Preface

Ribosomes are essential protein-RNA complexes that synthesize proteins according to the gene library of the cell in all living organisms. In this book, the authors present current research in the study of the molecular structure, biological functions and implications for genetic disease of ribosomes. Topics include ribosomal alterations and immune-related illness; the role of ribosomes in mediating hypoxia response and tolerance in eukaryotes; the potential biomedical use of ribosome-inactivating proteins; coarse-graining the nano-machine ribosome to elucidate its functional dynamics; and structural aspects of ribosomal ligands interactions providing the work of the human translational machinery.

Chapter 1 - The chapter is devoted to the review of structural principles of molecular processes providing functioning of the protein synthesizing system in higher organisms. Site-directed cross-linking and related data on structural elements of the human ribosome that form binding sites of mRNA, acceptor terminus of tRNA and hepatitis C virus IRES, and on peptides of the translation termination factor eRF1 participating in the organization of the stop codon recognition site are considered. Comparison of the structural information gained with translational system from higher organisms with the respective information on bacterial and lower eukaryotic systems is given. New insights into molecular mechanisms of translation based on the discussed data are presented.

Chapter 2 - Specific ribosome-directed stressors have the capacity to damage 28S ribosomal RNA and interfere with ribosomal functions during translation and cellular homeostasis. This can provoke so-called ribosomal stress responses that are closely associated with various disease processes including immunosuppressive or hypersensitivity-linked dysfunctions in humans and domestic animals. Since the primary toxic actions of most ribosomal stress-inducing agents generally inhibit global protein synthesis, highly dividing cells, including leukocytes and epithelial cells, are the most susceptible targets. Here, responses of immune cells and systems to acute and chronic exposure to ribosome-inactivating stress in various experimental models are discussed. This review will specifically focus on modulation of immune suppression and hypersensitivity diseases caused by ribosomal dysfunction. Evidence-based approaches characterizing ribosome-inactivating stressor and their implication as critical etiological factors of immune-associated diseases are also presented.

Chapter 3 - Ribosome biogenesis and protein synthesis are highly energy-consuming processes and therefore, key control points for cellular growth and division under various conditions of stress. Hypoxia is a state of poor oxygen supply that is experienced by diverse

organisms under numerous physiological and disease conditions. Hypoxia greatly reduces cellular energy production and hence, organisms must develop effective strategies to cope with this low oxygen state. One common strategy that eukaryotes, ranging from yeast to mammals, employ to adapt under hypoxia is the conservation of cellular energy via suppression of metabolism and protein synthesis. These biological changes occur as a result of coordinated cellular responses orchestrated by various signal transducers and regulators, such as HIFs in higher eukaryotes and Mga2 and Hap1 in yeast. Studies in various eukaryotes have indicated that ribosomes have an essential role in mediating responses to hypoxia. In yeast the expression of various ribosomal and related proteins are altered under hypoxic conditions. Interestingly, it appears that deletion of certain genes involved in ribosome biogenesis, including *NSR1, BUD21, RAI1* or *RPL20A*, minimizes such alterations and enhances the survival of yeast under hypoxic conditions. Such deletions also diminish the effect of key transcriptional regulators, like Swi2 and Swi3, whose targets are altered under hypoxia in the wild-type strain. Similarly, in *C.elegans* reduced activity of arginyl-transfer RNA (t-RNA) synthetase confers hypoxia tolerance. Mammals acquire hypoxia tolerance via global reduction in RNA translation and ribosome biogenesis. In this chapter, the authors will review the role of ribosomes in conferring tolerance to hypoxia and discuss future directions to elucidate the molecular mechanisms underlying hypoxia tolerance.

Chapter 4 - Analyses suggest that protein coding genes occur within ribosomal RNA genes, enabling to code for additional proteins without increasing genome size. Stop codons can be translated by antisense antitermination (suppressor) tRNAs with anticodons matching stop codons, hence overlap coding might not be limited to stopless 'open reading frames'. Alignment analyses detect several putative protein coding genes within mitochondrial rRNAs and the mitochondrial D-loop region that include stops. These putative overlapping genes are probably functional because corresponding RNA abundances in GenBank's EST database increase with the length of the putative overlap coding region (but not for regions not involved in overlap coding), and predicted protein secondary structures resemble those formed by their presumed homologues from GenBank. In addition, numbers of putative overlap coding genes increase with predicted capacities of antisense tRNAs to match stop codons when comparing mitochondrial genomes from each primate and *Drosophila* species. In human mitochondrial genomes, single nucleotide polymorphisms are less frequent in putative rRNA-encoded overlapping protein coding genes than in the rest of each 12S, 16S rRNAs and the D-loop, as one would expect if the coding function of these regions cause excess purifying selection against polymorphisms. However, further analyses based on polymorphism frequencies and proportions of pathogenic mutations among these yield partially inconclusive results, probably because of weaknesses intrinsic to indirect estimation of purifying selection as indirect evidence for gene function. Previous analyses showed that overlapping protein coding genes in frameshifted and antisense sequences of regular mitochondrial protein coding genes coevolve with capacities of antisense tRNAs to translate stop codons, reassigning stop codons to code for amino acids: UAR are reassigned to glutamine in primates, tryptophane in turtles, and serine in *Drosophila*; AGR stops are reassigned to arginine in primates and glycine in the olive ridley (*Lepidochelys*) and lysine in other turtles. Analyses of mitochondrial rRNAs converge with analyses of protein coding genes and strengthen the hypothesis that mitochondrial genomes code according to a stopless genetic code for previously undetected, cryptic overlapping protein coding genes. It is

probable that pathogenicity of at least some polymorphisms is due to the previously unsuspected function of rRNA and D-loop regions as protein coding regions.

Chapter 5 - Temperature up-shift or down-shift is one of the major environmental stresses encountered by microorganisms. Especially, one of the main consequences of temperature down-shift on cells is conformational changes of secondary structures of RNAs. These cold-shock induced structural changes of RNAs have significant effect on the global gene expressions, i) by improper termination of transcription, ii) inefficient RNA degradation, iii) translation of alternatively-structured mRNAs, and iv) biogenesis of ribosome and processing of rRNA. Several cold-shock proteins are produced to counteract these effects and thus allow cold acclimatization of the cell. This chapter describes how specific cold shock proteins modulate the ribosome biogenesis and eventually translation, to ensure effective protein production at low temperature.

Chapter 6 - Ribosome is crucial for protein synthesis in cells. Ribosome-inactivation proteins (RIPs) are a large family of unique enzymes that inhibit protein synthesis by site-specifically and irreversibly modifying the ribosomal RNA. According to their different enzymatic activity, RIPs can be classified into ribonuclease RIPs and N-glycosidase RIPs. RIPs have drawn the attention of many researchers due to their potential use as antivirus and anticancer reagents and as other biological and biomedical tools. In this chapter, the progress in the study of RIPs and the problems encountered in biological and biomedical research are discussed.

Chapter 7 - Coarse-grained computational techniques can provide information on conformational dynamics of the supramolecule ribosome, for which full-atom approaches become computationally demanding. Elastic network models and coarse-grained molecular dynamics simulations are the most commonly used methods for simple descriptions of the biomolecular structure. Usually, an amino acid or a nucleotide residue is represented by a single node, and simplified potential energy functions are used for interacting nodes. These approaches have been successful in reproducing the micro-to-milliseconds dynamics information, such as the experimentally observed ratchet-like rotation of the ribosomal subunits. Furthermore, they have provided insights on the structure-dynamics-function relationship of the ribosomal complex. This review summarizes various coarse-graining approaches on the nano-machine and recent studies that focus on its different functional sites.

Chapter 8 - In living cells and organisms, proteins are often made through decoding genetic information stored as sequences of nucleotides. The central machinery in the process is ribosome. Every protein is translated from the 5' end to the 3' end of an mRNA. Starting from the translation initiation site, every three consecutive nucleotides are considered as a codon and read as a single amino acid. Cellular proteins, which are made from 61 sense codons of a total 64 codons, typically comprise 20 natural amino acids. However, there are unusual cases existing in nature. Under certain circumstances, nature can use codons that are typically nonsense to code natural as well as unusual amino acids (*e.g.* selenocysteine and pyrrolysine). In addition, ribosomal frameshifting is also not uncommon, in which case one or two nucleotides in a coding sequence are skipped to make proteins based on a shifted frame. In the past decade or so, researchers have invented procedures in laboratories to biosynthesize proteins containing unusual amino acids. Such exploration has made a big impact. This chapter summarizes methods used in nature and in laboratories to incorporate unusual amino acids into proteins. In particular, recent studies, which re-engineered ribosome and other

related translational factors, are emphasized. Therapeutic applications of this technology are also discussed.

Chapter 9 - Bowen-Conradi syndrome (BCS), a lethal autosomal recessive disorder which affects infants in the Hutterite population, is caused by a point mutation in *EMG1*, a highly conserved gene necessary for biogenesis of the small subunit of the ribosome. Research on EMG1 function has centered on yeast models, due to their genetic tractability for examining a cell-essential protein. The effects of the BCS-causing mutation on human EMG1 protein function are therefore unknown. The authors previously showed that EMG1 protein levels in BCS patient fibroblasts were severely reduced compared to control cells, and the authors therefore went on to examine EMG1 stability. Using pulse-chase metabolic labeling in cells transiently transfected with wild type or mutant EMG1, the authors found that the mutant protein rapidly associated with a detergent-insoluble fraction. Inhibition of the proteasome resulted in the accumulation of a higher-molecular weight band likely corresponding to ubiquitinated EMG1, suggesting that EMG1 is normally degraded by this pathway. Using immunofluorescence in fibroblasts, the authors found that both wild type and mutated EMG1 were localized to the nucleolus, and that proteasome inhibition resulted in the accumulation of EMG1 in nucleoplasmic foci which may correspond to sites of proteasome-mediated degradation. These data suggest that the mutated EMG1 protein is unstable, leading to a reduced level of EMG1 in the cell. Although the mutated protein localizes normally, the reduction in its level is likely to impact downstream pathways that significantly alter cell function in BCS patients.