
Preface

A large portion of the human genome encodes for long noncoding RNAs (lncRNAs), transcripts longer than 200 nucleotides and without an open reading frame, which play widespread roles in gene regulation and other cellular processes. They can be transcribed from intergenic regions, but many of them are associated with annotated protein-coding genes, instead.

Noncoding and coding transcripts at a given locus can overlap, and this phenomenon is called transcriptional forest. It includes sense-antisense transcripts coming from transcription of both strands, sense noncoding RNA overlapping mRNA from the sense strand but not encoding for protein, or totally intronic RNAs.

Other long ncRNAs linked to coding genes are associated with enhancers (eRNAs) or promoters (paRNAs). They are generally low expressed but involved in important cellular mechanisms, like the dynamics of nuclear architecture, chromatin remodeling, and transcriptional regulation. They exert their function *in cis* affecting neighbor gene expression.

Both eRNAs and paRNAs can interact with DNA by several mechanisms, forming triplex with dsDNA, displacing a single strand of DNA to form RNA:DNA hybrid (so-called R loops), interacting with nascent RNA or with DNA-binding proteins in a sequence-specific manner. As many other long ncRNAs, they can interact with proteins important for transcriptional regulation. In those RNP complexes, they often are the scaffold which brings two or more proteins together and enable their co-localization and interaction. Another interesting role is the assignment of specificity to the binding of one or more proteins to a given genomic locus. Their regulated expression, for instance, can recruit certain protein complexes to an allele but not to the other.

Many of their interactors are epigenetic regulators. Epigenetic processes, such as DNA methylation and histone post-translational modifications which influence chromatin remodeling, contribute to the pathogenesis of many diseases, in particular human cancers, and impact on disease progression, treatment responses, and clinical outcome.

Promoter-associated RNA of key disease genes may represent a natural switch to exploit in order to manipulate dysregulation of relevant coding transcripts. PaRNAs expression, under certain conditions, can enable or not the binding of specific proteins to a promoter and selectively modulate the transcription of adjacent gene. According to this, they can be considered valuable regulatory elements to investigate further.

The main goal of this book is to summarize methods of molecular biology, biochemistry, and bioinformatics, useful to explore the expression and functions of promoter-associated RNA, which, among the classes mentioned till now, are still less characterized.

The book is subdivided into four parts. In the first part, genome-wide approaches are described to identify functionally relevant elements in noncoding regions and to detect transcription in correspondence of promoters. Importantly, bioinformatics chapters are included to help the reader to use publicly available data as a source of information about noncoding transcriptome.

In the second part of this book, techniques useful to deeply characterize paRNA structural features are described. Accurate investigation of physical features of a noncoding RNA, such as extension, secondary structure, and binding affinity for RNA-binding proteins, can help in understanding its putative function. Promoter-associated RNAs can be

considered a new class of molecules which play a key role in transcriptional regulation. In the third part is described how selecting good therapeutic target among paRNAs relevant in diseases and how impeding their function by RNA interference. Strategies to investigate transcriptional gene silencing mechanism are described. Further, some methods are reported to study R-loop structures, RNA:DNA hybrid structures used by noncoding RNAs to regulate gene expression, changing local chromatin environment. The last part of the book is dedicated to paRNA therapeutic potential. This part also describes how siRNAs directed against paRNAs can be applied in vivo to modulate transcription of important genes controlled by paRNAs.

I hope this book will help the reader to appreciate the potential of paRNAs as a new class of regulatory molecules to further investigate and value as tools for fine transcriptional tuning.

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Promoter Associated RNA

Methods and Protocols

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