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Protein binding cannot subdue a lively RNA

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Abstract

Ribosomes, the cell's protein-synthesis machines, are assembled from their components in a defined order. It emerges that the first assembly step must overcome dynamic structural rearrangements.

In cells, RNA molecules typically associate with proteins to form ribonucleoproteins. The ribosome is the large ribonucleoprotein responsible for protein synthesis, and is assembled by the sequential binding of its constituent proteins to ribosomal RNA. In a paper published on *Nature*'s website today, Kim *et al.*¹ describe the events that occur as the ribosomal protein S4 binds to a fragment of rRNA. The resulting complex is the first to form in the assembly of the small ribosomal subunit, and it must guide the assembly of the rest of the sub unit. The authors show that the complex is an extremely dynamic structure, rather than a static building block.

Sequential association of different proteins with an RNA molecule could be a mechanism enabling RNA to adopt its functional conformation, with each protein acting as a molecular 'chaperone' to direct the folding pathway or remodel misfolded structures. As first reported² in 1974, the small ribosomal subunit (30S) of the bacterium *Escherichia coli* can be reconstituted *in vitro* by the sequential addition of 21 small ribosomal proteins to the 16S rRNA (1,541 nucleotides in *E. coli*). S4 is the first protein to bind, and it triggers a conformational change in the RNA that facilitates subsequent protein association.

Kim and colleagues' study is the first single-molecule analysis of S4 binding to 16S rRNA, and reveals the details of the interactions involved. Using fluorescence-based experiments, the authors watched in real time as an S4 protein bound a five-way junction in 16S rRNA, in which five RNA arms (duplexes) radiated out from a single node (the junction). They observed that the five-way junction is not a static structure passively waiting for S4 to bind and remodel it (Fig. 1). By attaching fluorescent probes to two of its arms, Kim *et al.* saw those arms move on widely different timescales. In one experiment, the arms were closed for 100 seconds, then rapidly fluctuated between open and closed states for 50 s, before closing again for another 100 s. The authors also observed the arms jump from a closed state lasting 30 s to an open state that persisted for 15 s.

Kim and colleagues discovered a further level of complexity when they added magnesium ions (Mg^{2+}) to the system. In solutions containing concentrations of Mg^{2+} ions close to physiological levels, most of the RNA molecules existed in an open state, with the two arms far apart. But at a higher concentration, two populations of RNA existed, one in an open state and the other, slightly larger population in a closed state; the RNA molecules alternated stochastically between these states.

S4 specifically recognizes the junction and contacts an arm³, but if the RNA structure changes on millisecond and second timescales, as seen in the authors' real-time fluorescence data, how can the protein find its binding site? Proteins that bind to specific sequences in RNAs do so by recognizing single-stranded regions of the molecules. Such regions are intrinsically flexible, and the difference between their free and protein-bound structures is often dramatic. The mechanism of RNA binding by a protein must therefore include a means of catching a conformation that displays the RNA nucleotides in a geometry that the protein can recognize.

One widely used model of how a protein might bind to a flexible RNA is called conformation capture^{4–6}. This model acknowledges that RNA in solution is best described as an ensemble of conformations that have an unknown population distribution. Assuming that the structures are nearly equal in energy, and that they interconvert, then only some of the molecules will be able to bind to the protein. When the protein encounters an RNA with a binding-competent structure, it captures it, forming a complex. After capture, the RNA's structure changes to accommodate the protein's binding site. Such a mode of complex formation, in which the protein manipulates the RNA to complement its surface, is known as induced fit^{7–9}.

The conformation-capture and induced-fit models neglect the probability that the protein also undergoes conformational changes, so that in reality the capture and fitting processes are mutual. The authors show that S4–rRNA binding requires that S4 select among rRNA conformations (conformational selection) but that, when S4 is bound, new patterns of RNA dynamics appear in the complex (induced conformational changes). Such dynamics might be essential for the addition of the next protein.

Kim *et al.* describe the kinetics of the changes in the RNA, and the populations of molecules in each conformational state (in this case, two free and two bound states predominate). They also show how those kinetics and populations are altered by Mg^{2+} : S4 binds most efficiently at high Mg^{2+} concentrations, which hold the RNA in a closed conformational state. However, the authors observe that the RNA in the bound state is not a static prisoner — at least one of its arms waves frantically around the protein, as if in protest at its capture.

The authors make these experiments look easy. They are not. But they show the heart of RNA–protein interactions in a way that other methods cannot. In this case, Kim *et al.* have demonstrated that S4–rRNA mutual recognition is not a simple lock-and-key process, but a much more challenging one that could be described as conformational capture followed by mutual induced fit, and which thereby allows both protein and RNA to retain dynamic motion. S4–rRNA recognition may well be an archetype of the structure and dynamics of

ribonucleoproteins. If so, these experiments illustrate what is really meant by “the protein binds the RNA”.

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Figure 1. Binding of the S4 protein to an RNA five-way junction

a, Kim *et al.*¹ studied a fragment of 16S ribosomal RNA, which consists of five duplex 'arms' radiating from a central junction, by attaching fluorescent probes to two of the arms. They observed that the RNA's structure is highly dynamic, with the labelled arms (red) adopting a wide array of conformations. The other arms are also likely to move, but were not studied. **b**, The S4 protein forms a complex with 16S rRNA in the first step of ribosome assembly by binding to its preferred RNA conformation (binding step not shown). Once bound, S4 restrains, but does not fix, the movements of the RNA arms.