Yuhong Wang, Transcriptomics 2016, 4:2 (Suppl)
http://dx.doi.org/10.4172/2329-8936.C1.006

High efficient "-1" and "-2" ribosomal frame shiftings revealed by a mechanomagnetic force spectroscopy

Yuhong Wang
University of Houston, USA

The ribosomal frame shifting is a rare but ubiquitous process. The putative "-1" frame shifting motif includes a slippery sequence, a spacer, and a secondary mRNA structure. We report a new force-based method to direct observation of "-1" and "-2" frame shiftings with single nucleotide resolution. We reveal that EF-G•GTP is indispensable to frame shifting. The biological relevance of the *in vitro* results is verified by protein translations in the cell. The mechanistic insights provided by our assay demonstrated the application of this method to study the ribosome system. The ribosome pre- and post-translocation complexes are tethered to the surface by biotinylated mRNA. The 3'-mRNA uncovered by the ribosome forms duplexes with DNA probes with known sequences. The probes are labeled with magnetic beads. Under external forces, the duplexes dissociate step-wise according to the base pairs in the duplexes. The dissociation is detected by an atomic magnetometer and reflects the ribosome position with single nucleotide resolution. Three consecutive translocation steps were tracked to unambiguously identify the total of nine possible ribosome positions on the mRNA under *in vitro* conditions. Mechanistic studies were carried out by modifying the motif, introducing a secondary structure and varying other experimental conditions. Meanwhile, *in vivo* and *in vitro* protein synthesis experiments were performed to demonstrate the biological significance of the frame shifting results.

Biography

Yuhong Wang has completed her PhD in 2002 from the Johns Hopkins University and Post-doctoral studies from Caltech and UPenn. She is an Associate Professor in University of Houston. She develops new biophysical tools, such as single molecule FRET and mechanomagnetic force spectroscopies. Her research interests are ribosome mechanism and non-invasive detection of microRNAs.

ywang60@Central.uh.edu

Notes: