METHODS IN MOLECULAR BIOLOGY

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Protein Acetylation

Methods and Protocols

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Cover caption: Detection of acetylated histones on newly replicated DNA using Click-It reaction to attach biotin molecules to EdU bases in DNA, and Proximity Ligation Assay to generate fluorescent signal at sites where EdU bases and acetylated histones are close to each other.

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Preface

Over the past several decades, modulation of protein function by posttranslational modifications has attracted great interest in multiple areas of biology. The covalent and enzymatic modification of proteins post their biosynthesis provides an often reversible and acute means of regulating vital processes in cells. The functional roles of proteins can be modulated by various covalent modifications including phosphorylation, sumoylation, ubiquitination, glycosylation, and acetylation. Such protein modifications affect various aspects including protein folding, subcellular targeting, interactions with other molecules, catalytic function, and signaling capacity. This book is dedicated to protein acetylation, which has emerged as a highly provocative means of functional regulation. The leading experts in the field of protein acetylation were recruited for this book to contribute experimental strategies and useful laboratory protocols that represent a comprehensive collection of great utility and significance. I anticipate that this *Methods in Molecular Biology* book on Protein Acetylation will be widely read and applied in almost every biological setting of experimentation, as it is increasingly evident that acetylation of chromatin-associated proteins and nucleic acid-interacting and nucleic acid-modifying proteins plays extremely important roles in not only genome metabolism but more broadly cellular homeostasis, tissue integrity and function, and organismal fitness.

This book on protein acetylation describes both fundamental and more elaborate protocols to measure and assay protein acetylation and its consequences in biological systems. The book begins with three chapters on molecular and cellular techniques to detect, quantify, and isolate acetylated protein targets. Schilling et al. describe a high-resolution and high-throughput mass spectrometry approach using minimal protein lysate and an optimized affinity enrichment strategy with a label-free quantification workflow to assess protein acetylation (Chapter 1). This is followed by a contribution from the Parthun lab that describes experimental procedures to evaluate acetylation of histones during chromatin assembly, a highly relevant topic in the burgeoning field of epigenetics (Chapter 2). The combinatorial approach involves state-of-the-art iPOND, quantitative mass spectrometry, and SILAC methodologies to characterize acetylation in nascent chromatin. This first section of the book is wrapped up with an exemplary chapter from the Sidorova lab describing experimental procedures to detect and quantify acetylated histones that occur during DNA replication using an in situ Click Chemistry to label DNA and Proximity Ligation Assay to specifically visualize the labeled DNA with a modified histone of choice that is recognized by a modification-specific antibody (Chapter 3). The approach is valuable to measure not only histone modifications during active replication but also acetylation when the replication fork stalls due to damage or pharmacological nucleotide depletion.

The focus of this unique collection of experimental procedures dealing with protein acetylation is then targeted in four chapters on measurement and analysis of lysine acetylation and functional consequences. Lysine acetylation is enacted by a group of enzymes designated lysine acetyltransferases (KATs). This section begins with a chapter from the Balakrishnan laboratory which describes a reverse-phase HPLC-based strategy that assesses substrate consumption and product formation at the same time (Chapter 4). Its value

resides in the strong reproducibility and application toward kinetic analysis of KATs. Recognizing the importance of studying native KAT complexes, the Côté lab offers a useful protocol to purify KAT complexes from human cells based on genome editing and tandem affinity purification (TAP) (Chapter 5). They also describe experimental procedures to study the isolated KAT complexes using a lysine acetyltransferase assay and detection of acetylated lysines by small-scale affinity purification or anti-acetyl lysine antibody immunopurification. Finally, they present a general procedure to make homogeneous and site-specific acetylated recombinant protein in bacteria so that functional consequences of acetylation can be studied with a suitable quantity of purified protein. To address the difficulty of determining lysine acetylation stoichiometry across subcellular compartments in eukaryotic cells, the Denu lab provides a strategy of subcellular fractionation with offline prefractionation to determine acetylation stoichiometry using data-independent mass spectrometry (Chapter 6). This approach is highly valuable for investigation of acetylation in various organelles including mitochondria where the posttranslational modification may occur in low stoichiometry. This chapter is followed by one from the Orren lab that describes established and novel experimental approaches and methodologies to detect lysine acetylation or deacetylation of specific target proteins or groups of proteins and how these events are regulated (Chapter 7).

A unique class of deacetylation enzymes are the sirtuins, which are discussed in two chapters dedicated to protocols for detection and characterization of sirtuin targets. The Sinclair lab is especially interested in sirtuins as they represent a class of enzymes that have been implicated in healthy aging and longevity. In their chapter, Schultz et al. describe experimental approaches to identify and characterize sirtuin-activating compounds (STACs) (Chapter 8). This includes the purification of the prominent lysine deacetylase SIRT1, in vitro assays with recombinant SIRT1 and allosteric activators, and mitochondrial assays for SIRT1 activators in cells. Following this chapter, the Weiss lab presents a series of procedures to investigate small molecule inhibitors of another sirtuin known as SIRT2 implicated in tumorigenesis using genetically engineered and xenograft mouse models of cancer (Chapter 9).

Experimental procedures to detect and measure the consequences of acetylation for DNA repair and more broadly DNA processing enzymes and processes are presented in two modules. Beginning with protein targets that influence DNA repair, the Wyrick lab discusses techniques exploiting the model and genetically tractable unicellular organism yeast to investigate the functional consequences of histone acetylation on DNA nucleotide excision repair (NER) (Chapter 10). Procedures to measure the repair of cyclobutane pyrimidine dimers (CPDs) in bulk chromatin as well as individual chromatin loci in yeast that have been manipulated for their histone acetyltransferase (HAT) activity are described. Next, the Bhakat group presents experimental assays to assess the effect of acetylation on a key player in the base excision repair (BER) pathway, i.e., abasic endonuclease 1 (APE1) (Chapter 11). This narrative includes in vitro assays measuring APE1 DNA substrate binding and repair (catalytic) activity, as well as DNA damage repair in vivo as measured by comet assay and cell survival after genotoxic exposure, and subcellular localization of APE1 by immunostaining. Ononye et al. present experimental protocols to analyze the effects incurred by acetylation of the structure-specific nuclease known as flap endonuclease 1 (FEN1), a key processing enzyme implicated in BER and lagging strand maturation during DNA replication (Chapter 12). Following these chapters, the Tsai lab describes experimental assays to assess the effect of HDAC inhibition on DNA double-strand break (DSB) repair in mouse primary cortical neurons (Chapter 13).

Reaching beyond specific DNA repair proteins or pathways, the influence of protein acetylation on processes that affect genomic stability and the cellular stress response is described in two subsequent chapters. Song and Grummt present experimental protocols to assay acetylation of a target DNA helicase known as DDX21 that is implicated in the resolution of three-stranded nucleic acid structures designated R-loops composed of both DNA and RNA arising from transcription; such structures can be problematic when encountered by the replication fork (Chapter 14). Assays to measure the functional consequences of DDX21 acetylation on its helicase activity are presented. Aside from catalytic enzymes like helicases or nucleosomal proteins such as histones, certain DNA-interacting factors such as the tumor suppressor and cell cycle regulator p53 can be acetylated as well. Farkas and McMahon present a procedure for the rapid detection of p53 acetylation status that becomes modulated by cellular stress (Chapter 15).

Having covered various facets of acetylation and its modulation of biological processes at the molecular and cellular levels, as well as studies in model organisms including yeast and mouse, the final two chapters of the collection delve into two areas of disease and aging research of great interest, namely, neuroepigenetics and tumorigenesis. Myrum and Rapp present techniques to isolate and quantify brain-specific and cell subtype-specific acetylated histone proteins which play a role in the regulation of gene expression that profoundly influence long-term memory (Chapter 16). Hadley et al. describe functional tests of HDACs implicated in tumorigenesis which may be applied to assess the efficacy of HDAC inhibitors (Chapter 17). These final chapters emphasize the translational value of protein acetylation targets and their modulation via biological processes and pharmacological intervention.

Altogether, the compiled 17 chapters in this book provide a comprehensive set of experimental techniques and useful strategies to examine the molecular, cellular, tissue, and organismal consequences of protein acetylation. I firmly believe that this unique collection provides an extremely valuable resource to novices and experts alike who find that their research takes them in directions to characterize how the regulatory switches of protein acetylation events are manifested in significant ways and by diverse mechanisms in biological systems.

I wish to thank all the authors for their outstanding chapters. Their efforts are very much appreciated, and it was a great pleasure to work with them in producing this book. I also acknowledge John Walker, the series editor, for his helpful advice and insight as well as David Casey, the editor of Springer Protocols, for his assistance and guidance. Finally, I wish to thank my teachers and mentors throughout my academic and scientific career who have taught me the mechanics and usefulness of the scientific method, the practical value of keeping a good lab notebook, and the benefits of creative thinking. While the list extends beyond those mentioned here, I especially thank my grade school teacher Mr. Gerry Robinson (St. Vincent de Paul School (Wheeling)), high school teacher Mr. Carl Hocke (Central Catholic High School (Wheeling)), undergraduate professors Drs. Milton Smith and Robert Paysen (Bethany College), and graduate advisor Dr. Steven Matson (University of North Carolina at Chapel Hill).

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