Genetic Expression in the Cell Cycle

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Preface

An understanding of the molecular mechanisms that govern the expression of genetic information during the cell cycle requires full knowledge of how the genome is organized and the extent to which changes in its organization affect the ultimate synthesis and processing of RNA and other gene products. In this volume we have brought together investigators whose current research is directed toward several aspects of this central theme. The initial five chapters describe the intimate relationships between the supramolecular complexes that form the basic structure of chromatin. Emphasis is placed on the dynamics of cycle-dependent changes in the structural organization of some of these components.

The chromatosome, defined by neutron scatter, electron microscopy, and low resolution X-ray diffraction as a circular disk 11 nanometers in diameter and 5.5–6 nanometers in height, represents the primary subunit of chromatin. The first chapter introduces an extension of the details of our knowledge of this structure as a hexagonal bipartite disk stacked face-to-face and interconnected by axial histone H1, which has usually been considered as associated with the nucleosome linker region. The hexagonal bipartite disks appear to be aligned either in a continuous linear 100- to 140-Å-diameter nucleofilament or as 280-Å nucleofilaments achieved by a side-to-side association of the nucleosome hexagonal disks. This model proposes that the histone H1 is located at the axis in an optimum position to serve higher order packing and at the same time to provide postsynthetic modifications of histone H1 to accommodate the non-histone proteins, for example, the HMG proteins. The postsynthetic modifications of histone H1 and other histones are likely to play a major role in the transition from the extended state in interphase chromatin to the more contracted state in metaphase chromatin. These histone modifications are discussed in Chapter 2. This chapter reviews

the details of histone acetylation and its effect on the structure and function of chromatin during the cell cycle. These studies exploit the naturally synchronous cell cycle of *Physarum polycephalum* in which there appear to be substantial changes in both quantity and quality of transcription during the cell cycle. A convincing argument is made that many of the diverse observations on chromatin structural behavior represent the consequence of the kinases, acetyltransferases, and deacetylases to coordinate postsynthetic modifications of phosphorylation and acetylation of histones. The third chapter extends this theme to the role of the HMG proteins in relation to eukaryotic gene activity during the cell cycle. The importance of the HMG proteins is evidenced by the fact that they are associated with specific transcriptionally active chromatin fractions. The characterization of the HMG proteins in terms of intracellular concentration, distribution between the nucleus and the cytoplasm, and tissue and species specificity is reviewed. A molecular mechanism for their role in RNA transcription is proposed.

The ability to measure biochemical features of individual cells (Chapter 4) provides an opportunity to close the gap in our knowledge between cellular metabolic events at the molecular level, to study the behavior of cell populations at precise phases of the cell cycle, and to examine some of the dynamic aspects of chromatin structure discussed in the first three chapters. This is accomplished by means of newer techniques of flow cytometry to study synchronized CHO cells and cycling lymphocytes. A two-parameter frequency histogram has the capacity to classify cells on the basis of RNA versus DNA in G_1, S, and G_2 + M phases. Several lines of evidence indicate that G_1 phase cells can be further subdivided into G_{1A} and G_{1B}, which appear to be functionally distinct. The metachromatic properties of acridine orange also provide an index of chromatin structure on the basis of DNA stability. The mechanism of dye interaction (quinacrine dihydrochloride) as reviewed in Chapter 5 also provides an opportunity to monitor some specific cytological aspects of chromatin. These techniques are particularly useful in the analyses of the cell cycle blocks induced in temperature-sensitive mutants.

The relationships between transcriptional and posttranscriptional events and cell cycle regulation are examined in the next four chapters, with special reference to specific RNAs and inducible enzymes as probes of genetic expression. Chapter 6 presents evidence to demonstrate that small nuclear RNAs (SnRNA) are actively involved in gene regulation in eukaryotic cells. The implication of these studies is that active SnRNAs interact with nuclear proteins, possibly HMGs, to stimulate transcription. A key element of the proposed mechanism is the base-pair formation between SnRNA and DNA at the promoter region to facilitate the entrance of RNA polymerase. Chapter 7 focuses on the relationship be-

tween cell cycle regulation in the yeast *S. cerevisiae* and transcription of ribosomal RNA genes. A detailed description of the use of G_1-arresting compounds together with an analysis of their effects on the production of precursors of ribosomal RNA is presented. The relationship between this aspect of RNA metabolism and cell cycle regulation is also discussed. In Chapter 8 a detailed experimental account is provided to show that the expression of the gene for the ammonium-inducible isozyme of glutamate dehydrogenase in *Chlorella* is regulated primarily at the post-transcriptional level. The central element of this model is that in the absence of the inducer, subunits of the enzyme form dimers, which are degraded by endogenous proteases to nonantigenic products. The extent to which this model serves to extend our understanding of cycle-dependent regulation of gene expression in this cell is discussed by the authors. Chapter 9 introduces the reader to the use of conditional lethal mutants (e.g., cycle-specific *ts* mutants) to study the regulation of the cell cycle of eukaryotic cells. It is shown that these mutants are useful to study progression through the G_1 phase, particularly with regard to the involvement of RNA polymerase II.

The impact of specific gene products and other agents on specific phases of the cell cycle is considered in detail in subsequent chapters. Chapter 10 presents the concepts and methodologies employed to isolate and study specific cell cycle mutants of *Saccharomyces cerevisiae*. Extensive evidence is presented to show that the cell cycle of this yeast is uniquely regulated at one point through the action of several gene products. The authors discuss qualitative and quantitative differences between resting and actively dividing cells in terms of the concept of the G_0 state and regulation of the yeast cell cycle. In Chapter 11 we are introduced to the use of cultured *Drosophila* cells, which are unique in that they are arrested in G_2 under the influence of ecdysteroids. This is a promising new experimental system utilizing an organism whose genetics and morphogenetic attributes are well documented. The antiproliferative effect of interferon on cultured human fibroblasts is evaluated in Chapter 12 in terms of the effects of this potent cellular inhibitor on the cell membrane, cytoskeletal components, and synthesis of macromolecules. The authors develop the notion that the response to interferon, while manifestly heterogeneous, operates through a common pathway resulting in impaired proliferative capacity for the treated cells. This section of the monograph closes with a detailed analysis in Chapter 13 of the complex pattern of interaction between insulin, hydrocortisone, prostaglandins, and two growth factors as determined by the kinetics of initiation of DNA synthesis in cultured mouse cells. This analysis serves to illustrate the complex program of genetic expression that governs this particular phase of the cell cycle.

One of the challenging questions in cell biology is: To what extent are the cell membrane and related subcellular elements involved in the control of proliferation, differentiation, and cell cycle kinetics? To be sure, a question of this magnitude deserves an extensive and thorough discussion. Chapters 14–17, which complete this monograph, highlight some of the most recent experimental approaches to this complex problem. The extent to which the dynamic properties of the cell membrane have an impact on the cell cycle of neuroblastoma cells is the subject of Chapter 14. Of particular relevance is the relationship between changes in cation transport and the ability of cells to progress toward cell division. The authors make use of synchronized cells and exogenous growth factors to show that electrical and ionic events at the cell membrane, such as the electroneutral Na^+–H^+ exchange, are prerequisites for cell proliferation. An extensive review of what is known of the role of ionic fluxes, as well as the activity of the Na^+,K^+-ATPase in the regulation of cell proliferation, differentiation, and transformation, is presented in Chapter 15. Having evaluated the extensive literature on this subject, the authors present their own studies that show that amiloride, a drug which blocks passive Na^+ influx, has an inhibitory effect on rapidly proliferating cells (normal or transformed), suggesting that Na^+ influx may have a regulatory function. Chapter 16 focuses on the role of calcium levels on cell division in synchronized *Tetrahymena*. It would appear that changes in Ca^{2+} influx may exert their influence not only through an interaction of Ca^{2+} with calmodulin but through the activation of microtubule disassembly and cortical changes associated with actin-like proteins. The correlation between stimulation of cell growth and stimulation of monovalent cation fluxes is examined in the last chapter, which summarizes studies in rat hepatocytes, human T lymphocytes, mouse neuroblastoma cells, and mouse 3T3 fibroblasts in particular. This chapter not only serves as a summary of the work discussed in this section of the monograph but provides us with a synthesis of the events in G_0 cells and points to the directions of future research in this area of cell biology.

The primary objective of this monograph is to formulate new concepts of the control of genetic expression in the cell cycle.

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