

Methods in ENZYMOLOGY

Volume 549

Riboswitch Discovery,
Structure and Function

Edited by

Donald H. Burke-Aguero



Riboswitch Discovery Structure And Function 549 Methods In Enzymology

**Wayne M. Becker, Lewis J.
Kleinsmith, Jeff Hardin**



Riboswitch Discovery Structure And Function 549 Methods In Enzymology:

Riboswitch Discovery, Structure and Function, 2014-11-21 This new volume of Methods in Enzymology continues the legacy of this premier serial with quality chapters authored by leaders in the field This volume covers research methods in riboswitch discovery and validation synthesis and sample prep methods for large RNAs riboswitch structure and function methods folding pathways and dynamics and ligand interactions and thermodynamics Continues the legacy of this premier serial with quality chapters authored by leaders in the field Covers research methods in riboswitch discovery structure and function Contains sections on such topics as riboswitch discovery and validation synthesis and sample prep methods for large RNAs riboswitch structure and function methods folding pathways and dynamics ligand interactions and thermodynamics

DNA Repair Enzymes: Structure, Biophysics, and Mechanism, 2017-07-11 DNA Repair Enzymes Part B Volume 592 is the latest volume in the Methods in Enzymology series and the first part of a thematic that focuses on DNA Repair Enzymes Topics in this updated volume include MacroBac New Technologies for Robust and Efficient Large Scale Production of Recombinant Multiprotein Complexes Production and Assay of Recombinant Multisubunit Chromatin Remodeling Complexes Analysis of Functional Dynamics of Modular Multidomain Proteins by SAXS and NMR the Use of Single Cysteine Variants for Trapping Transient States in DNA Mismatch Repair and Structural Studies of RNases H2 as an Example of Crystal Structure Determination of Protein Nucleic Acid Complexes Includes contributions from leading authorities working in enzymology Focuses on DNA repair enzymes Informs and updates on all the latest developments in the field of enzymology

Riboswitches as Targets and Tools, 2015-01-12 This new volume of Methods in Enzymology continues the legacy of this premier serial with quality chapters authored by leaders in the field This volume covers research methods in riboswitches as targets and tools and contains sections on such topics as constructing and optimizing artificial riboswitches live cell imaging and intracellular sensors with artificial riboswitches conditional control of gene expression with artificial riboswitches using artificial riboswitches for protein evolution and pathway optimization and anti riboswitch drug screens Continues the legacy of this premier serial with quality chapters authored by leaders in the field Covers research methods in riboswitches as targets and tools Contains sections on such topics as constructing and optimizing artificial riboswitches synthetic biology live cell imaging and intracellular sensors with artificial riboswitches synthetic biology conditional control of gene expression with artificial riboswitches synthetic biology using artificial riboswitches for protein evolution and pathway optimization anti riboswitches drug screens

Current Developments in Biotechnology and Bioengineering
Sudhir P. Singh, Ashok Pandey, Guocheng Du, Sudesh Kumar, 2018-11-20 Current Developments in Biotechnology and Bioengineering Synthetic Biology Cell Engineering and Bioprocessing Technologies covers the current perspectives and outlook of synthetic biology in the agriculture food and health sectors This book begins with the basics about synthetic biology and cell engineering and then explores this in more detail focusing on topics like applications of synthetic biology

industrial bioprocesses and future perspectives Information on cell engineering is also presented and manipulation in endogenous metabolic network is studied alongside advanced topics such as fine tuning of metabolic pathways de novo biosynthetic pathway design enzyme engineering targeted to improved kinetics and stability and potential applications of the novel biological systems in bioprocess technology to achieve the production of value added compounds with specific biological activities Assists in developing a conceptual understanding of synthetic biology and cellular and metabolic engineering Includes comprehensive information on new developments and advancements Lists applications of synthetic biology in agriculture food and health **Exploring Protein Structure: Principles and Practice** Tim Skern, 2018-07-04

This textbook introduces the basics of protein structure and logically explains how to use online software to explore the information in protein structure databases Readers will find easily understandable step by step exercises and video trainings to support them in grasping the fundamental concepts After reading this book readers will have the skills required to independently explore and analyze macromolecular structures will be versed in extracting information from protein databases and will be able to visualize protein structures using specialized software and on line algorithms This book is written for advanced undergraduates and PhD students wishing to use information from structural biology in their assignments and research and will be a valuable source of information for all those interested in applied and theoretical aspects of structural biology Dissertation Abstracts International , 2008 *Molecular Biology* Nancy Lynn Craig, Rachel Green, Carol C. Greider, Carol W. Greider, Gisela Storz, Cynthia Wolberger, Orna Cohen-Fix, 2021 *Molecular Biology Principles of Genome Function* offers a fresh distinctive approach to the teaching of molecular biology It is an approach that reflects the challenge of teaching a subject that is in many ways unrecognizable from the molecular biology of the 20th century a discipline in which our understanding has advanced immeasurably but about which many questions remain to be answered KEY FEATURES A focus on the underlying principles equips students with a robust conceptual framework on which to add further detail from the vast amount of scientific information available to us today An emphasis on commonalities reflects the conserved molecular processes and components that we now know to exist between bacteria archaea and eukaryotes Experimental Approach panels demonstrate the central importance of experimental evidence to furthering our understanding of molecular biology by describing research that has been particularly valuable in elucidating different aspects of the subject Online resources for both instructors and students alike enhance the educational value of the text NEW TO THIS EDITION New content on epigenetics targeted genome editing and pre mRNA splicing Cutting edge scientific breakthroughs in CRISPR technology including a description of newly defined steps in the molecular mechanisms underlying CRISPR mediated adaptation in bacterial adaptive immunity and a description of a recently discovered transposable element family whose integration mechanism is closely related to and involves molecular relatives of the CRISPR Cas bacterial adaptive immunity system Enhanced coverage of DNA replication and regulatory RNAs Seven new Experimental Approach

panels This title is available as an eBook Visit VitalSource for more information or to purchase *The World of the Cell* Wayne M. Becker, Lewis J. Kleinsmith, Jeff Hardin, 2005 Accompanying CD ROM contains more than 80 animations and interactive activities numerous microscopy video sequences that show cellular processes in action a searchable glossary flashcards automatically graded practice quizzes annotated web links key terms a gradebook and access to Research Navigator

Structure and Function of Bacterial Riboregulators Robert Andrew Battaglia, 2020 Bacteria use cis regulatory RNA structures called riboswitches to control the expression of many genes Riboswitches are found in the 5' untranslated regions of mRNAs where they regulate transcription or translation of downstream genes by forming a stabilized conformation upon specific ligand binding These regulatory elements are important for bacterial survival because they quickly alter gene expression in response to changes in the intracellular environment Studying riboswitches in atomic detail using X ray crystallography has allowed me to reveal how the structure of these RNAs connects specific recognition of a ligand to subsequent changes in gene expression Riboswitch like regulatory elements called T boxes sense amino acid abundance by binding tRNA Conserved structural domains in the T box allow it to decode tRNA and determine whether it is aminoacylated To gain a mechanistic understanding of these processes and how they affect gene regulation I solved a high resolution crystal structure of a full length T box tRNA complex The structure reveals the organization of T box domains and their contributions to decoding and aminoacylation sensing This was the first glimpse of aminoacylation sensing by the T box which is facilitated by a three domain module that makes specific contacts to the uncharged tRNA terminus I solved another structure of the T box bound to a charged tRNA mimic to understand how the aminoacylation status of tRNA determines T box regulatory output Together these structures reveal how charged and uncharged tRNA influence T box conformation selection to affect gene expression Although atomic level analysis is necessary for understanding the relationship between structure and function in riboswitches understanding the biological contexts in which riboswitches are involved is an equally critical endeavor After solving the structure of a guanine riboswitch I became interested in studying the biological role of its ligand Guanine was not appreciated as a biologically relevant metabolite but the wide distribution of guanine riboswitches in bacteria suggests otherwise To understand the toxicity and intracellular source of guanine I used genetic approaches in the model bacterium *Bacillus subtilis* My results have uncovered novel guanine efflux pump genes and a potential non enzymatic route of guanine production Expanding beyond the study of riboswitch function to understanding the processes they regulate is a promising new avenue to undiscovered biology

Riboswitches Alexander Serganov, 2010-11-19 The transfer of hereditary information from genes to proteins is one of the essential processes in all living organisms on our planet Some genes are expressed without modulation throughout the life of a cell while many others require various degrees of control to precisely balance cellular metabolism with environmental conditions For many years researchers attributed this regulatory function to protein molecules which can direct gene expression at multiple levels in

response to various input signals and with different degrees of selectivity. Even when the control of gene expression was achieved via direct interactions between proteins and mRNAs, the active role was routinely assigned to proteins while RNAs were considered merely as recipient molecules. The discovery of RNA interference and multiple bacterial regulatory RNAs caused a shift from the perception of proteins as the predominant regulators of gene expression to the acknowledgement of the importance of RNAs in many regulatory circuits. Such a viewpoint received strong support several years ago after the discovery of riboswitches and related RNA sensors. mRNA regions capable of alternating their conformations in response to the presence of cellular metabolites and other physical or chemical cues. These classes of RNA pass on cellular and environmental information directly to transcription or translation machinery without the assistance of proteins. The riboswitches are commonly defined as evolutionarily conserved mRNA regions capable of specific binding to metabolite molecules and as a result adopting a particular RNA conformation that modulates gene expression.

Relating Riboswitch Structure to Gene Regulation in Live Cells Debapratim Dutta, 2019

The rise of antibiotic resistance calls for immediate focus on identifying novel drug targets. Riboswitches are a distinct class of RNA that have already been validated as drug targets. They take part in gene regulation in direct response to specific small molecule effectors. Levels. Studies on riboswitches have mostly focused on their ligand binding abilities in vitro, outpacing our understanding of the underlying mechanisms that link effector binding to gene regulation. To convincingly relate RNA structure to function, I analyzed the preQ1 II riboswitch, a well characterized riboswitch that recognizes the metabolite pre-queuosine. I used an RNA chemical modification analysis called in-cell SHAPE to compare flexibility changes of individual nucleotides in response to preQ1. The gene OFF conformational state data showed excellent correlation with our bound state crystal structure. We then developed a reporter assay in which GFPuv is controlled by a preQ1 II riboswitch to study its gene regulatory function. Added effector showed a 10 fold repression of GFPuv expression consistent with binding studies done by Isothermal Titration Calorimetry (ITC). The functional relevance of in vitro observations thus established, I sought to identify molecular interactions that connect preQ1 binding to gene regulation. We used our reporter assay and ITC along with site directed mutagenesis to study specific nucleobase interactions hypothesized to be on molecular signal transduction pathways. Analyses were conducted on 15 mutants flanking the preQ1 binding pocket. Several conserved elements of the riboswitch like the A minor bases and the helix P4 were found dispensable for effector sensing but are playing an immense role in gene regulation. I also observed several interactions that are essential for gene regulation inside live cells but were not for ligand binding. The results helped shed light on how riboswitches function in their natural environment inside cells. I also discuss the nucleoside dynamics and structural changes occurring to the preQ1 I riboswitch upon ligand binding. I used chemical probing methods like DMS and SHAPE and found bases proximal to the ligand binding site undergoing a change in their conformation or flexibility upon effector binding. Together with crystal structure, computational studies and 2AP fluorescence studies, we

confirmed that the dynamic L2 loop in the apo state riboswitch is key to functioning of the RBS for effective gene regulation

Decoding **Riboswitch Discovery Structure And Function 549 Methods In Enzymology**: Revealing the Captivating Potential of Verbal Expression

In a period characterized by interconnectedness and an insatiable thirst for knowledge, the captivating potential of verbal expression has emerged as a formidable force. Its power to evoke sentiments, stimulate introspection, and incite profound transformations is genuinely awe-inspiring. Within the pages of "**Riboswitch Discovery Structure And Function 549 Methods In Enzymology**," a mesmerizing literary creation penned with a celebrated wordsmith, readers embark on an enlightening odyssey, unraveling the intricate significance of language and its enduring affect our lives. In this appraisal, we shall explore the book is central themes, evaluate its distinctive writing style, and gauge its pervasive influence on the hearts and minds of its readership.

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