Preface

This book is not an attempt to review all of the knowledge accumulated to date in the fields of genetics and epigenetics. Its primary goal is to provide interested students, teachers and researchers with a framework that they can use to understand the basis of epigenetic regulation and to appreciate its derivation from genetics and its interdependence with genetic mechanisms. With rare exceptions, classical transmission genetics, chromosome mechanics and classical cytogenetics are not included. Rather, molecular genetic facts with particular relevance and impact on epigenetic observations and theory were selected for discussion. Another goal of the book is to highlight the role played by the three-dimensional organization of the genetic material, and its distribution within a functionally compartmentalized nucleus. This architectural organization of the genome plays a major role in the retrieval, interpretation and execution of genetic and epigenetic information. As a means of illustrating and emphasizing the evolution of the relationship among genetics, epigenetics and nuclear architecture, from its beginning to the present, wherever possible, references will be made to research articles that first described the new observations.

Approximately three decades ago, most geneticists felt that the mechanism of transcription that underlies all aspects of gene function was largely understood. After all, transcription could be reconstituted *in vitro* with purified factors and enzymes that were highly conserved in eukaryotes and therefore largely interchangeable. Yet, it was also eminently clear that although the fundamental aspects of the transcription process were necessary, they were woefully insufficient to explain the basis of cellular specialization and of organism development; the parameters that defined transcription did not explain the differential expression of genes in time and space during embryogenesis or in the course of adult life. The initial insights on this front had been provided by the observations of Vincent Allfrey and Alfred Mirsky that the chemical nature of the genetic material—the complex of DNA, histones and non-histone proteins referred to as chromatin—was different in metabolically active cells. The manifestation of this difference, namely the acetylation of histones, indicated that in order to be activated, i.e. to allow the retrieval of genetic information, "a change in the fine structure of the chromatin and in the capacity of the DNA to serve as a template for RNA synthesis" must occur (Pogo et al., 1966). Almost a decade later, Arthur Riggs and Robin Holliday proposed that the methylation of DNA, known to occur in a wide variety of eukaryotic organisms, could be responsible for the repression of gene activity associated with one of the two X chromosomes in female mammals, by altering protein–DNA interactions (Holliday and Pugh, 1975; Riggs, 1975); this suggested the existence of a general mechanism that would be responsible for repressing genes that should remain inactive or that should be made inactive under particular circumstances of cellular differentiation. A difference in the physical organization of active and inactive genes was first demonstrated by Harold Weintraub and Mark Groudine who discovered that the DNA associated with active genes in purified nuclei is more sensitive to enzymatic digestion (Weintraub and Groudine, 1976).

The modern era of research into the chromatin modifications that would allow and regulate gene expression was introduced by separate discoveries, each illustrating a fundamental class of regulatory mechanisms. Craig Peterson and Ira Herskowitz published the purification of a multiprotein complex of yeast that appeared to perform a general function in the transcription of many genes by assisting gene-specific activators (Peterson and Herskowitz, 1992). A few years later, James Brownell and David Allis isolated the first histone acetyl transferase from the highly transcriptionally active macronucleus of *Tetrahymena* and subsequently showed that it was the ortholog of a transcription co-factor long known to be necessary for the activation of numerous genes in yeast (Brownell and Allis, 1995; Brownell et al., 1996). Sealing the dynamic role of histone acetylation in the regulation of

gene function was the isolation of the first histone deacetylase by Stewart Schriber (Tauton et al., 1996). These discoveries marked the onset of a major research effort to identify additional covalent modifications of histones and the enzymes responsible for inducing or eliminating these modifications. In parallel, new multiprotein complexes that remodel the conformational state of chromatin were characterized. Such endeavors were aided immeasurably by bioinformatics approaches that revealed the extensive structural and functional conservation of these chromatin-modifying factors. With the advent of microarray technology initially, and subsequently of next-generation DNA sequencing, recent emphasis has been placed on describing global epigenetic patterns across whole chromosomes or entire genomes, with often surprising and illuminating results.

A fundamental insight into the relative position of epigenetic modifications in the overall process of gene function was provided by the discovery of sequence-specific transcription activators—the so-called **pioneer factors**—that are able to access their binding sites in compacted chromatin (Cirillo et al., 2002; Natarajan et al., 1999; Neely et al., 1999). In turn, these factors recruit the modifying complexes responsible for the epigenetic modifications that are associated with gene activation or repression. These discoveries have led to the formulation of some general principles governing the modulation of gene function—opportunistic transcription factors may insert themselves into the genome, attract other factors and initiate covalent modifications of histones that alter inter-nucleosomal interactions, or serve as platforms for transcription activators and repressors; protein complexes change the architectural state of chromatin by displacing nucleosomes or by modifying their association with DNA. The mechanistic basis responsible for these dynamic structural changes in the organization of chromatin is just now beginning to be addressed through a burgeoning effort to redefine them at the biophysical and structural levels.

The eukaryotic nucleus is a highly complex organelle that contains, in addition to the genetic information of the organism, a myriad of distinct subnuclear regions (compartments) and structures. It is not surprising that the orderly reading of the genetic blueprint for cellular maintenance and differentiation engages and affects many aspects of nuclear organization, and that the different nuclear processes involved in gene expression occur in particular nuclear locations. The first suggestion of this spatial dimension was the apparent differential localization of active and inactive regions of the genome, with the former usually more centrally located in nuclei, and the latter usually associated with the nuclear periphery—the so-called lamina. These observations were soon followed by the discovery that the activity of some genes was correlated with their association with nuclear pore complexes or with their presence, together with other genes, in intra-nuclear foci rich in all of the elements of the transcriptional machinery that were named transcriptional factories. Recently, some evidence is accumulating that PML bodies (named after the abnormal distribution of a nuclear protein in promyelocytic leukemia patients) may play a role in transcription and in the response to DNA damage. Nuclear speckles (a.k.a. inter-chromatin granule clusters) are enriched in RNA-splicing factors and seem to associate with active genes.

Clearly, the nuclear address of genes must depend on interactions between specific characteristics of the chromatin wherein they reside and on the properties of the organelles involved. A particularly well-studied case in point is the clustering of insulator sites to organize the genome into cell-specific, large chromatin domains thought to be important in establishing specific programs of gene expression.

It is abundantly evident that absence or malfunction of developmentally regulated transcription factors and errors in histone modifications or remodeling are associated with human diseases, including cancers, and that many cancers exhibit various levels of nuclear disorganization. One of the purposes of this book is to highlight how transcriptional regulation and the concomitant epigenetic modifications are integrated in, and impacted by, the parameters that relate to the architectural organization of nuclei. Beyond contributing to understanding basic cellular differentiation and development, this synthesis should suggest new avenues for therapeutic interventions and lead to more focused approaches to redirect stem cell differentiation for the purpose of regenerative medicine.

To achieve the aim embodied in the title of the book requires a substantial level of selectivity. To this end, fundamental aspects of molecular genetic and epigenetic regulation and of nuclear organization are reviewed in the context of higher metazoans, with an emphasis on mammals in general, and on humans in particular. The same approach is used for a general description of nuclear structures. Examples drawn from unicellular organisms and plants are used when particular regulatory parameters have been studied uniquely in these forms.

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