

Methods in ENZYMOLOGY

Volume 549

Riboswitch Discovery,
Structure and Function

Edited by

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Riboswitch Discovery Structure And Function 549

Methods In Enzymology

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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

Structures of Large RNA Molecules and Their Complexes, 2015-06-06 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 RNA folding and dynamics RNA protein interactions and large RNPs Continues the legacy of this premier serial with quality chapters on structures of large RNA molecules and their complexes [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 [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 prequeosine1 preQ1 I used an RNA chemical modification analysis called in cell ic 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

Artificial Riboswitches Atsushi Ogawa,2016-09-03 Artificial riboswitches and other ligand responsive gene regulators make it possible to switch protein synthesis ON or OFF with arbitrary ligand molecules Artificial Riboswitches Methods and Protocols focuses on the state of the art methods developed in recent years for creating artificial riboswitches therefore this volume could be regarded as a collection of recipes for the gene circuit elements in synthetic biology and metabolic engineering Chapters cover topics such as screening or rational design methods for obtaining artificial riboswitches that function in either bacterial or eukaryotic translational systems protocols for evaluating the activities of the resultant riboswitches as well as protocols for construction of ligand dependent trans acting gene regulators Written in the successful Methods in Molecular Biology series format chapters include introductions to their respective topics lists of the necessary materials and reagents step by step readily reproducible protocols and notes on troubleshooting and avoiding known pitfalls Authoritative and easily accessible Artificial Riboswitches Methods and Protocols seeks to serve not only bioengineers who aim to reprogram cell behaviors and molecular biologists who leverage these regulators for genetic studies but to all researchers interested in this fascinating field

Riboswitches Alexander Serganov,2009-04-17 The transfer of hereditary information from genes to proteins is one of the essential pr esses

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.

Intracellular Studies of Ribozymes and Riboswitches Peter Y. Watson, 2012. This work explores the biological functions of structured RNAs in the living cell. RNA folding studies of the hairpin ribozyme, functional exploration of the glmS ribozyme, and discovery of the ydaO ATP riboswitch are described herein. We expressed the hairpin ribozyme in a yeast model system to report intracellular folding outcomes. We determined that a threshold of structural stability exists: structures with stabilities weaker than 17 kcal/mol undergo exchange while more stable structures resist equilibration. Using the same system, we investigated the folding of a regulatory RNA or riboswitch that controls expression of glucosamine 6-phosphate synthase. Expanding upon the traditional view that riboswitches respond to a specific metabolite ligand, we determined that the glmS riboswitch is also subject to inhibition by endogenous metabolites. We further confirmed this finding using reporter experiments in *Bacillus subtilis* in the natural context of the glmS riboswitch. These insights increased our understanding of RNAs' regulatory capacity by demonstrating that they are capable of integrating information from multiple chemical signals. These results prompted an increased interest in the role of riboswitches in sensing central components of energy metabolism. We therefore performed a novel biochemical screen to determine whether orphan riboswitches of unknown function might bind ATP. We discovered that the ydaO riboswitch, a representative of a widespread class of riboswitches, binds ATP *in vitro* and responds to ATP levels *in vivo*. This motif controls diverse genes across a variety of Gram-positive bacteria, demonstrating that a single riboswitch can regulate disparate pathways in response to energy availability. These studies lay the framework for understanding the complex and diverse regulatory functions of RNAs.

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