Preface

In recognition of monumental impact of gene expression in producing normal and abnormal cellular states, we are pleased to offer the scientific community a volume on Gene Regulation, Methods and Protocols. The goal is to provide scientists in academia, food, and pharmaceutical industry, as well as public institutions, complementary technologies for investigating various facets of regulatory systems that contribute to the control of protein-coding genes in mammalian cells.

The technologies are broad in their scope. They include biochemical assays, methods in molecular biology, spectroscopic techniques, and high-throughput approaches for delineating key processes that contribute to the regulation of gene expression. The chapters are organized to offer a comprehensive, integrated, and coherent view of control systems and their associated components. The introductory chapter draws attention to the interconnectivity of regulatory circuits and provides examples of regulatory hubs. This chapter underscores the importance of protein networks in dynamics of gene activation, gene repression, and histone modifications.

Several techniques encompass chromatin structural features that influence gene activation. Two chapters detail methods for DNase hypersensitivity analysis, for high-throughput DNA sequencing to study chromatin accessibility and to identify regulatory DNA sequences on a global scale. Another chapter focuses on DNA derived from nucleosome-free regions for functional assays, to localize and study the activity of *cis*-regulatory modules dispersed within promoters and enhancers of genes.

A chapter highlights the utility of Heavy methyl-SILAC (Stable Isotope Labeling of Amino Acids in Cell Culture) to distinguish preexisting and newly generated methyl marks on histones. Coupling this technology with quantitative liquid chromatography and mass spectrometry (LC–MS) makes possible monitoring changes in site-specific histone methylation patterns.

Several chapters offer protocols for identifying regulatory sequences for functional assays and studies of protein–DNA interactions. Two chapters feature luciferase-based assays, including a dual luciferase reporter system to localize transcription factor binding sites within promoter segments of genes. Another chapter covers a functional assay that can be implemented for de novo identification of endogenous transcriptional regulatory modules in cultured mammalian cells.

Three chapters are devoted to technologies designed to examine genome-wide association of transcription factors with regulatory regions of genes. A protocol focuses on isolation, purification, and immunoprecipitation of DNA fragments associated with a transcription factor of interest, with the intention of massive parallel sequencing. A method (HaloCHIP) utilizes a HaloTag protein fusion and corresponding interaction resin (HaloLink) to capture crosslinked protein–DNA complexes directly from cell lysates. This approach effectively yields the DNA fragments bound to a protein of interest, circumvents the need for using antibodies, and facilitates downstream analyses including DNA amplification, use of microarrays, and massive DNA sequencing.

A powerful genetic method, a modified yeast one-hybrid system, is presented for analysis of DNA–protein interactions on a genome-wide scale. When compared to other methods, the modified system offers several advantages including low-cost, large-scale output, and ease of reagent handling.

Several chapters cover in vitro techniques for studies of protein–DNA interactions. A new application of mRNA display provides a method for in vitro selection of DNA-binding proteins. In a single experiment, using DNA as bait, the selection system can identify various DNA–protein complexes including those that contain hetero-oligomers of transcription factors bound to DNA.

A technology (SILAC-based quantitative proteomics) is included for identifying specific interactions between proteins and functional DNA elements in an unbiased manner. Another technique, electrophoretic mobility shift assay (EMSA) is useful for characterization of nucleoprotein complexes formed with short DNA fragments. This technique is particularly convenient for determining whether a DNA fragment of interest includes binding site(s) for transcription factors present in nuclear extracts prepared from a given cell type. When used in conjunction with specific antibodies, EMSA offers a strategy (supershift assay) for identification of transcription factors that associate with a DNA fragment.

Several chapters cover spectroscopic techniques for studying protein–DNA and protein–protein interactions. Fluorescence Resonance Energy Transfer (FRET) is useful for measuring changes in DNA conformation due to protein binding because small changes in the distance between two fluorophores (2–10 nm) translate into large changes in energy transfer. With Fluorescence Cross-Correlation Spectroscopy (FCCS), it is possible to assess interaction of target molecules in aqueous condition. When applied to cultured cells, with FCCS one can directly observe dimerization between transcription factors in living cells. Another spectroscopic technique, Fluorescence Anisotropy/polarization Microplate assay (FAMA), provides a powerful tool to investigate the interaction of transcriptional coactivators with transcription factors that bind DNA.

Two schemes are covered for isolation and characterization of relatively large multiprotein complexes. One scheme was designed to purify multisubunit complexes gently and quickly from crude extracts prepared from mammalian cells. An example describes isolation of the mammalian Mediator complex from cultured cells. The other scheme uses a single-step FLAG affinity purification to isolate chromatin modifying complexes for subsequent characterization by sucrose gradient equilibrium centrifugation and mass spectrometry.

Several complementary approaches are offered for studies of histone acetylases (i.e. p300) and deacetylases. A chromatin immunoprecipitation (ChIP) protocol is described for examining p300-dependent regulatory elements in genomic DNA. A mammalian two-hybrid system is presented to detect interactions between two proteins in vivo. This approach overcomes limitations inherent to the yeast two-hybrid system. As an example, the mammalian system was applied to study interactions of p300 with a transcription factor associated with hypoxia (HIF-1α) and a transcription factor relevant to inflammation (NF-κB-p65). Another protocol uses a HDAC inhibitor and takes advantage of different types of p300 to study the interplay of bromodomain and histone acetylation in p300-dependent gene expression. Use of HDAC inhibitor Valproic acid is also described for inducing differentiation of pluripotent stem cells.

To examine gene repression, a chapter details strategies for studies of protein interactions with different DNA methyltransferases. Furthermore a combination of sedimentation and immunoprecipitation assays is presented for analysis and characterization of complexes that repress transcription.

A chapter deals with peptide microarray technology used to identify substrates for recombinant kinases, to discover and evaluate kinase-inhibitors, and to examine changes in activity of kinases in cell lysates and lysates from fresh frozen (tumor) tissue. The method described was developed for examining dynamics of peptide microarrays with real-time read out, and for determining the influence of assay parameters on optimization experiments.

To investigate multicomponent transcriptional complexes, an experimental approach assesses the function of each component on chromatin reconstructed in vitro. Another technique, promoter-independent abortive initiation assay, exploits the intrinsic ability of RNA polymerases to initiate transcription from nicked DNA templates. These assays can be used to measure the effect of transcription factors such as TFIIB and RNA polymerase mutations on abortive transcription. A protocol (nuclear recruitment assay) details a strategy to validate transcription factor interactions in mammalian cells. In addition, a method is described for isolating cell lines with multicopy arrays of reporter transgenes, for real-time high-resolution imaging of transcriptional activation dynamics in single cells.

I hope that the breadth and scope of methodologies offer a beneficial resource for studies of gene regulation. In principle, the protocols and experimental strategies could be applied by researchers in diverse fields including molecular biology, genomics, biochemistry, biomedicine, nutrition, and agricultural sciences.

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