly show the importance of ligand structure in the interaction of chromium(III) complexes with proteins.

Depending on the ligand some chromium(III) complexes have been found to bring about apoptosis in lymphocyte cells, while some other complexes did not bring about any appreciable cell damage. These studies clearly demonstrate that under certain ligand environment the chromium(III) complex can be biotoxic.

Chapter XIII - Eukaryotic RNA polymerase II (RNAP) transcribes mRNA and microRNA genes. RNAP consists of 12 subunits of which RPB5 is an active one and serves as one of targets of transcription regulators in signal transduction. RPB5 mediating protein (RMP), which associates with RNA polymerase (RNAP) II subunit RPB5, was cloned from human hepatocellular carcinoma (HCC) cell HepG2 and have multiple variants, including the unconventional prefoldin RPB5 interactor (URI). It has been shown that RMP/URI participates in transcription regulation, nutrient-related signaling pathway and maintenance of genomic stability. Recently RMP/URI has been demonstrated to be an oncogene by two groups simultaneously. In ovarian cancer, RMP/URI is overexpressed and/or amplified, which is oncogenic for the tumor growth. Meanwhile, in the carcinogenesis of hepatocellular carcinoma, RMP has been found to play an important role in the antiapoptosis, which is required for the proliferation of HCC cells. RMP is ocogenic for the growth of HCC xenograft tumors.