Eukaryotic Gene Expression

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Preface

The recent surge of interest in recombinant DNA research is understandable considering that biologists from all disciplines, using recently developed molecular techniques, can now study with great precision the structure and regulation of specific genes. As a discipline, molecular biology is no longer a mere subspeciality of biology or biochemistry: it is the *new* biology. Current approaches to the outstanding problems in virtually all the traditional disciplines in biology are now being explored using the recombinant DNA technology. In this atmosphere of rapid progress, the role of information exchange and swift publication becomes quite crucial. Consequently, there has been an equally rapid proliferation of symposia volumes and review articles, apart from the explosion in popular science magazines and news media, which are always ready to simplify and sensationalize the implications of recent discoveries, often before the scientific community has had the opportunity to fully scrutinize the developments. Since many of the recent findings in this field have practical implications, quite often the symposia in molecular biology are sponsored by private industry and are of specialized interest and in any case quite expensive for students to participate in. Given that George Washington University is a teaching institution, our aim in sponsoring these Annual Spring Symposia is to provide, at cost, a forum for students and experts to discuss the latest developments in selected areas of great significance in biology. Additionally, since the University is located in Washington, D.C., there is ample opportunity to draw on the input of policy makers and political leadership, which significantly influences the support of biological research.

Eukaryotic gene expression was a logical choice as a topic, since much of the scientific progress in this field directly or indirectly influences the course of development in other areas of biomedical research. In selecting the topics to be included in this volume, my primary concern was to avoid a narrowly specialized set of articles that are quite often overreviewed and instead include

topics that are currently of interest, including promising new technical approaches. The chapters in this volume represent three main areas of interest. In the first group, the emphasis is on selected approaches to the organization of genetic material that have proved to be most promising—for example, studying the organization of active sites using the nuclease sensitivity of specific genes in chromatin (Chapter 1), the role of methylated regions in DNA in controlling gene expression such as that of the thymidine kinase gene (Chapter 2), and the significance of histone types synthesized in the regulation of gene expression during the cell cycle (Chapter 3). The structure and evolutionary divergence of an inducible gene, the prolactin gene, is emphasized in Chapter 4.

A second series of chapters concentrates on the expression of specific genes, in each case emphasizing an important technical approach. In Chapter 5, the structural analysis of histocompatibility class I and class II genes cloned by a sensitive method utilizing synthetic oligonucleotides is described by Sood and colleagues. The next two chapters describe gene-transfer experiments in which the primary aim is to study the regulation of their expression during the development of the intact organism. Chapter 6, by Gordon and Ruddle, discusses the general rationale for producing transgenic mice with specific reference to the expression of the herpes thymidine kinase gene and the human leukocyte interferon gene. Chapter 7 focuses on the expression of globin genes in the transgenic mouse.

The final series of chapters is concerned primarily with *in vivo* and *in vitro* transcriptional studies of specific genes. In Chapter 8, Gorman and colleagues describe a most sensitive and unique assay system in which the expression of the gene for chloromphenicol acetyltransferase is utilized to study the eukaryotic transcriptional enhancer elements. Chapter 9, by Schell and colleagues, discusses a very promising approach utilizing the Ti plasmid vector system for the regulation of plant genes. The remaining chapters focus on the organization and the transcriptional control elements of chicken collagen gene (Chapter 10) and the ribosomal genes of mouse and frog (Chapter 11) and rabbit (Chapter 12).

In all, these articles represent important fields of current research in molecular biology, and it is hoped that *Eukaryotic Gene Expression* will be a useful reference volume for students and experts alike.

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Contents

Some Observations on DNA Structure and Chromatin Organization at Specific Loci in *Drosophila melanogaster*

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1. INTRODUCTION

Within the eukaryotic nucleus, the DNA is packaged in a complex fashion by association with histones and other chromosomal proteins. One may suggest *a priori* that differential protein packaging of coding sequences at the broad level of the chromomere, or in the specific vicinity of a gene, or both, might be an important determinant in the selective expression of these sequences. Our goals have been to map features of chromatin structure relative to known functional sequences, to establish the presence of alternative patterns of structure during development, and to look for alterations in structure that might occur as part of the process of gene induction and repression. To this end, we have recently conducted a series of studies utilizing several different

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DNA-cleavage reagents to examine the patterns of DNA–protein interaction at a number of *Drosophila* genes. Concurrent studies using immunofluorescent staining of polytene chromosomes have identified several presumptive structural nonhistone chromosomal proteins, including some the distribution pattern of which indicates a preferential association with loci that are to be expressed at some point in the development of the salivary gland cells of *Drosophila*. We anticipate that the synthesis of this information may ultimately lead to a better understanding of the process of gene activation and hence provide insights into the regulation of this event during development. For a more thorough review of many of the issues raised herein, see Cartwright *et al.* (1982).

2. DEOXYRIBONUCLEASE-I-HYPERSENSITIVE SITES

Initial studies using deoxyribonuclease (DNase) I to digest chromatin suggested that this enzyme did not recognize any particular structural features, although a more rapid digestion of active loci relative to the genome as a whole was observed (Weintraub and Groudine, 1976). However, an experiment that allowed observation of the initial cleavage event for a given locus indicated an interesting specificity. Aliquots of nuclei isolated from *Drosophila* embryos were briefly digested with increasing amounts of DNase I, and the DNA was then purified and separated on agarose gels. A uniform smear of DNA fragments of decreasing average molecular weight was observed on staining with ethidium bromide. However, if the DNA fragments were then transferred to nitrocellulose by the Southern (1975) blotting technique and visualized by autoradiography using a specific radioactive DNA probe, a reproducible pattern of discrete bands was visualized (Wu *et al.*, 1979a). These bands apparently result from cleavage of the chromatin at particular sites hypersensitive to DNase I. It has been possible to unambiguously determine the position of such hypersensitive sites with respect to the restriction map of a particular region by utilizing an indirect end-labeling procedure as illustrated in Fig. 1 (Wu, 1980; Nedospasov and Georgiev, 1980). In these experiments, purified DNA from nuclear digests is cleaved completely with an appropriate restriction enzyme; *Bam* HI is used in the example shown. The DNA fragments are size-separated on an agarose gel, a Southern blot is prepared, and the filter is hybridized with a recombinant DNA fragment abutting the restriction site of interest, in this case the *Bam–Sal* fragment at the left-hand end. Only fragments to the right of this site will be visualized, as shown by the lines below the map. The largest is the parental *(Bam–Bam)*