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SnapShot: RNA Structure Probing Technologies

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Chemical probing coupled to high throughput sequencing offers a high throughput and flexible approach to uncover many aspects of RNA structure relevant to its cellular function and interactions. Chemical probes work by covalently modifying RNAs preferentially in regions that are unconstrained – i.e. those that are flexible, unstructured, unpaired and unbound by other RNA/protein/ligand interactions. These covalent modifications can then be mapped by sequencing to obtain structural information across a complex pool of RNAs all at once. A wide range of inputs can be used in these experiments, ranging from *in vitro* purified RNAs to RNAs from whole cells and tissues. Following the probing reaction, RNAs are reverse transcribed into cDNAs, formatted into high throughput sequencing libraries, and sequenced to detect modifications either as truncated cDNA products (RT-stops) or modification-induced mutations (RT-mutations) (Strobel et al., 2018). The distribution of modifications across a molecule is then used to calculate a 'reactivity' value for each nucleotide in the RNA, with higher reactivities typically corresponding to more unconstrained positions (Strobel et al., 2018).

Chemical probing reactivities can be used to uncover many layers of RNA structure. Since the probing reaction is sensitive to the general structural environment of each nucleotide (McGinnis et al., 2012), reactivities reflect base-pairing interactions, complex tertiary interactions and other environmental effects such as ion-mediated interactions, proteins/RNA/ligand interactions, and the change of RNA flexibility with different temperatures. Moreover, different probes access different structural information (Strobel et al., 2018): some modify the backbone of the RNA, while others preferentially modify specific bases (Ehresmann et al.). The choice of probe is an important consideration in the design of a chemical probing experiment (Strobel et al., 2018). For example, probes with short-half lives are advantageous for experiments that require rapid sample handling to capture out of equilibrium structures that may be present when RNAs are folded

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contranscriptionally (Watters et al., 2016), while probes with long half-lives are better suited to probing RNA structures inside cells (Spitale et al., 2015).

Chemical probing can be used to address many biological questions about RNA function, and give insights into the structural mechanisms behind these functions. For example, a protein-RNA binding interaction can be characterized by performing parallel probing experiments on purified RNA alone and RNA folded in the presence of the RNA-binding protein (RBP). By looking for changes in reactivities between the two conditions, RBP binding sites can be uncovered as well as other structural changes that result from the RBP-RNA interaction (Smola et al., 2015). Similar comparisons can reveal where ligands bind RNAs (Stoddard et al., 2008), how RNA folds change in the complex cellular environment (Spitale et al., 2015), how RNA folds change during transcription (Watters et al., 2016), and many others.

Chemical probing data can also be leveraged alongside computational methods to yield higher-resolution RNA structural models. Single-structure methods can use generated or existing databases of reactivity information (Yesselman et al. 2017) to increase the accuracy of 2- or 3-dimensional structural models of the RNA (Lorenz et al., 2016). However, RNAs often fold into an ensemble of different structures in solution which are captured at a population level by the bulk nature of chemical probing experiments. Multistate methods have been recently developed to extract this population-level information from probing data to predict the ensemble of distinct folds of an RNA molecule, as well as their relative distributions within the population (Li and Aviran, 2018). Beyond analysis of individual RNAs, comparative methods have been developed to ask questions about the conservation of structural elements between different sequences, and genome-wide tools are useful for linking reactivity patterns to genomic elements (Spitale et al., 2015, Mustoe et al., 2018, Strobel et al., 2018).

High throughput chemical probing offers a powerful and growing suite of experiments to uncover the RNA structure-function relationship. However, there are several limitations to keep in mind when designing these experiments. In some cases, it can be difficult to unambiguously assign structural changes from reactivity changes, as many different changes in structural context can lead to the same observed change in reactivity. Second, low amounts of input RNA can result in low signal and inaccurate reactivity estimates; in these cases, increasing the amount of starting material and/or increasing sequencing depth is necessary. Third, different steps in probing experiments can introduce bias into the data (Strobel et al., 2018). Protocols are being continuously developed to remove these biases, but careful processing of samples is important for producing high-quality libraries for sequencing. Other best practices include general considerations of sequencing depth. Genome-wide probing (Mustoe et al., 2018), cotranscriptional probing (Watters et al., 2016), and studies of long RNAs require increased sequencing depth compared to single-length experiments to produce robust reactivity estimates. Additionally, the use of multiple probes to study to same RNA is often advantageous to leverage the complementary information offered by different probes.

Chemical probing of RNA structure continues to increase in power and resolution. There are also exciting opportunities to continue to merge these techniques with other high-throughput methods such as CLIP, ribosome profiling and others to study the structural basis of RNA-protein interactions, the impact of RNA structure on translation and many other features of the RNA structure-function relationship. Overall, we anticipate these techniques will help uncover new RNA functional roles and the structural aspects of their mechanisms across the cell.

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