RNA systems biology:

uniting functional discoveries and structural tools to understand global roles of RNAs

RNAs form intricate shapes that play key roles in many cellular activities. This article showcases recent discoveries of functional RNAs—like catalytic ribozymes, regulatory riboswitches, and small RNAs—that influence how cells manage genes and metabolism. It also covers new genome-wide methods for analyzing RNA structure, offering fresh perspectives on the transcriptome's architecture. These "structomic" tools are helping scientists tackle big questions in RNA biology, such as how long non-coding RNAs work and how RNA structures may contribute to disease.

RNA plays a central role in biology due to its ability to carry genetic instructions and form complex structures. Its single-stranded nature enables it to act as a template for protein production while also folding into shapes that influence cellular functions. Catalytic RNAs, known as ribozymes, are vital for processes like protein synthesis, tRNA processing, and RNA splicing. Additionally, non-coding RNAs have a wide range of roles in controlling gene activity and expression

Broadening RNA Roles: RNA molecules are now known to perform a wide range of tasks beyond coding for proteins, such as guiding protein placement, serving as structural frameworks, and silencing genes. New functions are being uncovered rapidly, revealing RNAs as key players in complex cellular networks.

Structure Drives Function: Since RNA activity is closely linked to its shape, new technologies that map RNA structures across entire transcriptomes in living cells are crucial for understanding how structure influences function.

Innovative Techniques:

Advanced methods now allow researchers to examine RNA structures on a large scale.

High-throughput experiments and computational tools help analyze vast RNA datasets.

A Systems-Level View:

This review brings together recent discoveries and technological breakthroughs to offer a holistic perspective on RNA biology. It also explores the kinds of questions that can now be asked—and answered—thanks to this integrated understanding of RNA structure and function.

These approaches are shedding light on how RNA shapes contribute to their diverse roles.

Emerging Global Roles of RNA in Cell Regulation

Recent discoveries have revealed that RNA plays a broader role in controlling essential cellular activities, including metabolism and gene expression. These findings suggest that many RNA-based regulatory mechanisms remain undiscovered within the transcriptome.

Newly Identified Ribozymes: Twister and Its Relatives

Until 2013, only ten natural ribozyme classes were known. Using comparative genomics and RNA structure prediction, Roth et al. uncovered a new self-cleaving ribozyme called twister, found in over 2,700 sequences across various organisms. Its structure—a compact, double-pseudoknot—has been validated through biochemical and crystallographic studies.

Although its structure is well understood, the function of the twister ribozyme is still unknown. Building on this work, Weinberg et al. identified three additional ribozyme classes—twister sister, pistol, and hatchet—located near genetic elements linked to known ribozymes. Their biological roles also remain to be determined.

Riboswitches: Small-Molecule Regulators of Gene Expression

Structural Functionality

- Riboswitches, like ribozymes, rely on their RNA structure for function.
- Unlike ribozymes (which catalyze reactions), riboswitches change conformation upon ligand binding to regulate gene expression.

Regulatory Mechanisms:

- These conformational changes act as feedback sensors, influencing transcription, translation, and splicing.

- Riboswitches help maintain cellular homeostasis by responding to metabolite concentrations.

Big Roles for Small RNAs in Bacteria

Key Points

sRNAs (small RNAs) in bacteria are crucial regulators of gene expression, especially under stress or changing environmental conditions.

The Hfq protein acts as an RNA chaperone, facilitating interactions between sRNAs and their target mRNAs.

Hfq enhances sRNA stability, promotes base pairing with mRNAs, and can influence translation and mRNA degradation.

Recent research shows Hfq may also directly repress translation by binding near ribosome binding sites, with sRNAs guiding or stabilizing this repression.

These mechanisms allow bacteria to rapidly adjust their physiology, contributing to processes like virulence, metabolism, and antibiotic resistance.

RprA: Regulates plasmid conjugation in Salmonella via a feed-forward loop with AND gate logic.

RsmZ: Binds and releases RsmE dimers in Pseudomonas fluorescens, influencing cellular state.

SroC: Acts as a sponge for GcvB in Salmonella, forming a feedback loop from mRNA decay.

CpxQ: Derived from CpxP mRNA, represses inner membrane protein mRNAs.

MicL: Downregulates Lpp in E. coli during membrane stress, transcribed from a σE-dependent promoter.

RNA Structomics: A New Field

RNA structomics uses high-throughput methods to study RNA structure-function relationships across the transcriptome:

Challenges: Traditional phylogenetic approaches are less effective in complex eukaryotic genomes.

Techniques:

FragSeq & PARS: Map RNA cleavage sites using NGS.

PIP-seq: Profiles RNA-protein interactions via structure probing and crosslinking.

Chemical Probes: Enable in vivo RNA structure mapping by penetrating cell membranes.

Transcriptome-Wide RNA Structure Insights

- Genome-wide mapping of RNA structures reveals consistent patterns across species.
- Meta-analyses show structure-function relationships by averaging chemical reactivity across many RNAs.

Three-Nucleotide Periodicity

- A three-nucleotide periodicity in chemical reactivity is observed in mRNA coding regions, reflecting codon-based organization and suggesting structural modulation during translation.

What Are lncRNAs?

lncRNAs are RNA molecules longer than 200 nucleotides with minimal protein-coding potential.

They are abundant but remain one of the least understood RNA types.

Structure-Function Insights

- Structure Matters: Emerging evidence shows that lncRNA structure is crucial for their regulatory roles.

- HOTAIR: Its secondary structure enables it to regulate epidermal development and repress tumor suppressor genes.

- Firre: Functions as a scaffold for organizing trans-chromosomal interactions.

- Extra-coding CEBPA: Uses structured RNA elements to recruit DNMT-1 and control DNA methylation at its locus.

Future Directions

- High-throughput structural studies are beginning to reveal how lncRNA architecture drives diverse biological functions.

- These findings suggest that many more lncRNAs may have structure-dependent roles yet to be discovered.

How does RNA misfolding contribute to human disease?

The growing appreciation for the role of RNA structure in cellular activities has lead to intriguing questions about the role of RNA structure in human disease.

- riboSNitches are RNA elements where a single nucleotide variant (SNV) can drastically change RNA structure, sometimes leading to diseases like β-Thalassemia or COPD.

- Wan et al. used PARS to analyze RNA structures in a family trio, finding that 15% of SNVs altered RNA folding.

- Corley et al. used this data to test RNA folding algorithms, aiming to predict riboSNitches from sequence alone.

- This research highlights a promising frontier in understanding RNA structure-function relationships and their role in human disease.

Conclusion:

RNA is no longer seen as just a passive messenger or catalyst—it plays active, diverse roles in regulating key cellular processes. Recent discoveries reveal RNAs in unexpected places with surprising functions, prompting scientists to reconsider RNA's significance. This evolving understanding supports the need for a new field: RNA systems biology, aimed at exploring RNA's full impact in modern biology.