Why ribosomes shift gears

Unveiled by measuring one molecule at a time

Translation is the biological reaction of protein synthesis in all living cells. The ribosome, the enzyme in charge of the reaction, reads codes from messenger ribonucleic acids (mRNA) and catalyzes polymerization of the corresponding amino acids. Each code consists of three bases, named a codon. In general, the ribosome firmly follows the reading frame of consecutive codons for translation of cellular mRNAs. Otherwise, the produced amino acid sequences will vary from one reaction to another, resulting in malfunctioning proteins. In virus-infected cells, however, some viral proteins can be expressed only through stimulating the ribosome to shift the reading frame during translation, a process called ribosomal frameshifting (RF). A region of the mRNA that forms base-pairs and folds into specific secondary or tertiary structures is required to stimulate RF, but the structural features essential for stimulation remain unclear. Here, we applied single-molecule approaches to investigate a model RF stimulator, DU177, and the results have provided great insights into the molecular mechanism underlying this important biological reaction (*Chen et al.,* 2017).

The RF stimulator DU177 consists of two stems (helices) and two connecting loops, which are bonded through several base triples formed between stems and loops. The overall structure is very stable, and thus, such structures were, and still are, thought to work as a road block to hinder translocation of the ribosome and to induce frameshifting. However, the first stem (instead of the whole structure) encountered by a translocating ribosome is likely to play a pivotal role in determining whether frameshifting occurs. To this end, we designed a DU177 mimic in which the ribosome target stem can be independently measured while the original base-base interacting network is preserved. Then, we used "optical tweezers" to capture the two ends of a single RNA molecule, and by pulling the two ends apart, we can measure how much force is required to break the structure. The force is a realistic approximation of what the translocating ribosome may experience. In addition, the stem stability is determined by the base-base interacting network. We can dissect the network by mutating specific bases and then measuring how the structural stability and conformation are changed accordingly. To this end, we labeled two interacting fluorescent probes on the RNA, whose conformational changes would be reported by the fluorescent signals measured using another single-molecule approach called smFRET. With measurements at the single-molecule level, we can dig deeper into the dynamic nature of a reaction or a structure without the interference of mixed signals from other molecules.

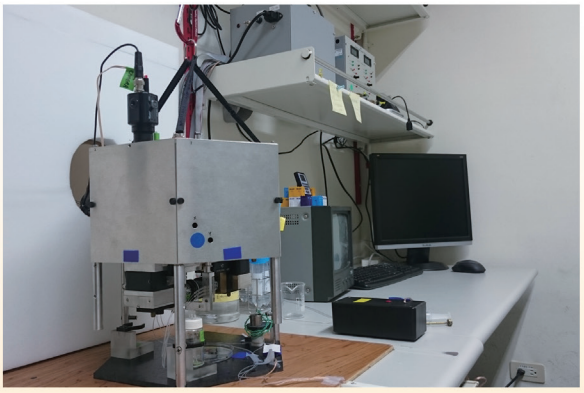

Figure 1. Optical tweezers. The instrument is used to apply a mechanical force on a single molecule to measure its structural stability.

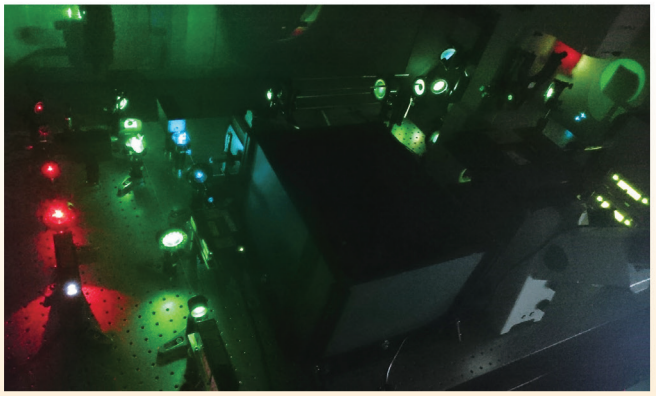

Figure 2. smFRET. An inverted microscope equipped with various lasers and a specialized charge-coupled device (CCD) camera to detect fluorescence from the reaction of single molecules in real time.

Based on these experiments, we found that the ribosome target stem of DU177 exhibited enhanced resistance against helix unwinding, such that the forward movement of the ribosome was hampered, resulting in an increased likelihood of frameshifting. Several base-base interactions (including base pairs and base triples) inside the DU177 structure contributed to the unwinding resistance. The base triples, which are distal to the ribosome target stem, formed the core of stabilization. The stabilization effect was then propagated to other base triples, including those proximal to the ribosome target stem and further to several specific bases in the loop juxtaposed to the stem. Thus, the unwinding resistance directly resulted from intercalation of the loop bases with the stem helix, but the intercalation was not established without other base triples. Such a hierarchical coordination among base-base interactions in an RF stimulator accounts, at least in part, for the nature of frameshifting.

In conclusion, by measuring one RNA molecule at a time, we detangled the complex interaction network inside RNA and identified the structural features that can lead to ribosomal frameshifting. The study helps us understand this fundamental biological reaction with insightful details.

Reference

Chen, Y., Chang, K., Hu, H., Chen, Y., Lin, Y., Hsu, C., Chang, Cheng-Fu, Chang, Kung-Yao, Wen, J. (2017). Coordination among tertiary base pairs results in an efficient frameshift-stimulating RNA pseudoknot. *Nucleic Acids Research, 45*(10), 6011 - 6022. DOI:10.1093/nar/gkx134

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Overfishing induces aggregation of fishes through altering life history traits

Even distribution in diverse habitats can reduce the risk of extinction for a species. Understanding how a species is spatially distributed, including heterogeneity (variance), distribution range, and habitat types, is critical for the management, conservation and restoration of biodiversity. Previous studies have found that the geographical distribution of a population is affected by various factors, including the environmental and biological traits of the species.

One aspect of distribution that is rarely studied is the "aggregation potential" of a species. In 1961, ecologist Lionel Roy Taylor proposed that when the mean density of a population (M) increases, the population will be more aggregated in fewer places (higher variance V). He quantified and formulated this relationship as $V = aM^b$, which is known as Taylor's power law. The exponent b in Taylor's power law represents the aggregation potential – how the spatial variance (V) varies with a unit change in the mean density (M) of a population. For two species living in the same place, the species with a larger b will distribute more unevenly when its population size increases than the species with a smaller b.

What factors may cause the differences in the aggregation potential of a species? Previous theatrical studies have predicted that b is related to the life history traits of a species, such as the average body size, fecundity, maturation age, etc. For example, a species with a small body size and high fecundity may be able to produce a large number of offspring, but the offspring cannot travel far in a short time. Therefore, it is predicted that when the density of this species increases, it will distribute less evenly as most of the newborns will aggregate in a few places. However, empirical evidence for such a theory has been lacking.