1

Introduction

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The sequencing of the human genome more than 20 years ago revealed that it encodes just ~20,000 proteins, comprising only about three percent of its ~3 billion bases [1]. However, we now know that more than 80% of the genome is transcribed into RNA at some point [2]. In addition to RNAs that encode polypeptide sequences and facilitate protein biogenesis, diverse classes of non-coding RNA such as microRNAs, long non-coding RNAs (lncRNAs), small nucleolar RNAs (snoRNAs), and others continue to be discovered and characterized [3]. Numerous genome-wide association studies and other broad genomics efforts have pointed toward non-coding RNAs as relevant to diverse diseases including neurodegenerative conditions, infectious diseases, and cancer [3]. Furthermore, recent estimates indicate that only ~15% of the human proteome has been successfully targeted with small molecules, which is partly due to the lack of suitable binding pockets for small molecules on many proteins [4]. Challenges with targeting a large part of the human proteome, coupled with widespread non-coding functions of RNA in both humans and pathogenic organisms, point toward RNA and related regulatory processes as intriguing alternative drug targets for novel therapeutics.

A key promise of developing small molecules that target RNA or regulate RNA function is that, by doing so, one could potentially pharmacologically control the function of genes or signaling pathways that are challenging to modulate at the protein level [5–9]. However, understanding RNA as a small-molecule drug target comes with a number of challenges. Many RNAs are structurally dynamic, more closely resembling intrinsically disordered proteins than the ordered protein domains that are commonly associated with "traditional" drug targets [10]. Limitations in biophysical techniques to characterize RNA–ligand complexes and structural ensembles at atomic resolution complicate efforts to rationally design new potential drug molecules. In addition, the majority of RNAs are bound to and interact with RNA-binding proteins, often in a time- or stimulus-dependent fashion [11]. The RNA biopolymer consists of just four bases in contrast to the 20 canonical amino acids found in protein sequences. In addition, the chemical

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nature of the anionic phosphodiester backbone of RNA contrasts starkly with that of the polypeptide backbone of proteins, resulting in considerably different biophysical properties of the two polymers. Still, fundamental studies aimed at understanding the targetability of RNA indicate that it is capable of folding into complex, three-dimensional structures with hydrophobic pockets that are likely suitable for small-molecule binding [7, 12]. Moreover, naturally occurring aptamers (as seen in riboswitches) and lab-evolved aptamers provide validation that RNA can interact with and sense low-molecular-weight species ranging from metal cations or halide ions to drug-like small molecules and even complex metabolites like cobalamin (vitamin B12) with exquisite selectivity [13].

Indeed, the history of drug discovery has already demonstrated the potential and significant impact that RNA-targeting medicines can have. Ribosome-targeting antibiotics, exemplified by macrolides, aminoglycosides, and tetracyclines, have been known since at least the 1940s and represent the largest class of clinically used drugs to treat infections [14]. In general, these compounds make specific contacts with ribosomal RNA (rRNA) within the large ribonucleoprotein complex of the ribosome and modulate protein synthesis. The development of these compounds has had a remarkable impact on human health. However, despite the massive clinical success of such antibiotics, efforts to develop compounds that target specific transcripts apart from the ribosome itself have proven challenging. Nevertheless, the approval of risdiplam in 2020 for the treatment of spinal muscular atrophy demonstrates that small molecules that target RNA can indeed be developed as powerful and mechanistically novel therapeutics [15].

One important factor that represents a barrier to developing RNA-targeted small molecule therapeutics is selectivity. Antisense oligonucleotides and related sequence-based probes such as peptide nucleic acids (PNAs) and locked nucleic acids (LNAs) are now routinely used as tools and provide high sequence specificity [16]. Antisense molecules have also been approved as therapeutics; however, challenges with delivery, biodistribution, and cell permeability continue to be barriers impacting the broader application and development of such molecules as therapeutics. As a complementary modality, small molecules remain highly attractive as drugs due to their ability to passively diffuse across cell membranes, achieve high metabolically stability and oral bioavailability, as well as the potential for penetration into the central nervous system (CNS). From a patient perspective, these characteristics can translate into significant benefits such as the convenience of once-a-day oral administration. The widespread recognition that RNA can directly drive disease, coupled with the potential to modulate it with small-molecule drugs, has driven significant interest in this emerging field.

This book represents a collection of perspectives from leading experts on the state of the art in understanding RNA as a target for small molecules. The chapters included represent a broad overview, covering topics ranging from how to think about and understand RNA structure, how to identify and understand druglike small molecules that bind to RNA, various approaches for controlling RNA function with small molecules, to overviews of RNA–protein interactions and

post-transcriptional modifications of RNA. Finally, we provide an outlook chapter that adds perspective to the future of RNA-targeted drug discovery, including specific challenges that need to be overcome to develop RNA-targeted therapeutics.

In Chapter 2, Incarnato provides a discussion on how to think about RNA structure at the level of individual base pairs. This chapter covers methods and applications for utilizing structure probing to characterize individual RNAs (including both well-defined structures and conformationally diverse ensembles). In Chapter 3, Braun et al. report on the history and progress in determining atomic resolution structures of RNA. This space has historically been highly challenging due to the flexible nature of the RNA biopolymer. However, advances in cryoelectron microscopy and techniques in X-Ray crystallography have resulted in exciting progress in recent years. The increasing availability of atomic resolution structures of RNA ligand complexes is bound to significantly enhance the ability to effectively design high-quality small molecules that bind RNA selectively.

Chapter 4 provides a discussion on lead generation techniques for identifying small molecules that bind to RNA. These methods, including both target-based and phenotypic screens, represent diverse approaches to discover starting points for identifying lead structures for medicinal chemistry programs. In many cases, lead generation techniques used for identifying compounds that target proteins can be adapted for RNA targets, and the authors discuss specific challenges and considerations when applying these methods to RNA. This chapter also discuss several RNA-specific approaches for establishing cellular target engagement. In Chapter 5, Hay et al. discuss the types of chemical matter that binds to RNA, how to characterize it, and how it differs from (and is similar to) protein binding small molecules. Here, it is becoming clear that diverse chemotypes can interact with RNA, both within traditional druglike chemical space and beyond.

The next series of chapters describe examples of different classes of RNA targets. In Chapter 6, Duca provides an overview of microRNAs and efforts to target them. These small RNAs are an intriguing class of non-coding RNA targets that have received considerable interest from both industrial and academic groups as they play a key role in regulating mRNA levels and as a result, control expression levels of proteins. In Chapter 7, Barraza et al. describe efforts to modulate pre-mRNA splicing with small molecules. This important process is the target of clinically validated therapies for spinal muscular atrophy and holds promise for a variety of other mechanistically novel therapies for other diseases including Huntington's diseases and various cancers. While early work has focused on rare monogenic diseases, there is considerable promise in the development of splice modulators as therapeutics for a variety of diseases. Chapter 8, by Mohsen et al. describes riboswitches, which are naturally occurring, ligand-responsive RNA aptamers. These intriguing structures have been the subject of considerable study in both structural/biochemical contexts as well as drug discovery – primarily through the lens of identifying novel antibiotics. However, they also provide promise in synthetic biology as well, particularly in the exciting area of gene therapy as a potential on/off switch for gene expression.

In Chapter 9, Meyer et al. describe examples of small molecules that degrade RNA. By a mechanism analogous to targeted protein degradation strategies such as PROTACs, these molecules recruit nucleases to target RNAs for cleavage and degradation. While some RNA targeting molecules are now FDA approved, RNA-targeting chimeras (RiboTACs) are still at an earlier stage of development. However, RiboTACs may offer specific advantages over monofunctional ligands that target RNA and present opportunities to harness induced proximity pharmacology in the RNA target space and also function in a sub-stoichiometric matter.

In Chapter 10, VanGraafeiland et al. discuss how modulation of programmed frameshifting, primarily by targeting sequences in viral genomes, is an intriguing therapeutic strategy with significant potential. With the development of several potent molecules, cellular proof of concept has now been established for this unique mechanism of controlling gene expression with small molecules. In Chapter 11, Soueid et al. broadly discuss RNA–protein interactions as targets for therapeutics. RNA-binding proteins represent nearly 10% of the human genome and regulate a wide range of cellular and disease relevant processes. Here, understanding structure and patterns of recognition is key to both unraveling biology and developing therapeutics. This chapter also describes the attributes and limitations of a several assay formats that have been developed for identifying compounds that modulate RNA–protein interactions. Chapter 12, by Eggert et al. covers epitranscriptomics. In this context, epitranscriptomics is defined as chemical modifications of RNA, and how these modifications alter biological processes. Although a wide variety of RNA modifications are known, only a few have been studied extensively. Nevertheless, this is an active area of research where small molecules have already entered clinical development.

Finally, in Chapter 13, we provide an outlook on the field and discuss some of the challenges and opportunities facing the development of RNA-targeting molecules as therapeutics. Continued efforts to develop the fundamentals of medicinal chemistry and molecular design principles within this challenging space, coupled with the importance of target validation, establishing relevant functional assays, and understanding selectivity are key topics. The latter stands to benefit tremendously as more and more atomic resolution structures of RNA–small molecules complexes become availed. While many challenges remain, the potential for RNA-targeting medicines to make a broader impact on human health stands as a compelling rationale for continued efforts. Together, we hope that these chapters provide an exciting perspective on how to think about and prosecute RNA as targets for small molecules.

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