interesting and difficult biological problems across scales. ❐

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The author declares no competing interests.

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RNA structure: a renaissance begins?

Advances in cryo-EM technology will open a new era of RNA-only 3D structure determination.

Rhiju Das

Where are all the RNA structures? For decades, RNA has been known to act as both a genetic script and a molecular machine, and the amount of RNA sequence transcribed in organisms like humans exceeds the number of protein sequences by at least tenfold[1]. Numerous RNA structures have been deposited in the Protein Data Bank (PDB), which, despite its name, remains the biology community's storehouse of structural information for both RNA and proteins. But a quick search in the PDB reveals a serious imbalance tipped in favor of protein structures: the fraction of RNA-only structures remains below 1%. Biologists—even RNA structural biologists—would be forgiven for assuming that most RNAs don't fold. Or, if RNAs do fold, their structures might be ill-defined in three dimensions unless organized by partners like proteins. As a molecule, RNA has been called 'floppy', 'flexible' and 'conformationally heterogeneous', and this reputation has scared off all but the boldest from attempting crystallography or NMR on RNA-only structures. Is determining RNA three-dimensional (3D) structure a lost cause?

A couple of years ago, the field began to revisit the determination of RNA-only structures with cryogenic electron microscopy (cryo-EM), not expecting much but excited by the technique's clear power in imaging ribosomes, spliceosomes and other RNAs complexed to proteins. To everyone's surprise, protein-free, RNA-only complexes in fact did lend themselves to imaging by cryo-EM. Newly visualizable molecules included the *Tetrahymena* ribozyme, the first RNA-only enzyme discovered in nature (Fig. 1a)[2]; its global structure had remained intractable for four decades. Other RNAs studied using cryo-EM were smaller than 30 kDa, well below the ~100 kDa detection limit expected for proteins[3], and maps with sub-4-Å resolution even allowed detection of small-molecule ligands[4]. In 2020, the COVID-19 pandemic prompted testing of even smaller RNAs, including a 28 kDa frameshift stimulation element from the SARS-CoV-2 genome—and cryo-EM delivered again (Fig. 1b)[5]. Each of these structures revealed homologies to other RNAs; some structures revealed holes and pockets that might be targeted by drugs for potential biomedical disruption. For RNAs, as for proteins, structure truly can illuminate function.

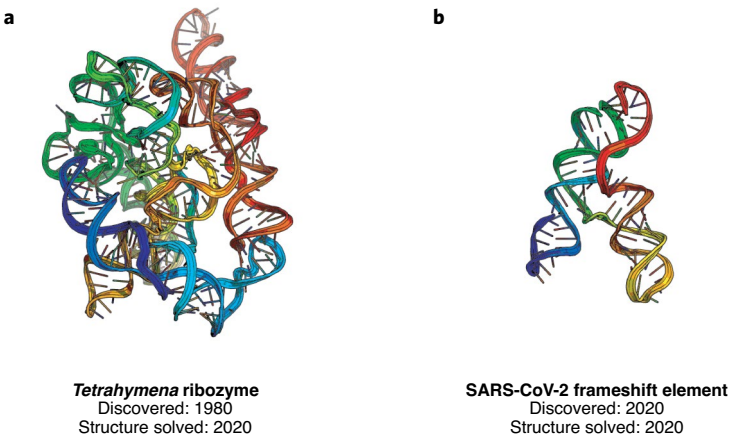

Fig. 1 | Coordinates of RNA molecules with complex folds revealed through cryogenic electron microscopy. a, *Tetrahymena* ribozyme, first RNA-only enzyme discovered (PDB 6WLS). **b**, SARS-CoV-2 frameshift stimulation element, a highly conserved potential target for COVID-19 antivirals that reprograms the ribosome (PDB 6XRZ).

These results suggest that RNA 3D structures are there for the finding. Indeed, RNA may be the perfect molecule for cryo-EM, and cryo-EM may be the perfect technique for RNA. At the time of writing, however, it is still early days for RNA-only cryo-EM. How far can the resolution of cryo-EM be pushed for RNA-only systems, especially if they form multiple biologically important states? How many RNA domains with well-defined, biologically relevant 3D structures are there really in the human transcriptome—or the Earth's transcriptome? If the answers are favorable, an RNA structure renaissance has begun, and in another 50 years our 3D structural database may very well be dominated by RNAs, not proteins. Perhaps the Protein Data Bank will need another name. ❐

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