# PREFACE

Ribosome is an important cellular organelle that is necessary for the basic cellular process, protein synthesis. Furthermore, ribosomal protein is a group of proteins that is important in biomedical research. The authors of this book present and review important data on ribosomal proteins, important in tropical diseases such as malaria and tuberculosis. In addition, some functions of ribosomal proteins, other than translation, are called extraribosomal function or extraribosomal activity. In this book, the extraribosomal functions of ribosomal protein S19 (RPS19) is discussed in detail. Other chapters in this book examine the structures of the free and bound forms of proteins that have experimentally been found to be essential for the first steps of ribosome assembly. A summary of ribosomal protein mutations generated in eukaryotes is also provided and their proposed roles in the control of cell growth and proliferation, as well as their impact in human diseases. Important challenges we face in explaining life from the genome and proteome viewpoint is also discussed, including how to measure a living protein/RNA/DNA, how to restore the missing relation cross generations and how to correlate structure with function. Finally, the authors suggest possible solutions for such challenges.

Chapter 1 - Recombinant proteins are widely used in industry (pharmaceutical, chemical, food, cosmetics…), research and diagnostics; therefore, interest in their production is increasing. Due to low cost, high productivity, simple cultivation and rapid use, bacterial systems remain very interesting for recombinant protein production. One of the most commonly used bacterial hosts is still *Escherichia coli*. However, upon overexpression in *E. coli*, recombinant proteins tend to aggregate into inclusion bodies (IBs). For a long time, IBs were considered to be deposits of biologically inactive target proteins. The denaturation and renaturation step of protein isolation from IBs have to be optimized. However, the isolation process often results in poor recovery of biologically active proteins and accounts for a major cost in protein production, thus IBs were regarded as a bottleneck in protein production.

The temperature and induction regime were recognized as very important factors affecting the growth of the bacteria *E. coli*, as well as the recombinant protein yield and protein folding and thus protein quality inside IBs. After optimization of the bioprocess, the authors were able to identify and explore a new sub-type of inclusion bodies. These new IBs contain correctly folded protein. They show several significantly different properties from IBs previously described (solubility, fragility, irreversible contraction at low pH); and the authors have therefore proposed that they be given the designation "non classical inclusion bodies" (ncIBs).

Non-classical IBs are easily soluble in mild detergents, thus proteins can be extracted under non-denaturing conditions in biologically active form without applying any renaturation steps. Thus the isolation process is faster and economically advantageous and can be exploited for environment-friendly biotechnological production.

Such IBs composed of active proteins could also be used as pure nanoparticles in diagnostics, as biocatalysts in enzymatic processes, or even as biopharmaceuticals. Furthermore, studying IBs' formation inside prokaryotic cells could provide useful knowledge of aggregation mechanisms, applicable also to aggregates formed inside eukaryotic cells, responsible for numerous degenerative diseases.

Chapter 2 - Some ribosomal proteins play roles in activities other than translation. Such functions of ribosomal proteins are called extraribosomal function or extraribosomal activity in common. Ribosomal protein S19 (RPS19) is a component of the small subunit of eukaryotic and archaeobacterial ribosomes having 145 amino acid residues. RPS19 exhibits various extraribosomal functions, which basically correlate to the apoptosis or programmed cell death. In the apoptosis-initiated cells, RPS19 molecules are cross-linked intermolecularly with an isopeptide bond between Lys122 and Gln137 by a transglutaminase-catalyzed reaction. This results in homo-dimerization gaining a new molecular conformation capable of binding the C5a receptor, a G protein-coupled heptahelical cell surface receptor. The dimer is then released from the apoptotic cells. Meanwhile, the apoptosis-initiated cells *de novo* synthesize the C5a receptor. The C5a receptor was initially identified as a leukocyte chemotactic receptor for the complement-derived C5a. Due to a molecular mimicry between the RPS19 dimer and C5a, both molecules can ligate the C5a receptor exhibiting some difference between them. The RPS19 dimer has a potency to cause at least three cellular events through the C5a receptor ligation. One is apoptosis promotion by an autocrine/paracrine effect. On the other hand, the RPS19 dimer recruits monocytes/macrophages by ligating the C5a receptor on these leukocytes. The monocytes/macrophages thus infiltrated engulf the apoptotic cells and digest them. The macrophages then move to regional lymph nodes *via* lymphatics and present apoptotic cell-derived antigenic peptides to T lymphocytes. The acquired immune response may then start on demand. The apoptotic cell clearance is basically an event outside the acute inflammatory response in which neutrophils play major roles. The RPS19 dimer inhibits neutrophil infiltration by ligating the neutrophil C5a receptor apparently as an antagonist. Other extraribosomal functions of the RPS19 dimer have been observed. There is a hypothesis that some elements of the apoptotic program would be used in terminal differentiation of erythroblasts when they lose their nuclei, and macrophages would be responsible for clearing the erythroblast-derived nuclei. The pro-apoptotic effect and macrophage recruiting effect of the RPS19 dimer seem to also function in the erythropoiesis. Interestingly enough, there is a molecule indistinguishable from RPS19 in plasma. The RPS19-like molecule is cross-linked by coagulation factor XIIIa, the plasma transglutaminase, in the blood coagulation process. The RPS19 dimer-like molecule seems play a role in resolution of coagula due to recruiting monocytes/macrophages.

Chapter 3 - The recent crystallographic structures of the ribosomal subunits have revealed the picture of the final product of a complex assembly process that condenses the rRNA and the ribosomal proteins into active ribosomes. The folding of rRNA is the rate-limiting step of this complicate assembly pathway. Some ribosomal proteins are required for facilitating the structural rearrangements of rRNA and avoiding the kinetic traps that frequently impede RNA

folding. It is thought that the long basic r-protein extensions that penetrate deeply into the subunit cores play a key role in this process through disorder-order transitions and/or co-folding mechanisms. A current view is that such structural transitions may facilitate the proper rRNA folding. In this paper, the structures of the free and bound forms of proteins that have been experimentally found to be essential for the first steps of ribosome assembly have been compared. It is shown that the extensions of L3, L4, L20 and L22, have different structural and dynamics properties that probably correlate with different functions. This study also suggests that the specific coil-helix transition that occurs in a phylogenetically conserved cluster of basic residues of the L20 extension is strictly required for the large subunit assembly in eubacteria. In contrast, in showing that the ordering of the inner loop of L4 upon rRNA binding is not required for the assembly function of L4, this study indicates that different categories of disorder-order transitions are associated with different biological functions.

Chapter 4 - There is much concentration in recent years in understanding the coordinated role of ribosomal protein expression in the regulation of ribosome biogenesis, translational control, and cellular functions. Although ribosomal proteins are dispensable for catalyzing the formation of peptidic bonds, they provide a conformational environment essential for the process of protein biosynthesis. Imbalance in the production of ribosomal proteins results in non-equimolar amounts of protein for each subunit and induces defects in ribosome assembly. For these reasons, delineation of the precise function of specific ribosomal protein gene products is rendered difficult as their gene targeting is accompanied by severe growth defects resulting in cellular death. The objective of this commentary is to provide a summary of ribosomal protein mutations generated in eukaryotes and discuss their proposed roles in the control of cell growth and proliferation and impact in human diseases.

Chapter 5 - The extent of current research in ancient biology is not restricted by the presence of fossil records, owing to the developments in computational analysis and engineering techniques of proteins. Ancestral sequences of extant genes/proteins can be inferred with the molecular phylogeny reconstruction methods, which are mainly based on maximum-likelihood estimation. Once an ancestral sequence is determined, a reconstruction of the ancestral protein is not difficult by using the current protein engineering techniques, and reproduced protein can be experimentally tested either biochemically or biophysically. To date, such 'experimental molecular archeology' has been examined on several enzymes, elongation factor, photo-reactive proteins, nuclear receptor, and lectin, and largely helped to elucidate evolutionary processes of stability, specificity, and structure of the proteins. Although this method would promise a new field in protein engineering, several problems still remain to be solved, the largest among which should be accuracy of inferred ancestral sequence as a whole. In this chapter, the reported studies of ancestral protein reconstruction are reviewed with a focus on the authors' recent results on the ancestral congerin, which is the β-galactoside specific lectin of fish involved in the biological defense system. The reconstruction of ancestral congerin is important because two extant isoforms of this protein, congerins I and II, have been rapidly evolving, and their molecular properties and structures have been differentiating due to the natural selection pressures operating on the genes.

Chapter 6 - The ribosome is an important cellular organelle that is necessary for the basic cellular process, protein synthesis. Ribosomal protein is a group of proteins that is presented with a focus on biomedical research. In this article, the author will review and present

important data on the ribosomal proteins in important tropical diseases including malaria and tuberculosis.

Chapter 7 - In this chapter, the authors discuss three challenges we face in explaining the life from genome and proteome viewpoint, that is, how to measure a living protein/RNA/DNA, how to restore the missing relation cross generations and how to correlate the structure with its function. Finally, the authors suggest the possible solutions for these three challenges.

Chapter 8 – Self-similar properties of the proteins in the ribosome, the ribosomal proteins, are investigated in terms of various types of fractal dimensions. The authors especially estimate both the chain fractal and capacity dimensions of backbones of the ribosomal proteins, together with the mass fractal dimension of the ribosomal proteins at the atomic level. The chain fractal and capacity dimensions of the ribosomal proteins are compared with that of the self-avoiding walk, which is a typical model of a polymer without interaction between monomers. These fractal dimensions of the ribosomal protein support the existence of the long and extended domain, which is hardly seen in the globular protein. The authors demonstrate that the mass fractal dimension of the ribosomal proteins is pertinent to explain their structural characteristics. The self-similarity also upholds the fact that the ribosomal proteins function primarily to stabilize the structure of the ribosome by both the long-extended domain of the protein penetrating into the inside of the RNA, and the globular domain interacting with the RNA on the exterior of it. These results partially, if not whole, unravel the structural characteristics of the proteins in the ribosome.

Chapter 9 - For tissue growth, organisation and repair, interaction between cells and their extracellular matrix (ECM) is critical. Fibronectin ECM renders a polyvalent ligand display for cell adhesion through binding to cell membrane integrin receptors. Mimicking this polyvalent display and designing ligands which bind specific integrin receptor subtypes will be discussed in this chapter. The central peptide motif for integrin binding is RGD (arginine-glycine-aspartic acid) but the integrin $\alpha5\beta1$ subtype requires the synergy site on the $9^{th}$ type III fibronectin domain (FIII) in addition to RGD on the $10^{th}$ FIII domain. Polyvalent integrin $\alpha5\beta1$ ligands have been synthesised using a protein engineering approach, designing chimeras consisting of a N-terminal $9^{th}$-$10^{th}$ FIII domain pair, IgG-derived hinge and leucine zipper-derived helix; the latter mutated to yield di-, tri- and tetrameric coiled coils and thus self-assembling, multimeric integrin $\alpha5\beta1$ ligands. A unique C-terminal cysteine was appended to the helix to facilitate 'anchoring' of the chimeras with a defined orientation on a surface. Careful characterization of such self-assembling chimeras is required and can be achieved through the use of complementary techniques including size exclusion chromatography, analytical ultracentrifugation and circular dichroism. Surface adsorption and specific immobilization can then be unambiguously characterized by neutron reflectivity, relating the determined models of the adsorbed protein layers to their biological function. Application of these engineered proteins and cell adhesive surfaces as scaffolds or other biomedical devices has the potential to promote tissue repair and regeneration, for a wide variety of tissues including bone and skin. These applications will be discussed in the context of the future outlook for these ECM protein engineering approaches.

Chapter 10 - Halophilic organisms thrive in a wide variety of environments, ranging from mildly saline to saturated brine. Extremely saline conditions are very harsh to proteins and can result in problems such as loss of flexibility, which leads to loss of catalytic activity, or aggregation and precipitation due to salting-out effects. Halophilic proteins have therefore

acquired specific adaptations to prevent such problems, and that is clearly reflected in their amino acid composition. As a consequence, halophilic proteins do not function properly in low salt conditions, and many even unfold and denature without sufficient amounts of salt. One of the most obvious adaptations found in halophilic proteins is an excess of acidic residues, while other adaptations include a low lysine content and an increase in small hydrophobic amino acids, the latter of which is at the expense of large hydrophobic residues. Different functions have been suggested for the role of the acidic residues. They are, most likely, important for binding of essential water molecules, but may play also a critical role in binding of salt or prevention of aggregation. The other changes in amino acid composition in halophilic proteins are probably important for the reduction of hydrophobicity, in order to prevent aggregation and maintain flexibility in high salt concentrations. Structural features that may be important for halophilic adaptation are also found, such as an increased number of salt-bridges. That is, however, not used by all halophilic proteins, indicating that different strategies have evolved to cope with very saline conditions.

Chapter 11 - Alkaliphilic microorganisms (alkaliphiles) produce a variety of proteins, mostly enzymes, that are stable and optimally active at alkaline pH. The alkaline enzymes represent most of the currently known proteins adapted to high pH. Alkaline enzymes are widely used for industrial or commercial purposes, for example, as additives to laundry detergents. They have been improved in the course of evolution to be resistant to low proton density conditions that would cause protein denaturation or dissociation. In order to elucidate the mechanism of high-pH adaptation, the alkaline enzymes have been characterized and their structures have been compared with homologous non-alkaliphilic proteins. These comparative studies, together with several experimental and theoretical studies, have suggested three major strategies for alkaline adaptation: p*K*a modulation, Asp+Glu gain, and Asp+Lys loss–Glu+Arg gain. The history of representative alkaline enzymes, proposed strategies for high-pH adaptation, and protein engineering studies performed in order to adapt proteins to alkaline conditions will be reviewed in this chapter.