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# Regulatory Mechanisms through RNA Conformational Switching and Dynamics

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The last few years have deepened our appreciation of RNA as a magical molecule. RNA serves as an enzyme (ribozyme) and receptor (riboswitch). It is essential for gene editing (CRISPR), regulation of mRNA levels (ncRNA), and is the basis of customized vaccines (mRNA vaccines). RNA plays the central catalytic roles in translation (ribosome) and splicing (spliceosome), and it has been implicated as the Rosetta Stone in the origin of life (RNA world) where it simultaneously serves functional and genetic functions. These breathtaking properties of RNA have led to profound advances in molecular medicine and in humanity's understanding of how life can begin and evolve. Key to these astounding functions is the ability of RNA to adopt exquisite secondary and tertiary structures and to switch between different conformations.

It has become increasingly clear that RNA molecules can adopt more than a single fold. The partition function, which describes the major folds and their populations, is the most realistic way to describe an RNA sequence. This molecular view of RNA, which is dictated by the energetic landscape, is powerful because it also provides a deep functional view of RNA, as each of the different folds can have a different biological function. The 16 articles in this special issue illustrate just how general and important the multi-fold concept of RNA is. We see that diverse RNAs such as riboswitches, thermometers, ncRNAs and viral genomes, adopt multiple folds, and that the populations of these folds change through conformational switching to alter gene expression. The conformation of the RNA can be controlled by diverse factors such as the binding of small molecules and proteins, covalent modifications of the RNA, flanking sequence, and temperature, and the energetic landscape for a given RNA molecule can be re-shaped on the fly as the RNA is transcribed and nascent flanking sequence interacts with the upstream portion of the transcript. Adding to the richness of this picture, the atoms and helical regions of an RNA fold have motions, ranging from the picosecond to the millisecond timescales, that also play critical roles in gene expression.

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Computational techniques, such as molecular dynamics (MD), Nearest-Neighbor theory and bioinformatics, have combined with experimental ones to enhance our understanding of RNA conformational switching and allowed predictions to be made.

Lucks and co-workers set the stage for the issue (and provide the cover for the issue) by asking the central question, “How does RNA fold dynamically?” [1] First, they consider the framework of conformational switching and dynamics, and the role of co-transcriptional folding that leads to an “ever-shifting energy landscape”. Second, they consider strand switching as a ubiquitous and energetically feasible way to undergo RNA conformational switching. And third, they remind us of the complexity of RNA folding in vivo in which cellular factors can influence the free energy landscape. Perhaps there is no better example of RNAs that undergo conformational switching than riboswitches, whose very name connotates RNA switching. There are three contributions on riboswitches in this issue. First, Lennon and Batey review how gene expression is controlled in cobalamin (vitamin $B_{12}$) riboswitches [2]. Once the start codon is transcribed, these riboswitches end up populating one of two major folds; however, they first go through additional folds as transcription is occurring. The population and structures of these folds is dictated by the cobalamin small molecule cofactor, which itself interacts directly with the strands of a four-way junction to stabilize it. The co-transcriptional landscape of ligand binding and regulation is also shaped by several transcriptional pause sites on the RNA. These sites function to allow the RNA to fold without interference from downstream flanking sequence and to regulate expression by switching exposure of the ribosome binding site. Second, Hoetzel and Suess describe a technique to enhance engineering of synthetic riboswitches [3]. The authors take a two-pronged approach: 1.) Reviewing pertinent literature wherein selected aptamers are found to generally fail to conformationally switch and 2.) Harnessing these data to propose a fresh experimental approach to isolate switching RNAs. Specifically, the authors offer an innovative experimental technique called “Capture-SELEX” to facilitate isolation of only those aptamers that switch conformation. Their technique requires the aptamer to undergo a conformational change to be released from solid support and thus be selected. Promising data in support of this approach are provided and drive home the key point that ligand binding can induce RNA refolding in a general way. In the third article, Fürtig and coworkers investigate the mechanisms by which ribosomes load onto the Shine-Dalgarno sequence following ligand-induced conformational change in the adenine-sensing riboswitch [4]. By developing a clever 19F NMR approach that allows sensitive monitoring of intra- and intermolecular interactions, the authors show that the primary binding site of the 30S ribosome localizes downstream of the expression platform in the 3’-tail region. Loading of the 30S ribosome onto the 3’-tail is followed by backsliding in the 5’-direction towards the Shine-Dalgarno sequence. The authors suggest that this mode of ribosome loading might provide an additional layer of control over translational riboswitches.

Analogous to Riboswitches, RNA thermometers (RNAT) control translation via mechanisms that include temperature-induced changes to RNA structure. Narberhaus and colleagues describe how two RNATs regulate translation of genes encoding the YscJ and YscT proteins, essential components of the type III secretion system (T3SS) from *Yersinia* pseudotuberculosis [5]. Using a combination of reporter constructs and structure probing, the authors demonstrate that the yscJ and yscT thermometers regulate translation efficiency

by a temperature-induced opening of the Shine-Dalgarno region. Constructs designed to stabilize the temperature-induced opening of the thermometers validate that RNA structure controls the downstream expression the YscJ and YscT proteins. When combined with previous work, the authors reveal that the assembly of the T3SS apparatus is translationally controlled at multiple levels via RNA-based temperature sensors. In a related manuscript, Bevilacqua and coworkers delve into the mechanisms by which flanking RNA structure modulates the folding stability of the ROSE thermometer [6]. ROSE controls the expression of heat shock protein A, whose 5'UTR is highly structured and consists of three stem loops upstream of the thermometer. By combining UV thermal denaturation studies, modeling of co-transcriptional folding, and in-line probing, the authors conclude that the upstream SL domains likely act as folding guides for the ROSE thermometer. Both the Narberhaus and Bevilacqua papers further illuminate how RNA conformational landscapes can be modulated by cis or trans stimuli to regulate expression of temperature responsive genes.

Conformational dynamics of different folds are extremely important in RNAs as well, including in larger RNAs such as viral RNAs. Three contributions to this special issue illustrate this concept. First, Dayie and co-workers review the conformational dynamics of the hepatitis B virus pre-genomic RNA [7]. Using NMR relaxation techniques as well as MD calculations, they describe motions of the RNA on the picosecond-to-nanosecond and microsecond-to-millisecond timescales and their potential importance for replication. The authors discuss how these local motions, which often occur in conserved structural regions, may be critical for facilitating viral packaging, protein-priming, and DNA synthesis. Their studies emphasize that the motions of the various states in the energetic landscape may play some of the most important roles in RNA function. Second, Tolbert and co-workers describe conformational dynamics in the human immunodeficiency virus (HIV) that are important for an alternative splicing event that controls the levels of *tat* mRNA [8]. By applying a diverse combination of techniques—phylogenetic analysis, small angle X-ray scattering (SAXS), and NMR structure determination and dynamics analysis—these authors describe the energetic landscape of alternative splicing through a complex set of at least three folded states of the RNA whose populations are regulated by the binding of cognate proteins. In addition, the authors illustrate that dynamics on the micro-to-millisecond timescale are important for rearranging base pairing that ultimately lead to splicing. This article is notable for encompassing both RNA conformational switching and dynamics. Third, Bonilla and Kieft describe how cryogenic electron microscopy (cryo-EM) has the potential to inform simultaneously on RNA structure and dynamics [9]. The authors review how cryo-EM, owing to its ability to image and classify single particles, can in one fell swoop give the structures, populations, and motions of multiple folds of an RNA. This article is forward-looking and describes the potential and limitations of this method, illustrating this in the example of a tRNA-mimic from brome mosaic virus. Detailed analysis with cryoSPARC allowed multiple conformational frames of this system to be discerned.

MicroRNAs regulate gene expression events via the context of stably folded RNA hairpins that are further contextualized by elements of non-canonical structure. The various internal and apical loops that decorate miRNAs differentially modulate their processing efficiencies from their nascent transcripts via mechanisms that include tertiary and quaternary interactions. As such, the structures of miRNAs are directly linked to their

gene regulatory capacity. In a pair of manuscripts, the Keane and Sattler groups shed light on how structural rearrangements of RNA tertiary and quaternary interactions contribute to miRNA function. Keane and coworkers took aim at the NPSL2 non-precursor miR element, which helps to compact the oncomiR-1 locus via stacking interactions with pre-miR-19b [10]. By combining NMR and SAXS, the authors reveal that NPSL2 folds to form a compact tertiary structure where the nucleobases within the large internal and apical loops base stack. Notably, several adenosines located within the internal loop have elevated pKa's that the authors speculate to be important for the stability of NPSL2 and its ability to compact oncomiR-1. By comparison, the Sattler group investigated the influence of a single nucleotide polymorphism (SNP) on the quaternary structure of Pri-miR-30C [11]. Using a biophysical tour de force, the authors demonstrate that both the wild-type and G-to-A variant of pri-miR-30c adopt similar secondary structures, albeit the SNP shifts a pre-existing monomer-dimer equilibrium to favor the monomeric state. The auxiliary processing factor hnRNP A1 does not bind to the WT miR-30C as efficiently as it does to the G/A variant presumably because dimerization sequesters its binding epitope. This study provides an interesting example as to how quaternary interactions of an RNA folding landscape can be modulated by SNPs to in turn influence protein recognition.

Continuing with the theme of RNA folding and protein recognition, the manuscript by Woodson and colleagues describes smFRET study of the complex formed between the Hfq chaperone and OxyS small RNA (sRNA) [12]. The authors reveal that Hfq remodels the OxyS sRNA via a multi-step mechanism that includes compaction of the RNA structure, followed by local unfolding of the SLb domain to create a more open RNA conformation. Notably, the authors determined that the frequency of the OxyS RNA conformational transitions depends on the intrinsically disordered CTD of Hfq, suggesting that the CTD acts to gate the OxyS sRNA through its different structural states. This article eloquently demonstrates how charged and intrinsically disordered regions of a protein chaperone can influence the folding landscape of RNA.

There are powerful ways to describe the energetic landscape of RNA using computational techniques, typically coupled with wet bench experimentation. This issue features three different computational approaches to RNA conformational switching. One of the most important uses Nearest-Neighbor theory to provide free energy parameters for prediction algorithms. In recent years, it has become clear that RNA modifications play important roles not only in the structure and function of tRNA and rRNA, but also in mRNA and ncRNAs. Mathews and co-workers describe how the energetic landscape of RNA is reshaped by one of the most common and important RNA modifications, N6-methyladenosine (m6A) [13]. The authors report free energy parameters from nearly 100 different experiments having diverse structural contexts for the m6A, including various different neighboring Watson-Crick base pairs, positions along the helix, and imperfections such as loops and bulges. This study is important because it allows different folds and their populations to be determined for modified RNAs. A second set of computational approaches, reviewed by Aviran and Incarnato, use bioinformatics on a genome-wide scale to make sense of RNA structure probing, using chemicals such as dimethylsulfate (DMS) and SHAPE reagents, in terms of the multiple folds in the RNA folding landscape [14]. These authors describe how to deconvolute weighted averages of structure probing data in terms of the

underlying folds and their populations. They divide computational approaches into two categories: thermodynamic-dependent ones, such as the Nearest-Neighbor approach, and thermodynamic-independent ones, which take sequencing data and sort it into population-weighted clusters representing each fold. A third approach, developed by Prajapati and co-workers, uses MD to explore conformational switching of RNA at the tertiary structural level [15]. Given the massive size of the RNAs of interest, from riboswitches to the ribosome, simplifications of force fields are needed. The authors use enhanced sampling MD to query the changing between different RNA tertiary structures. They utilize a single collective variable called the “tertiary contacts distance” based on multiple tertiary contacts, to characterize the free energy landscape. The authors apply the approach to the SAM-I riboswitch under three different conditions and validate their outcomes with experimental ensemble data. They offer that their approach is all-atom and can be transferred to other types of biomolecules.

Given the ability of RNA to switch between different conformational states, its unique physical properties can be leveraged to design synthetic molecular logic gates. The manuscript by Contreras and coworkers reviews a class of riboregulators known as toehold-mediated switches [16]. The authors’ exposition highlights the range of applications of toehold switches as translational and transcriptional control devices. Concluding perspectives highlight the utility of toehold switches as reagents to detect endogenous transcriptional events. It also emphasizes the need for better RNA prediction tools and the ability to model the complex cellular environment. Toe-hold switches are certainly handy devices based on the intrinsic property of RNA to change its conformation.

The articles in this issue illustrate that RNA should be viewed as a molecular contortionist, a molecule that can adapt its shape in response to various combinatorial signals to manifest an array of biological outputs. By so doing, non-coding RNAs can “punch above their weight” by adopting different structures for different functions. This shape-shifting nature of RNA can occur locally via remodeling of base-base interactions or on a more global scale where entire domains refold to shift populations. This encoded plasticity of RNA centralizes its dominance in biology because of its ability to multiplex different biological inputs. Such physical features allow RNA to act like a combinatorial signaling hub whereby effector molecules and flanking sequence conspire to shift dynamic conformational landscapes toward multiple biological outputs. Adoption of this signaling hub paradigm allows conceptualization of how ncRNAs contribute to so many biological processes, each with the capability to be modulated through weak-to-strong interactions with effector molecules.

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