RNA FOLDING

Tracking RNA structures as RNAs transit through the cell

RNAs perform diverse cellular functions that are mediated at least in part by their structure. However, how RNA structure changes throughout the RNA lifecycle and how these changes affect RNA function remain incompletely understood. A detailed in vivo characterization of RNA structure in various cellular subcompartments now provides insights into how RNA structural changes influence translation, RNA decay, protein binding and RNA modification.

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RNAs serve important cellular roles by forming structures that regulate and coordinate key biological processes, such as transcription, translation, transcript turnover, splicing, polyadenylation and molecular scaffolding[1,2]. Recent technical advances that couple enzymatic or chemical probing with high-throughput sequencing have allowed the study of RNA structure in multiple conditions, including within the cell, and have provided unprecedented views of the RNA 'structurome'[3–5]. However, these techniques are typically applied to whole cells or isolated compartments, which blurs the view of how RNA structures change throughout the RNA lifecycle and how specific structures guide RNA function in different cellular subcompartments. Here, Sun, Fazal, Li et al. address this question by using in vivo click-selective 2′-hydroxyl acylation and profiling experiments (icSHAPE)[6] in mouse and human cells[7]. These data give a first glimpse into the connection between RNA structure and RNA function within cellular subcompartments.

By including a fractionation step between probing and sequencing, Sun, Fazal, Li et al. characterize RNA structures across chromatin, nucleoplasm and cytoplasm[7] (Fig. 1), which allows comparison of structural properties of the same RNA in distinct cellular subcompartments. Intriguingly, the authors find positive Pearson correlation between icSHAPE reactivities from the chromatin fraction and those from the nucleoplasmic and cytoplasmic fractions. This suggests that many RNA structures are stable and that they are established near chromatin, setting the RNA fold that guides RNA function in the nucleoplasm and cytoplasm. By further connecting the structural data to readouts of transcription and translation rates as well as RNA half-life, the authors establish links between RNA structure and the regulation of these processes.

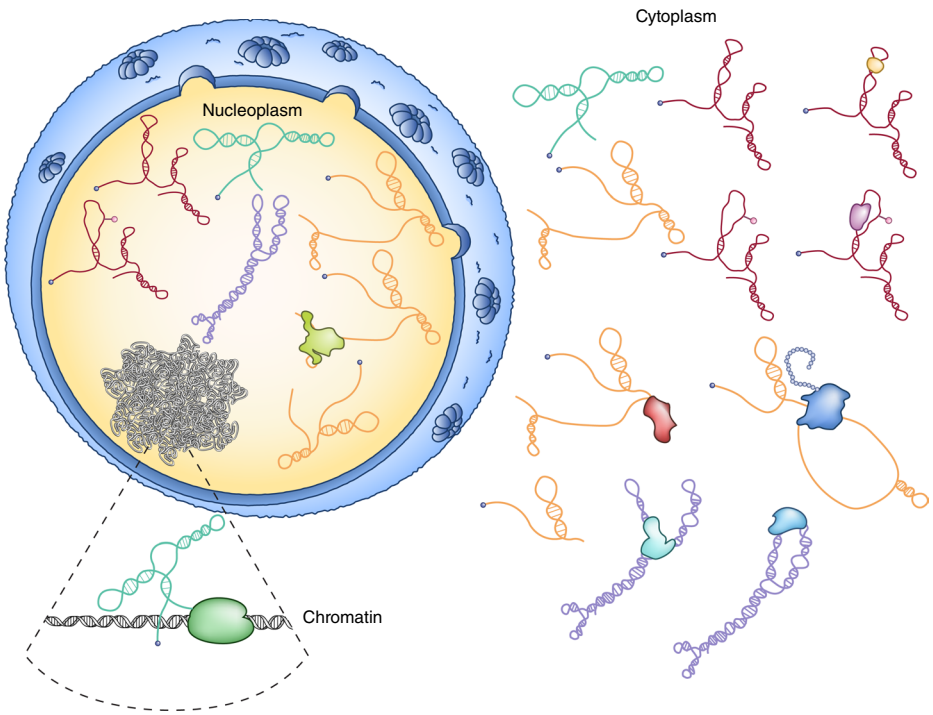

Fig. 1 | RNA structures throughout cellular subcompartments. The formation of RNA structure can initiate during transcription, and Sun, Fazal, Li et al. find that many RNAs preserve their structure when transiting from chromatin to the nucleoplasm and cytoplasm (green RNA)[7]. Some RNAs do change structure as they move between cellular subcompartments. This can be caused by interactions with RBPs (purple RNA) or m^{6}A modification (pink lollipop, red RNAs) or a combination of both. RNA structures in different cellular subcompartments are tied to RNA function, for example, by affecting translational efficiency and RNA degradation (orange RNAs). Translational efficiency can be affected by the structure of 5′ UTR RNA (blue elongating ribosome), and certain RNases (red RBP) can be sensitive to RNA structures in RNA processing and RNA degradation pathways (orange RNAs).

The observed negative correlation between the cytoplasmic structure of 5′ untranslated region (UTR) and translation efficiency in mouse cells suggests that RNA structure inhibits translation. This has been noted in several previous studies of other organisms (reviewed in ref. [8]), but here the effect is further defined, because the analysis is restricted to RNAs in the cytoplasmic fraction, where translation occurs. More interestingly, the authors also observe a negative correlation between 5′ UTR structure and transcription rate in the chromatin fraction. This provides new

transcriptome-wide cellular evidence of an interplay between newly formed RNA structure and transcriptional dynamics, a link that is fundamental to understanding the first steps of gene expression and RNA processing[9,10]. Finally, the authors find a negative correlation between RNA structure and RNA half-life in the nucleoplasm and cytoplasm of mouse and human cells, which suggests that RNA structure accelerates the degradation process. These three trends are all consistent with the observation that RNA structure is linked to these processes, although higher spatial resolution and statistical power will be needed to definitively establish the directionality of the link; i.e., whether RNA structure affects these processes or vice versa, or a mixture of both. The extent of these influences may also vary for different RNAs.

While many RNA structures are stable from one cellular compartment to the next, some RNAs do undergo substantial structural changes during their lifecycle. A particularly intriguing aspect of the study by Sun, Fazal, Li et al. is the ability to connect RNA structure changes in each cellular subcompartment to known RNA-binding protein (RBP) binding sites and RNA modification sites[7]. In fact, the binding sites of many known RBPs, including HNRNPs, STAU1, LIN28A/B and IGF2BP3, are associated with structural changes among the three cellular subcompartments.

A deeper look at sites of RNA structural changes between chromatin and cytoplasm reveals co-occurrences of RBP binding sites and RNA modification sites. Of all RNA modifications, m^{6}A is reported to be one of the most abundant and is added to nuclear RNAs by the RNA methyltransferases METTL3 and METTL14[11,12]. m^{6}A has been previously shown to affect RNA structure by causing otherwise structured regions to become less structured, which affects the binding of RBPs[13]. Sun, Fazal, Li et al. also observe that icSHAPE reactivities increase at known m^{6}A modification sites between chromatin and nucleoplasm/cytoplasm fractions[7]. Analysis of RBP binding sites, m^{6}A sites and icSHAPE changes between chromatin and cytoplasm further reveals candidate RBP binding sites at which protein binding could be affected by m^{6}A. Two patterns are found: (1) structural changes induced by m^{6}A allow binding of RBPs on different sites of the RNA, and (2) m^{6}A directly at the RBP binding site reduces RBP binding affinity (Fig. 1). Focusing on IGF2BP3, a RBP whose RNA-binding propensity was previously found to be affected by RNA modification[14], as an example of the former pattern, and on LIN28A as an example of the latter, the authors confirm differential binding to two modified target RNA oligonucleotides. The observation of antagonistic structural interaction of LIN28A and m^{6}A is particularly interesting because LIN28A has been implicated in functional roles opposite to those of m^{6}A, such as inhibiting primary microRNA processing and stem cell differentiation[15–18].

The RNA structure probing data obtained by Sun, Fazal, Li et al.[7] reveal a wealth of targets to pursue with further mechanistic studies to determine exactly how RNA structure across cellular subcompartments affects or is affected by cellular processes. Thus, these authors take a compelling next step in improving the understanding of how RNA structure changes between cellular subcompartments and how these structural changes influence the myriad roles that RNAs play across the cell. Their tour de force analysis of transcriptome-wide cellular subcompartment icSHAPE data, integrated with additional sources of '-omics' data to link changes in RNA structure to changes in function, reveals a complex system of RNA regulation. RNA structure in each cellular subcompartment can affect or be affected by processes that occur in that subcompartment. For some RNAs, this is further affected by changes in RNA structure associated with RBP binding and/or RNA modifications. From our point of view, one of the most intriguing findings is that many RNA structures that form near chromatin persist as the RNA transits through the cell. This suggests that the establishment of these structures during the process of transcription — or cotranscriptional RNA folding[19] — may be a preeminent process that has a large role in determining the function and fate of cellular RNAs. As more systems are studied and technologies improve to allow models of cellular RNA structures of even higher resolution[20], even more fascinating in vivo details of the role of RNA structure in complex regulatory functions across the cell are likely to be discovered. ❐

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Competing interests

The authors declare no competing interests.