Trends in Biochemical Sciences | Technology of the Month

Mapping *In Vivo* RNA Structures and Interactions

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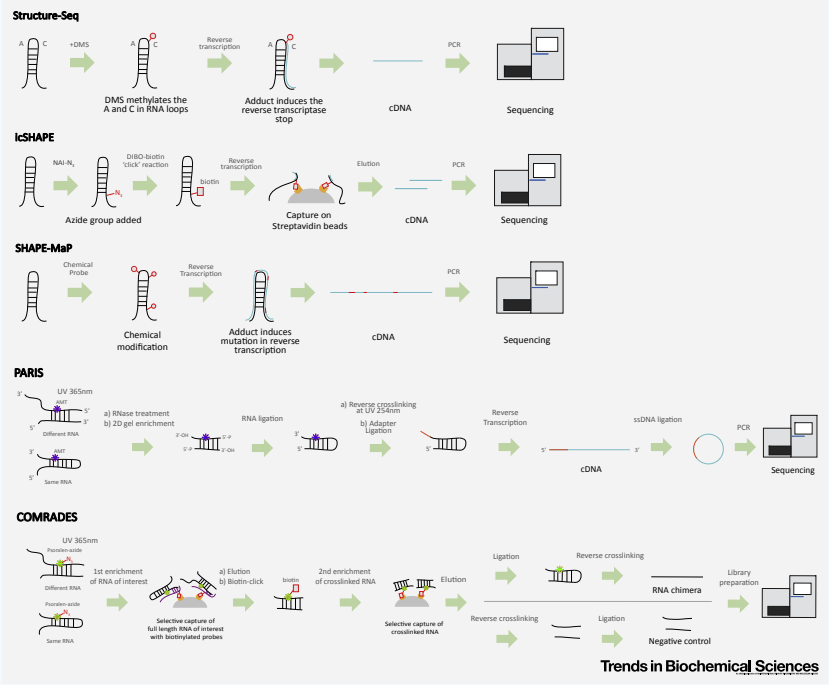
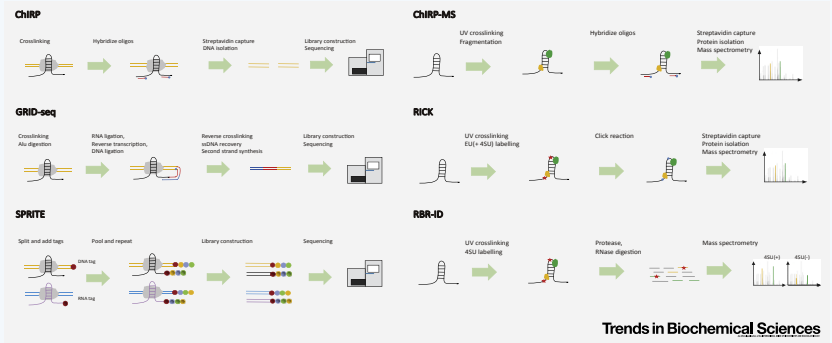


RNA folds to form diverse secondary and tertiary structures and often interacts with other biomolecules to function in cells. The technologies developed to map *in vivo* RNA structures and interactions can be broadly classified into four categories.

(i) RNA structure probing: Methods based on chemical probing to modify RNA, followed by reverse transcriptase (RT) stop or mutation readout.
(ii) RNA–RNA interaction (RRI) mapping: Methods based on chemical crosslinking agents to capture direct RNA base pairs, followed by proximity ligation and reverse crosslinking.
(iii) RNA–DNA interaction mapping: Methods based on antisense hybridization probes to capture RNA-interacting DNA, bivalent linkers to ligate DNA with RNA in the vicinity, or split-pool tagging to label RNA–DNA interactions with specific barcodes.
(iv) RNA–protein interaction mapping: RNA-centric methods based on the combination of mass spectrometry with antisense hybridization probe pulldown or nucleoside analog labeling.



ADVANTAGES:
Structure-seq yields the first *in vivo* transcriptome-wide RNA structure map at nucleotide resolution and icSHAPE achieves the first global profiling of *in vivo* RNA structure for all four bases. SHAPE-MaP provides unbiased profiling based on RT mutation rate instead of RT stop.

PARIS is one of the first methods to map RNA helices and RRIs at near-base resolution and COMRADES provides a global view of multiple coexisting conformations for the selected RNA.

ChIRP is one of the earliest methods to achieve unbiased high-throughput maps of long non-coding RNA (lncRNA)-interacted DNA and proteins. GRID-seq enables the global discovery of the *in situ* RNA–chromatin interactome, while SPRITE reveals simultaneous RNA–DNA long-range interactions.

RICK systematically captures the binding proteins of newly transcribed RNA and RBR-ID identifies *in vivo* protein–RNA interactions in a high-throughput manner with peptide-level resolution.

CHALLENGES:
All of the structure-probing methods obtain RNA structure ensemble information only.

Methods to compare RNA structures among different conditions or across methods are required.

Transcriptomic RRI detection methods are commonly limited by poor crosslinking and ligation rate.

Transcriptomic protein–RNA interaction detection methods cannot provide direct interaction sites.

Putative information is lost in all of these transcriptomic structure or interaction detection methods, especially for transcripts with low abundance, such as long non-coding RNAs.

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