RNA CONFORMATION

Lightening up invisible states

The versatility of RNA is achieved in part through its ability to adopt various shapes of structures. A new technology called X-ray scattering interferometry enables the detection of 'invisible' states by lighting up gold pairs tagged to RNA molecules.

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Nobel laureate Paul Flory once wrote: "The number of configurations which a long chain molecule may assume is very large. Consideration of each of them individually would be a task of scope beyond all possibility of realization. This brings us at once to the necessity of adopting a statistical point of view. Therefore, the deduction of appropriate average over the total population of configurations for an ensemble of chain molecules will be our goal, rather than description in detail implied by some of the formulation"[1]. For the same reason, the number of potential RNA conformers with multiple flexible regions that serve as pivot points is also very large[2,3]. A description of an RNA with a single conformation thus is insufficient to elucidate the multifunctional roles of these molecules in biology. Not only do multiple conformations coexist, but they also change with time, while being largely 'invisible' using conventional sets of tools.

Ever since the genesis of structural biology, people have been looking for various ways to characterize those invisible conformations, the vast majority of whose structures could not be determined. The most notable techniques among many are NMR spectroscopy, single-molecule Förster resonance energy transfer (smFRET)[4] and small-angle X-ray scattering[5–7]. Shi *et al.*[8] now report an application of X-ray scattering interferometry (XSI) to study the conformation space of two kink-turn RNA motifs, KtA and KtB. These RNA structural elements are ubiquitous and can exist in multiple conformation states depending on solution environments.

XSI exploits interference signals between two electron-super-rich particles such as nanogold particles. The more electrons an atom has, the shinier and more sensitive it is in the eyes of X-ray detection methods (**Fig. 1**). The scattering of a nanogold particle can be viewed metaphorically as rippling waves centered around where a stone is thrown in a tranquil pond. The interference occurs where the waves caused by two stones meet. The pattern of the interference can be mathematically interpreted using Fourier transformation to yield the distance information between the pair of the stones. If many isolated pairs of stones, with various distances between each pair of stones, are thrown into the pond simultaneously, the Fourier transformation of the collective interference pattern results not in a single distance but in a distribution of distances between pairs of nanogold particles, reflecting the heterogeneity in distances among the various pairs. The fact that the pairs are isolated means that the solution is sufficiently diluted and there is no aggregation taking place. In simple words, XSI can be used to directly measure distances and the distance distributions between two points with high sensitivity without any assumption.

By attaching a pair of nanogold particles to the two ends of each KtA or KtB RNA molecules, Shi *et al.*[8] measured the distance distributions of the particles using XSI and showed the dependence of distance distribution and equilibrium between the kinked and unkinked conformations on salt concentrations and types of salts. They found that not only did KtA become more 'kinked' in both the kinked and unkinked populations, but the kinked population also increased, and the distance distribution became narrower, as the ionic

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Figure 1 | A technology called X-ray scattering interferometry (XSI) looks for invisible 'ghost' states. Tagged with nanogold particles, those invisible states of RNA molecules are lit up under XSI so that their behaviors can be characterized.

strength increased. The coexistence of two conformation states with the distributed distances reinforces the notion that such RNA molecules in solution can only be sufficiently described by two ensembles of conformation states. These findings are consistent with the results of a previous smFRET study[9] and can be explained perfectly by a compacting effect, a direct consequence of increased condensation screening by counterions.

If counterions are considered to be mild 'tuners' of RNA conformation states, one might expect that ribonucleic binding proteins, RNA natural cognate regulators, would be able to 'tune' RNA conformations into a more restricted conformation space, if not a single conformation. However, this perception is not necessarily true in every case. In two examples studied by the authors, KtA complexed with L7Ae, a kink-turn binding protein, still exhibited relatively large end-to-end distance variance in low- or intermediate-salt conditions, and was converged ('collapsed') into that of a single conformation only in the high-salt condition. In contrast, the KtB–L7Ae complex showed much narrower distance distributions that were comparable to those expected for single kinked conformation under the range of salt conditions. The difference here is that only KtB is the natural target of L7Ae, even though both KtA and KtB bind L7Ae with very high affinities.

XSI was dubbed a 'molecular ruler' when it was first introduced to accurately measure structural parameters of a DNA duplex[10]. The potential of this technology, due to its sensitivity and accuracy, is very significant. One such potential application is determining the arrangements of components in a multicomponent complex by triangulation. This approach can be very powerful when combined with data from conventional small-angle X-ray scattering (SAXS), which can provide the global shape of a complex. A second possible application is correlating and 'calibrating' an ensemble calculation restrained by SAXS and residual dipolar couplings[7] with direct ensemble measurements using XSI. Another potentially very exciting application is single-molecule scattering using an X-ray free-electron laser (XFEL), which is about a billion times brighter than the latest synchrotron sources and may potentially be used to 'visualize' the behaviors of a single molecule in various 'invisible' states.

In summary, XSI may be the tool of choice for studying behaviors of biomacromolecules and complexes that are not accessible by other conventional methods. ■

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Competing financial interests

The author declares no competing financial interests.

TRANSLATION

Ribosomes make sweeping arrests

The arrest peptides that function with the macrolide antibiotic erythromycin stall translating ribosomes in the presence of the antibiotic, leading to remodeling of the downstream mRNA and enhancement of the translation of resistance genes. Current work suggests that small changes in the nascent peptide dictate the ability of ribosomes to respond to this and other small molecules.

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Regulation of gene expression is a vital process for all organisms. One regulation mechanism uses arrest peptides, sequences that pause translation *in cis*, stalling the ribosome that just synthesized them. The arrested ribosomal complex changes the conformation or localization of its mRNA, modulating its translation. Most arrest peptides are active only in the presence of specific environmental cues and modulate the cell's response to those cues, such as the upregulation of antibiotic-resistance genes upon exposure to antibiotics. In this issue, Gupta *et al.* show how exquisitely tuned the arrest peptides associated with erythromycin are: varying a single amino acid can make an arrest peptide active with both erythromycin and telithromycin, make it selective for one or the other, or inactivate it altogether[1]. The simplicity of this switching to generate useful, functionally distinct arrest peptides highlights the possibility that these peptides could be more widespread than currently thought.

Although only a handful are known, ribosome arrest peptides have been found in several prokaryotes and in eukaryotes from yeast to human[2]. They translationally control genes involved in a variety of functions, including balancing expression of the protein translocation machinery, stress response and regulation of metabolite biosynthesis. Despite a similar first step, in which sequence-specific contacts between the nascent peptide and the ribosome result in an arrested translation complex, the downstream mechanisms vary markedly. In eukaryotes, these range from the formation of a physical barrier to further ribosomal scans, to relocalizing mRNA for a splicing event that gives a desired open reading frame. The current study illuminates the classic prokaryotic mechanism, whereby the stalled translation complex changes the structure of the mRNA to expose an obscured ribosome-binding site (RBS) to increase translation from it.

The road to understanding arrest peptides started nearly four decades ago. Using Maxam-Gilbert sequencing, the Weisblum[3] and Dubnau[4] groups deduced a mechanism in which antibiotic resistance depends on control of the conformation of the mRNA encoding the rRNA methyltransferase ErmC. This mRNA contains a small