PREFACE

Histones are DNA-binding proteins that help in the packaging of DNA to fit into the cell nucleus in a hierarchy of organization. During the packaging and unfolding process, histones are involved in crucial biological activities at the molecular level. This book presents research in the study of histone class, structure and function, including the roles of linker histones in chromatin structure, the post-translational modifications of histones and the importance of chromatin structure and epigenetics in gene regulation and carcinogenesis.

Chapter 1 - The H1 linker histone family of proteins is the most divergent heterogeneous class of histone proteins. In mammals, there are eleven different subtypes. Histone H1 binds to the DNA entering and exiting the nucleosomal core particle and has an important role in the establishment and maintenance of higher order chromatin structure. As such, this histone class was traditionally regarded as being the architectural proteins of chromatin involved in chromatin condensation. This led to the speculation that this class of histone proteins functions as global repressors of transcription. However, their evolutionary conserved heterogeneity, as well as more recent data, has led to the hypothesis that the H1 subtypes are also involved in chromatin remodeling and genomic integrity and function not only as negative regulators, but also as positive regulators of certain genes. The major epigenetic modification (post-translational modification) of the H1 histones is phosphorylation. The results of many investigations have also implicated phosphorylation of H1 in the regulation of chromatin remodeling. Changes in the H1 subtype composition of chromatin have been extensively studied in many cell and tissue systems during various biological processes, such as development and differentiation. However, whether there is a functional relevance in these subtype switches had not been addressed in these classic studies. More recent results now indicate that specific subtypes may have unexpected and discrete functional roles, not only during development and differentiation, but also during DNA replication, DNA repair, apoptosis and aging. In this light, the aim of this review is to present the highlights of the accumulated available data which strongly support that specific H1 subtypes may have functionally significant roles in certain biological processes.

Chapter 2 - The manner in which linker histone globular domains associate with the nucleosome is a key issue in understanding the location of the linker histone tails, and thus their functional roles in regulating gene expression. Understanding of the location of the linker histone globular domain within the nucleosome can provide insight into the nucleosome architecture in the context of the location relative to the dyad on a statistically significant nucleosome. It can also indicate any possible association between linker histones

and different nucleosomes in the higher order chromatin structure. Therefore, it would contribute to the implication of gene regulation and higher-order structure models. However, studies on the binding of the linker histone globular domain to the nucleosome have not yielded a unified picture. In this chapter, I will discuss the current findings in this field including the linker histone variants, the structure of the linker histone globular domain, the location of the linker histone within a chromatosome, the functional domains of the linker histone tail, the contribution of linker histones to higher order chromatin structure and its connection to gene expression.

Chapter 3 - The advantages of using *Saccharomyces cerevisiae* as a model for chromatin studies imply its relatively small number of genes, about 6000, which enables uncomplicated genome-wide study. On the other hand, its short cell cycle - around two hours for genome duplication is another benefit for research. It has been shown that *S. cerevisiae* cells possess about 28% homology of genes with human. Interestingly 20% of genes known to take part in the development and progression of certain human diseases have their homologs in yeast cells. Furthermore, yeast genome possesses a full complement of histones and other chromatin proteins which express vast homology with the respective proteins in human cells. These characteristics of *S. cerevisiae* allow obtained results on them to be easily approximated for human cells. Chromatin has always been a matter of extensive scientific research. A good part of the knowledge of the authors on its structure and function has been gathered by experimenting on *S. cerevisiae*. Strangely enough, among the plethora of proteins which build up yeast chromatin the linker histone catches a specific attention with its odd features. *S. cerevisiae* linker histone, Hho1p, is the only histone known to possess two globular domains instead of one. Previous hypotheses suggested that it is responsible for the organization of yeast chromatin mostly in euchromatin. Nonetheless, recent data, including ours, have shown that Hho1p functions in yeast chromatin are far more diverse. It has been proved to be involved in the building and maintenance of the higher-order chromatin organization in yeast, from the 30 nm fiber, chromatin loop organization up to further levels of chromatin compaction. Altogether these data brings this histone protein closer to linker histones of higher eukaryotic cells and points out that linker histones and higher-order chromatin structures are more evolutionary conserved than it is generally believed.

Chapter 4 - Recent quantitative proteome analyses of human metaphase chromosomes have revealed various types of histones and their amounts involved in metaphase chromosomes. Core histones, and their variants such as H2A.Z and MacroH2A account for 70 % of the total amount of chromosome proteins. Significant amounts of ubiquinized-H2A are also found in chromosomes. In addition, different types of linker histone H1 and its variants, including H1.X, are found in total 6 % of chromosome proteins. Unlike authentic linker histones that localize over the entire chromatin region, histone H1.X accumulates in the nucleolus during interphase and is at the chromosome periphery during mitosis, suggesting a distinct structural role for H1.X. Knockdown analysis of H1.X results in the failure of alignment and/or segregation of mitotic chromosomes. HP1-BP74 which has an H1 globular domain at the central region has been identified from proteome analysis. The HP1-BP74 globular domain binds to the linker DNA between nucleosomes, facilitating the formation of the higher-order structure, chromatosome. Proteome analyses of metaphase chromosomes shed light on the importance of histones in the formation of higher-order chromosome structure.

Chapter 5 - Histones are subjected to post translational modifications such as acetylation, deacetylation, methylation, phosphorylation, ubiquitination, sumoylation, ADP ribosylation, glycosylation, biotinylation, and carbonylation. The first four post-translational modifications (acetylation, deacetylation, methylation, and phosphorylation) have been studied extensively in comparison with the other types of modification. Histone modification activity has been shown to participate in a variety of cellular processes including, but not limited to, gene activity. In this chapter, the authors will discuss current findings of histone modifications and its connection to gene activity.

Chapter 6 - Histone modifications play a crucial role in the epigenetic regulation of various aspects of eukaryotic development. Histone modifications such as methylation and acetylation of histone tails are regulated by histone modifiers which are specific enzymatic complexes including histone methyltransferases, histone demethylases, histone acetyltransferases and histone deacetylases. Histone modifications result in the modulation of chromatin structure, a process which leads to the optimal gene expression programmes that eukaryotic organisms require during development and in response to changing environments. The action of histone modifiers leads to open (active) or closed (inactive) forms of chromatin, which are associated with activation or silencing of genes, respectively. Whereas histone modifiers have been well investigated and reviewed in the model plant *Arabidopsis*, comprehensive reports regarding the study of histone modifiers in plants of high economic value, such as cereals, are limited. This review will present the current knowledge on epigenetic histone modifiers in cereal model plants and crops such as *Brachypodium*, rice, maize, and barley.

Chapter 7 - Chromatin condensation and subsequent decondensation are processes required for proper execution of various cellular events including cell division and DNA replication. During mitosis, chromatin compaction is at its highest, whereas relaxation of chromatin is necessary for DNA replication, repair, recombination; and gene transcription. Since histone proteins are directly complexed with DNA in the form of a nucleosome, great emphasis is put on deciphering histone post-translational modifications that control the chromatin condensation state. Histone H3 phosphorylation is a mark present in mitosis, where chromatin condensation is necessary, and in transcriptional activation of genes, when chromatin needs to be decondensed. There are four characterized phospho residues within the H3 N-terminal tail during mitosis: Thr3, Ser10, Thr11, and Ser28. Interestingly, H3 phosphorylated at Ser10, Thr11, and Ser28 has been observed on genomic regions of transcriptionally active genes. Therefore, H3 phosphorylation is involved in processes requiring opposing chromatin states. The level of H3 phosphorylation is mediated by opposing actions of specific kinases and phosphatases during mitosis and gene transcription. The cellular contexts under which specific residues on H3 are phosphorylated in mitosis and interphase are known to some extent. However, the functional consequences of H3 phosphorylation are still unclear. Likewise the role of epigenetic signaling events, regarding methylation and acetylation of histone proteins, that regulate histone modification during mitosis is still actively being explored. Several converging and reinforcing signals, including transcription factors, noncoding RNAs, DNA methylation, and histone modifications orchestrate complex epigenetic states. Although all of these pathways modulate transcription from chromatin in vivo, the mechanism by which epigenetic information is transmitted through cell division remains unclear.

Chapter 8 - A nuclear genome physically exists as a nuclear DNA molecules or a set of chromosomes. The structural parameters of a nucleus or chromosome would determine the functional basis of a genome, i.e. regulation of gene expression and transmission of the genome to the next generation. In plants, there are distinct upper limits of structural parameters for maintaining genome functions. The genomic DNA content in a nucleus has an upper limit of 3% (v/v, DNA/nucleus) for construction and maintenance of a functional nucleus. The length of a chromosome arm is also restricted to an upper limit that is less than a half of the length between the two spindle poles. Moreover each chromosome functionally differentiates region from to region which is characterized with specific combinations of histone modifications. The three-dimensional distribution of the each chromosomal region with specific patterns of histone modification and the functional differentiation of the nuclear space are also closely related to the plants with large-sized genomes. This fact clearly indicates that the higher structure of the nucleus and the chromosomes with different histone modification set play essential roles in genome functions.

Chapter 9 - Oxidative stress causes DNA and protein damage, which may compromise genomic integrity. While oxidant-induced DNA damage has been extensively studied, less is known about nuclear protein damage, particularly of histones, which support chromatin structure and influence gene expression. Among other mechanisms, protein damage may be caused via non-enzymatic glycation initiated by reducing sugars (e.g., glucose and ADP-ribose) or reactive dicarbonyl compounds (e.g., glyoxal). Glycation is a non-enzymatic reaction between the reducing sugar carbonyl and protein amino groups, which undergo rearrangements to stable ketoamines and advanced glycation end products (AGEs) such as fluorescent (e.g., argpyrimidine) and non-fluorescent (e.g., N^{ε}-carboxymethyllysine, CML) protein adducts and crosslinks. Protein glycation, induced by hyperglycemia, has been implicated in diabetic complications, although the contribution of histone glycation to these processes is unknown. High local concentrations of ADP-ribose are produced in the chromatin microenvironment during DNA repair, potentially contributing to histone glycation. This chapter describes the authors' detection of non-enzymatic modifications occurring *in vivo* on histone H1 from calf thymus (Part I) and the basis for inhibition of these processes. In particular, the authors will discuss the roles of rutin metabolites as alternative, effective natural product AGE inhibitors for prevention of histone H1 modification and resulting disease complications (Part II).

Chapter 10 - The organisation of DNA and histone proteins into chromatin plays a central role in the regulation of eukaryotic gene expression. The coordinated regulation of gene expression programs is critical in directing developmental programs and in enabling cells to respond to extracellular signals. The combination of transcription factor binding sites found in gene promoters and enhancers, and the availability of these transcription factors, is important in co-ordinating these gene expression programs. However transcription factors must operate within the chromatin landscape, which regulates their ability to access their binding sites and drive transcription. The chromatin architecture of a gene can vary dramatically in different cell types, with differences in histone density, composition, modification and the presence of chromatin remodelers. While there would appear to be almost unlimited permutations of chromatin architectures, recent data generated using genome-wide technologies has begun to define chromatin signatures that are associated with particular gene states, and provide insight into how these architectures interact with extracellular signals. In this chapter the authors

review current information on the role of chromatin in regulating inducible gene expression, using the immune system as a model.

Chapter 11 - The production of ribosomal RNA (rRNA) accounts for more than half the transcriptional activity of an actively growing eukaryotic cell. Not surprisingly given its importance to the cell's well-being, the entire system of rRNA production is subject to several levels of regulation that often distinguish it from those of the protein-coding genes. The regulation of rRNA gene expression occurs at many levels, including that of chromatin structure. The authors' current understanding of rRNA gene chromatin (r-chromatin) in higher eukaryotes is that it exists in several forms ranging from a nucleosomal and silenced chromatin in which promoter DNA is methylated at several positions to a non-nucleosomal, methylation-poor chromatin associated with the ribosomal transcription factor UBF. The roughly equal ratio of silenced to active rDNA copies usually changes very little between resting and actively growing cells, and most of the expression regulation is due to the rate of transcription initiation and elongation at the active rDNA copies. This peculiar observation suggests that the organization of r-chromatin plays an important role in more than gene expression regulation, and is likely to involve the stability of the recombination-prone ribosomal RNA genes. In this chapter, the authors describe different structural aspects of the r-chromatin and their possible relation to the regulation of its expression.

Chapter 12 - Cancer development is a multi-factorial event, involving nuclear processes that promote the expression of genes linked to cell survival and proliferation. A clear connection has been established between epigenetic events and cancer biology. However, the nature of this relationship as it relates to disease progression and treatment remains unclear. With a particular emphasis on breast cancer, here the authors review advancements in the fields of DNA methylation, histone acetylation and histone methylation as they relate to tumor development, progression, and treatment.