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Therapeutic Applications of Ribozymes and Riboswitches

Methods and Protocols

Edited by

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Preface

The RNA field is currently undergoing a revolution. In the past years, several unexpected functions have been uncovered for RNA molecules including phosphodiester backbone cleavage and gene expression regulation, both in a cis- and trans-acting fashion. Upon those discoveries, the idea that RNA could be used as a molecular tool was rapidly considered. For instance, considerable efforts have been put to use RNA molecules either as drugs or as drug targets. Today, therapeutic applications employing RNA molecules include the use of ribozymes as silencing tools, while riboswitches are involved in the development of novel antimicrobial agents. This volume presents essential protocols for the development of RNA-based therapeutic strategies using ribozymes and riboswitches as a way to treat various diseases.

Ribozymes are autocatalytic RNAs mainly found in viroids and viral genomes. Small ribozymes such as the one found in the hepatitis delta virus are important for the replication of the host. The main characteristic of ribozymes which is to specifically recognize and cleave RNA molecules was rapidly applied to target undesirable messenger RNAs. Thus, over the years, various ribozyme candidates have been engineered, optimized, and characterized to cleave target mRNAs with high specificity and efficiency. The degradation of targeted mRNAs and the associated shutdown expression are the direct consequences of the ribozyme cleavage activity.

Several chapters of this book describe recent and promising techniques designed to identify accessible regions in RNA molecules. This step is the premise for the development of good ribozyme candidates. Technical approaches such as SHAPE and nuclease probing, analyzed jointly with bioinformatic tools, are still relevant methods. However, the challenge is now to apply those methods simultaneously on a genomic scale and to determine the accessibility of each nucleotide directly in vivo. Moreover the catalytic activity of ribozyme candidates has to be evaluated both in vitro and in vivo. This is important because it is known that ribozymes possessing a great in vitro cleavage activity do not necessarily show the best activities in vivo. Upon the design and evaluation of ribozyme candidates, the choice of the expression system and the cellular model will greatly depend on the target RNA.

In order to offer a general idea of protocols used, specific strategies associated to various biological targets will be presented. For example, researchers have now been able to construct ribozymes against mRNAs important for prions, viruses, and cancer progression. Also, recent experiments have shown the efficiency of delivery systems, which are nevertheless still limited by the inherent cellular instability of RNA. Relevant modifications to increase the half-life of ribozymes in specific conditions are also important issues that researchers will have to improve in the next few years.

Riboswitches are genetic regulatory elements mostly retrieved in the 5′ untranslated regions of bacteria. The capacity of riboswitches to specifically recognize small metabolites and theirs derivatives makes them an attractive target to develop novel antimicrobial agents.

Antibiotics against riboswitches are promising for therapeutic use since none of those RNA switches have been identified so far in humans. The first step in the antibiotic development consists in the identification of riboswitches controlling essentials genes. It is also important to characterize the regulation mechanism used by each targeted riboswitch. Bioinformatic analysis used in combination with biochemical and activity assays is the principal method needed to validate a riboswitch candidate. Upon riboswitch confirmation, the determination of the most promising therapeutic molecule can then be performed. Various methods to characterize affinity properties and intrinsic riboswitch characteristics were developed in the last few years. Efficient small molecules targeting resistant bacterial riboswitches constitute a new class of antibiotic, which is exciting considering that only one new antibiotic class has been found since 1985. New challenges, including rapid, efficient, and high-throughput methods to easily identify riboswitches and screen a diversity of ligands, have now emerged from those recent discoveries.

This book offers a complete overview of protocols used in the development of RNA molecule as drugs and drug target. All chapters describe a recent and precise RNA molecule approaches or studies in the development of an RNA therapeutic tool. We are convinced that these methods will help researchers from various domains of life sciences, including clinicians, biochemists, and virologists.

We want to thank all authors who participated in the production of this book. Their contributions will hopefully inspire many researches in the development of new therapeutic applications implicating RNA molecules or directly targeting harmful RNA.

Sherbrooke, QC, Canada

Daniel Lafontaine
Audrey Dubé

Contents

Preface . . . . . *v*
Contributors . . . . . *ix*

1 Identification of Regulatory RNA in Bacterial Genomes by Genome-Scale Mapping of Transcription Start Sites 1
Navjot Singh and Joseph T. Wade

2 Screening Inhibitory Potential of Anti-HIV RT RNA Aptamers 11
Margaret J. Lange and Donald H. Burke

3 Design and Evaluation of Clinically Relevant SOFA-HDV Ribozymes Targeting HIV RNA 31
Robert J. Scarborough, Michel V. Lévesque, Jean-Pierre Perreault, and Anne Gatignol

4 Directing RNase P-Mediated Cleavage of Target mRNAs by Engineered External Guide Sequences in Cultured Cells 45
Xiaohong Jiang, Naresh Sunkara, Sangwei Lu, and Fenyong Liu

5 Design and Analysis of Hammerhead Ribozyme Activity Against an Artificial Gene Target 57
James R. Carter, Pruksa Nawtaisong, Velmurugan Balaraman, and Malcolm J. Fraser Jr.

6 Knockdown Strategies for the Study of Proprotein Convertases and Proliferation in Prostate Cancer Cells 67
François D'Anjou, Frédéric Couture, Roxane Desjardins, and Robert Day

7 Use of Tumor-Targeting *Trans*-Splicing Ribozyme for Cancer Treatment 83
Seong-Wook Lee and Jin-Sook Jeong

8 Characterization of Hairpin Ribozyme Reactions 97
Preeti Bajaj and Christian Hammann

9 Finding Instances of Riboswitches and Ribozymes by Homology Search of Structured RNA with Infernal 113
Amell El Korbi, Jonathan Ouellet, Mohammad Reza Naghdi, and Jonathan Perreault

10 Structure-Based Virtual Screening for the Identification of RNA-Binding Ligands 127
Peter Daldrop and Ruth Brenk

11 Probing Riboswitch Binding Sites with Molecular Docking, Focused Libraries, and In-line Probing Assays 141
Francesco Colizzi, Anne-Marie Lamontagne, Daniel A. Lafontaine, and Giovanni Bussi

12 Discovery of Small Molecule Modifiers of microRNAs for the Treatment of HCV Infection 153
Valerie T. Tripp and Douglas D. Young

13 Bacterial Flavin Mononucleotide Riboswitches as Targets for Flavin Analogs 165
Danielle Biscaro Pedrolli and Matthias Mack

14 Construction and Application of Riboswitch-Based Sensors That Detect Metabolites Within Bacterial Cells 177
Casey C. Fowler and Yingfu Li

15 Screening Assays to Identify Artificial glmS Ribozyme Activators 199
Christina E. Lünse and Günter Mayer

16 Analysis of Riboswitch Structure and Ligand Binding Using Small-Angle X-ray Scattering (SAXS) 211
Nathan J. Baird and Adrian R. Ferré-D'Amaré

17 Use of SHAPE to Select 2AP Substitution Sites for RNA–Ligand Interactions and Dynamics Studies 227
Marie F. Soulière and Ronald Micura

18 Cell Internalization SELEX: In Vitro Selection for Molecules That Internalize into Cells 241
Amy Yan and Matthew Levy

19 DNA Electronic Switches Based on Analyte-Responsive Aptamers 267
Jason M. Thomas, Hua-Zhong Yu, and Dipankar Sen

Index *277*

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