Structured mRNA induces the ribosome into a hyper-rotated state

Peiwu Qin¹, Dongmei Yu^{2,3}, Xiaobing Zuo⁴ & Peter V Cornish^{1,2,3,*}

Abstract

During protein synthesis, mRNA and tRNA are moved through the ribosome by the process of translocation. The small diameter of the mRNA entrance tunnel only permits unstructured mRNA to pass through. However, there are structured elements within mRNA that present a barrier for translocation that must be unwound. The ribosome has been shown to unwind RNA in the absence of additional factors, but the mechanism remains unclear. Here, we show using single molecule Förster resonance energy transfer and small angle X-ray scattering experiments a new global conformational state of the ribosome. In the presence of the frameshift inducing *dnaX* hairpin, the ribosomal subunits are driven into a hyper-rotated state and the L1 stalk is predominantly in an open conformation. This previously unobserved conformational state provides structural insight into the helicase activity of the ribosome and may have important implications for understanding the mechanism of reading frame maintenance.

Keywords helicase; ribosome; smFRET

Subject Categories RNA Biology; Protein Biosynthesis & Quality Control

DOI 10.1002/embr.201337762 | Received 15 July 2013 | Revised 20 November

2013 | Accepted 25 November 2013 | Published online 8 January 2014

EMBO Reports (2014) 15, 185–190

Introduction

High-resolution structures of the ribosome have shown that the average diameter of the mRNA entrance tunnel (~15 Å) is smaller than the average diameter of an RNA double helix (~21 Å) [1–3]. Therefore, mRNA with any secondary or tertiary structures needs to be unwound in order for proper translation to continue. Certain mRNA structures, such as RNA pseudoknots, have been shown to block the mRNA entrance channel and stall the ribosome [4]. The ability to unwind RNA appears to primarily reside within the ribosome [2,5]. The entry point of mRNA into the ribosome begins in a portion of the 30S subunit formed by proteins S3, S4 and S5 [2,5]. Due to the presence of positively charged residues, it has been

suggested that these proteins may passively unwind double-stranded RNA [2,4]. Alternately, it may be that these proteins function as a processivity clamp [2,5,6]. However, the precise role that these proteins and the ribosome as a whole play in the overall mechanism of RNA unwinding has not been established.

We designed single molecule Förster resonance energy transfer (smFRET) experiments using fluorescently labeled ribosomes in an attempt to observe the dynamic and conformational perturbations resulting from the presence of structured RNA on intersubunit rotation and L1 stalk dynamics. Previously, we employed the same fluorescently labeled ribosome constructs in several different configurations representing states along the translocation pathway in the presence of unstructured mRNA only, which lacked higher ordered structure downstream of the ribosome [7]. To maximize any potential differences between structured and unstructured mRNA on ribosome motions, we employed a series of mRNA molecules with wild-type and mutant hairpin structures based on the highly efficient frameshift stimulating hairpin (~50% occurrence) from the *dnaX* gene (Fig 1A) [8]. We subjected a similar set of ribosome complexes to small angle X-ray scattering (SAXS) experiments to monitor and compare the global conformational changes of the ribosome to results generated from the smFRET experiments.

Results

Visualization of hyper-rotated state

Fluorescently labeled *E. coli* ribosomes (S6(Cy5)/L9(Cy3)) were assembled on a short mRNA transcript containing a *dnaX* hairpin where the hairpin structure is positioned just outside the entry tunnel of the ribosome due to a 6 nucleotide linker (Fig 1A). RNA-ribosome complexes were then immobilized onto the surface of quartz slides through a biotin-neutravidin interaction for visualization via total internal reflection fluorescence (TIRF) microscopy (Fig 1B and supplementary Fig 1A and 2). The introduction of the *dnaX* hairpin allows us to directly compare ribosome conformational dynamics with our previous studies where the downstream RNA sequence is predicted to be primarily unstructured [9]. The

1 Department of Biochemistry, University of Missouri, Columbia, MO, USA

2 Department of Biological Engineering, University of Missouri, Columbia, MO, USA

3 Informatics Institute, University of Missouri, Columbia, MO, USA

4 X-ray Science Division, Argonne National Laboratory, Argonne, IL, USA

*Corresponding author. Tel: +1 573 882 0443; Fax: +1 573 882 5635; E-mail: cornishp@missouri.edu

Figure 1. Secondary structure of *dnaX* hairpin RNA and experimental setup.

- A** 2D representation of the *dnaX* hairpin construct.
- B** Ribosomes (green sphere for Cy3 and red sphere for Cy5) were immobilized by hybridization of the 3' tail of the mRNA with a 5' biotinylated DNA strand that was bound via neutravidin to a quartz slide.

binding of the ribosome to the *dnaX* RNA was confirmed by fluorescence anisotropy, association of tRNA to the ribosome was verified by filter binding, and translocation efficiency was determined by a

puromycin sensitivity assay (supplementary Methods, Table 1 and Fig 2C). Also, no significant change was observed for either the quantum yields or dye anisotropy in the fluorescently labeled ribosomes used in this study (supplementary Methods).

Similar to our previously published results, S6(Cy5)/L9(Cy3) labeled ribosomes with unstructured RNA adopt two primary conformations in the presence of tRNA in the P site and/or the A site; classical/non-rotated (high FRET efficiency) and hybrid/rotated (low FRET efficiency) states (Fig 2A–C) [9]. Note that FRET histogram counts presented in this work are normalized as contrasted to those presented in our previous work (see supplementary Methods) [7,9]. When the same labeled ribosomes were prepared with the *dnaX* mRNA construct and tRNA^{fMet}, the presence of the *dnaX* hairpin reduced the frequency of intersubunit motion and drove the ribosome into a approximately 0.22 FRET state (compare Fig 2B and E, supplementary Fig 3A and B, and supplementary Table 2). Addition of N-Ac-Phe-tRNA^{Phe} to the A site caused a reduction of the approximately 0.22 FRET state with a concomitant increase in the rotated (0.4 FRET) state (Fig 2D). In contrast to this, the presence of fMet-tRNA^{fMet} in the P site facilitated the motion of the ribosome into the non-rotated/classical state, however, the 0.22 FRET state is still apparent (Fig 2F). This lower FRET state (~0.22) (Fig 2D–F) indicates a further increase in the distance between the two fluorescent dyes (S6(Cy5)/L9(Cy3)). An estimate based on the center peak of the lower FRET population (0.22) suggests that a counterclockwise rotation of the 30S subunit is approximately 22° relative to the non-rotated state. This compared to reported values ranging from approximately 4° to 10° for the rotated state (supplementary Fig 1B and Discussion) [10–13]. This FRET state was not observed with ribosomes prepared with unstructured mRNA and has not been reported to date (Fig 2A–C) [9].

Figure 2. FRET histograms with normalized counts of structured and unstructured RNA ribosome complexes.

- A** Fluorescently labeled S6(Cy5)/L9(Cy3) ribosomes were assembled with m291 with tRNA^{fMet} in the P site and N-Ac-Phe-tRNA^{Phe} in the A site.
- B** Ribosomes as in (A) assembled with m291 with tRNA^{fMet} in the P site.
- C** Ribosomes as in (A) assembled with m291 with fMet-tRNA^{fMet} in the P site.
- D** Ribosomes as in (A) assembled with *dnaX* hairpin RNA with tRNA^{fMet} in the P site and N-Ac-Phe-tRNA^{Phe} in the A site.
- E** Ribosomes as in (A) assembled with *dnaX* hairpin RNA with tRNA^{fMet} in the P site.
- F** Ribosomes as in (A) assembled with *dnaX* hairpin RNA with fMet-tRNA^{fMet} in the P site.

Data information: Schematics (insets) for all panels are intended to illustrate the number and aminoacylation state of tRNAs present in the ribosome and not localization of the tRNA in hybrid or classical state. The number of traces (n) and percent showing fluctuations are indicated in each panel (supplementary Table 2).

To confirm that this is indeed an authentic conformation of the ribosome and does not just arise from donor only molecules, we prepared S11(Cy5)/L9(Cy3) labeled ribosomes with the same *dnaX* containing mRNA and tRNA^{fMet}. In previous studies, FRET changes arising from this construct were shown to anti-correlate with the S6/L9 labeled ribosomes since S6 and S11 are on opposite sides of L9 at the subunit interface (supplementary Fig 1A) [14]. If the approximately 0.22 FRET state observed with the S6(Cy5)/L9(Cy3) ribosomes represents a hyper-rotated state, we would expect a higher FRET state to emerge with the S11(Cy5)/L9(Cy3) ribosome construct. As expected, we observed an additional high FRET signal at approximately 0.8 (supplementary Figs 3E and F, 4A and B).

To further characterize the extent to which the *dnaX* hairpin influences the conformational dynamics of the ribosome, a series of *dnaX* hairpin mutants were made. The *dnaX*^{U9U10U11} mutant maintains the structure of the stem region but moves the hairpin structure three nucleotides away from the ribosome by insertion of 3 uracils between position +8 and +9 in the original *dnaX* hairpin construct (Fig 1A). We prepared this construct with S6(Cy5)/L9(Cy3) labeled ribosomes and tRNA^{fMet} and N-Ac-Phe-tRNA^{Phe} in the P and A site, respectively. Interestingly, a nearly complete reduction of the low FRET population was observed (Fig 3A). This result suggests that an additional three nucleotide spacer places the hairpin too far from the ribosome to allow for any productive interactions between the ribosome and hairpin structure consistent with a previous report by the Noller group [2]. Indeed, upon a single step of translocation with elongation factor G (EF-G) and GTP moving the hairpin closer to the ribosome, the low FRET state was restored (Fig 3B). To disrupt the secondary structure of the *dnaX* hairpin, two mutants (*dnaX*^{C13AC14A} and *dnaX*^{A29-40}) were constructed (Fig 1A). Ribosomes prepared with these constructs and either P-site only or P-site and A-site tRNA exhibited a reduction in the population of the 0.22 FRET state (Fig 3C–E). Finally, a DNA oligo (1 μ M) complementary to the downstream, unstructured region of m291 (+13 to +27) was added to imaging buffer and introduced to the same ribosome sample as in Fig 2A (P- and A-site tRNAs present). Remarkably, this resulted in induction of the approximately 0.22 FRET state (Fig 3F compare to Fig 2D). Two other oligos starting at +14 and +15 were also introduced to m291 (supplementary Fig 4E and F). While the approximately 0.22 FRET state was still apparent with the +14 construct, it was predominantly absent with the +15 oligo with only occasional formation of the 0.2 FRET state suggesting this is too far from the ribosome to induce stable hyper-rotated state formation (supplementary Fig 3G). These results collectively indicate that only when structured mRNA is positioned in the mRNA entrance tunnel that the ribosome is forced into the hyper-rotated state. The presence of the hyper-rotated state is independent of the identity of tRNA present in the P site (supplementary Fig 4C and D).

A reduction in the frequency of intersubunit motion was observed when structured mRNA was present whether ribosomes were prepared in the pre- or post-translocation state (supplementary Table 2). Previously, we observed frequent intersubunit motion in the absence of structured mRNA when ribosomes were prepared in the pre-translocation state with tRNA^{fMet} in the P site, but not in the post-translocation state with fMet-tRNA^{fMet} in the P site [9]. These results likely indicate that structured mRNA increases the energy barrier for the ribosome subunits to transition among the various conformational states. Indeed, there appears to be a negative

Figure 3. FRET histograms with normalized counts of mutant *dnaX* RNA ribosome complexes.

- A Fluorescently labeled S6(Cy5)/L9(Cy3) ribosomes were assembled with *dnaX*^{U9U10U11} RNA with tRNA^{fMet} in the P site and N-Ac-Phe-tRNA^{Phe} in the A site.
 B Ribosomes assembled as in (A) following incubation with EF-G-GTP for 20 minutes and subsequent washes (3 times) with binding buffer.
 C Ribosomes as in (A) assembled with *dnaX*^{C13AC14A} RNA with tRNA^{fMet} in the P site and N-Ac-Phe-tRNA^{Phe} in the A site.
 D Ribosomes as in (A) assembled with *dnaX*^{C13AC14A} RNA with tRNA^{fMet} in the P site.
 E Ribosomes as in (A) assembled with *dnaX*^{A29-40} RNA with tRNA^{fMet} in the P site.
 F Ribosomes as in (A) assembled with m291 with tRNA^{fMet} in the P site and N-Ac-Phe-tRNA^{Phe} in the A site. 1 μ M of the +13 oligo (5'-TTCAGCACTAGA TTT) was added to the imaging solution.

Data information: The number of traces (n) and percent showing fluctuations are indicated in each panel (supplementary Table 2).

correlation between percent occupancy of the hyper-rotated state and the number of traces that exhibit fluctuations for the +13, +14, and +15 DNA constructs (supplementary Table 2). While further investigation is required on this last point, it is clear that a substantial change is induced by structured mRNA on the equilibrium among these states (hyper-rotated, rotated, and non-rotated).

Visualization of L1 stalk dynamics

The influence that the *dnaX* hairpin has on the conformational dynamics of the L1 stalk was also investigated. The L1 stalk is known to be important in translocation by interacting with the elbow region of deacylated tRNA and assisting in the release of E-site tRNA [7,15]. Similar to our previous experiments, we found that the L1 stalk of L1(Cy5)/L33(Cy3) fluorescently labeled

ribosomes containing deacylated tRNA in the P site fluctuates between an open (low FRET) and closed (high FRET) conformation when unstructured mRNA was present in the mRNA entrance tunnel (Fig 4A) [7]. We have shown here that the presence of the *dnaX* hairpin reduces the frequency of intersubunit rotation (supplementary Fig 3B and D and supplementary Table 2). However, the L1 stalk of ribosomes containing tRNA^{fMet} in the P site with the *dnaX* hairpin RNA is still mobile as indicated by FRET traces (Fig 4B) similar to what was observed with EF-G bound to the ribosome [16]. Interestingly, in contrast to the FRET distribution of the L1 stalk in the presence of unstructured RNA (high FRET state ~ 0.6) (Fig 4C), the L1 stalk in hyper-rotated ribosomes exists primarily in the open conformation (low FRET state ~ 0.4) (Fig 4D). We observed two distinct behaviors of L1 stalk dynamics and intersubunit rotation, which may indicate that intersubunit ratcheting and L1 stalk motion have different contributions in tRNA translocation and dynamics of tRNA. Further experiments are necessary to investigate this in greater detail.

Structural changes inferred from small angle X-ray scattering (SAXS)

Structural changes induced by the presence of the *dnaX* hairpin were also studied using SAXS. Four different constructs and free 70S ribosomes were chosen to relate differences in SAXS to intersubunit rotation in hopes that a clear pattern or relationship would emerge. Wild-type ribosomes were initiated with either (Complex 1) m291 and N-Ac-Phe-tRNA^{Phe} in the P-site (~80% non-rotated), (Complex 2 and 3) m291 or *dnaX*^{U9U10U11} and tRNA^{fMet} in the P site (Fig 2B and 3A) (~70–80% rotated), and (Complex 4) *dnaX* hairpin RNA with tRNA^{fMet} (~70% hyper-rotated) (Fig 2E) [9]. The SAXS profiles for these constructs are shown (Fig 5A). The shapes and peak positions of the SAXS profiles are similar, and when normalized at low *q* values, the SAXS profiles superimpose well in the displayed *q* range indicating a similar global shape. However, the intensity of the first SAXS peak at *q* of 0.04–0.06 Å⁻¹ changed among the constructs (supplementary Fig 5A). The data from free 70S ribosomes were subtracted from each of the other constructs to emphasize the differences among the data ($\Delta I(q)$) (Fig 5B). This intensity change would not arise from the mRNA construct mass addition since the size difference is negligible compared to the size of the 70S ribosome, but correlates well with inter-subunit rotation. Non-rotated/classical state ribosomes (Complex 1) display the highest degree of similarity to free 70S ribosomes. This is followed by rotated/hybrid state ribosomes (Complex 2 and 3). Note that Complex 3 overlaps perfectly with Complex 2 while still containing the *dnaX* hairpin, yet it is too far from the ribosome to induce hyper-rotation as indicated by our smFRET data (Fig 3A). Finally, hyper-rotated ribosomes (Complex 4) have the largest degree of change relative to free 70S ribosomes. Thus, these data are consistent with the observation of a large-scale structural change in the ribosome and exhibit strong correlation between differences in SAXS intensity and intersubunit rotation angles (Figs 2 and 5).

To support our supposition that the first SAXS peak, 0.04–0.06 Å⁻¹, reports directly on intersubunit rotation, theoretical SAXS profiles were calculated using the program CRY SOL [17] for both the non-rotated state (3R8O and 3R8T) and the rotated state (3R8N and 3R8S) of the *E. coli* ribosome (supplementary Fig 5B) [11]. Within the *q* range of 0.04–0.06 Å⁻¹, the differences between the

Figure 4. FRET traces and FRET histograms showing L1 stalk conformational dynamics.

A Fluorescently labeled L1(Cy5)/L33(Cy3) ribosomes were assembled with m291 with tRNA^{fMet} in the P site and N-Ac-Phe-tRNA^{Phe} in the A site.
B Ribosomes as in (A) assembled with *dnaX* hairpin RNA with tRNA^{fMet} in the P site and N-Ac-Phe-tRNA^{Phe} in the A site.
C, D FRET histograms with normalized counts derived from ribosome samples as in (A) and (B), respectively, are shown. The number of traces (*n*) and percent showing fluctuations are indicated in each panel (supplementary Table 2).

Data information: Traces in (A) and (B) show fluorescence intensities observed for Cy3 (green) and Cy5 (red) with the calculated FRET trace in blue.

curves correlate with the degree of rotation of the 30S subunit similar to what was observed in the experimental data (Fig 5B and supplementary Fig 5C). Additionally, the small subunit of the ribosome was manually rotated $\pm 15^\circ$ relative to the large subunit (supplementary Fig 5D). CRY SOL was used to calculate *I* versus *q* plots based on these structures and resulted in the same pattern as in the experimental data; a decrease in *I* in the range 0.04–0.06 Å⁻¹ correlates with an increase in rotation angle (supplementary Fig 5E).

Discussion

The combination of our smFRET and SAXS experiments exhibit unambiguous support for the observation of a new hyper-rotated state of the ribosome in the presence of structured nucleic acid downstream of the ribosome's mRNA entrance tunnel. While large bodies of structural studies have been performed on the ribosome from various organisms, very few reports have been published specifically detailing the conformational changes in the ribosome that result from the presence of structured mRNA. In one study, a

Figure 5. SAXS data from ribosome complexes.

- A** The SAXS profiles ($I(q)$ versus q) of ribosome complexes with different RNA molecules as indicated. The SAXS profiles were vertically offset for clarity, otherwise, well superimposed in the displayed q range. The scattering intensity is displayed in the logarithmic scale.
- B** The scattering intensity difference ($\Delta I(q)$) against data of the free ribosomes for the same complexes as in panel (A). The SAXS profiles were normalized (supplementary Fig 5A) before doing the subtraction. The scattering intensity is in the linear scale. A free ribosome sample was used as the reference and appears on the abscissa (black line).

pseudoknot derived from infectious bronchitis virus (IBV) bound to mammalian 80S ribosomes was investigated by cryo-EM [4]. The ribosomes in this complex were suggested to remain in the rotated/hybrid state, which is different from our result. However, it is possible that mammalian ribosomes have a different mechanism for RNA unwinding or it may be due to the presence of eEF2 bound to the ribosome in that structure.

Our previous single molecule studies have shown that the ribosome can spontaneously fluctuate between just two rotational states with FRET values of 0.4 and 0.56 [9], which corresponds to the rotated and non-rotated state consistent with cryo-EM and X-ray studies [10,18]. Other structural studies have shown slight differences in the intersubunit rotation angle ($4\text{--}10^\circ$), which have depended on the source of ribosome, the types of binding ligands, and the method of structure determination [3,18–21]. In this study, we found a new stable state with a FRET value of 0.22 as a result of the presence of an RNA hairpin, which has not been observed in our previous study with unstructured mRNA (m291 and m301) or other single molecule studies [9,22,23]. This lower FRET state (0.22) represents a further rotation ($\sim 22^\circ$) that separates the

distance between the two fluorescent dyes. For these reasons, we propose, based on our smFRET and SAXS data, that this observation is different from all previous structural and single molecule studies.

Translocation and RNA unwinding are strictly coupled ribosomal functions [2,24]. The ribosome was shown to open three base pairs for each translocation cycle in a model where thermal fluctuations bias RNA towards an open state and mechanical force pulls RNA strands apart [5]. Our results suggest a possible mechanism for the role of ribosomal conformational dynamics in the helicase process. Upon hyper-rotated state formation, the mRNA is pulled further into the ribosome and held stably in this configuration. Ribosomal proteins S3, S4, and S5, which are positively charged, line the mRNA entrance tunnel [2]. Interactions between these proteins and RNA structure may thus provide the bias towards unwinding of RNA [5]. Since the frequency of intersubunit rotation is reduced in the presence of structured mRNA (supplementary Table 2), it is unlikely that this motion would provide the mechanical force necessary to pull apart RNA as in the Tinoco model [5]. Therefore, it may be that 30S head motion plays a crucial role in unwinding [25]. It is worth noting that S3 is located on the head and S4 and S5 on the body potentially setting up the role of head motion in grabbing RNA with proteins S3, S4, and S5 and pulling it apart. Indeed, Cryo-EM studies of the mammalian ribosome show different conformations of the 30S subunit in the presence of structured mRNA [4]. However, the precise role of the 30S head demands further study to completely describe its role in unwinding.

The L1 stalk of the ribosome is close to the E site and is important for tRNA movement and release from the ribosome [26]. Several groups have shown that there is a correlation between reading frame maintenance and E-site tRNA [27,28]. In some cases, the presence of tRNA in the E site has been shown to prevent frameshifting [28]. Our results have shown that in the hyper-rotated state the L1 stalk is forced into an extended, open configuration (Fig 4). This would preclude stable interactions forming between tRNA in the E site and the L1 stalk. While further experiments are necessary, it is tempting to speculate that restructuring of the L1 stalk in the hyper-rotated state has an influence on frameshifting efficiency.

Materials and Methods

More detailed procedures can be found in supplementary Methods. Fluorescently labeled ribosomes were prepared as previously described [9,29]. smFRET data were acquired using a home-built TIRF microscope. The relative occupancies in the various FRET states, as well as their rates of interconversion, were determined using established techniques including hidden Markov modeling (HMM) procedures [9].

SAXS data were collected at Beamline 12ID-B of the Advanced Photon Source (APS) at Argonne National Laboratory. Scattered X-ray intensities were measured using a Pilatus 2M detector (DECTRIS Ltd). The sample-to-detector distance was set such that the scattering angle of momentum transfer (q) of the SAXS experiments was $0.009\text{--}0.65\text{ \AA}^{-1}$. A flow cell with a cylindrical quartz capillary with a diameter of 1.5 mm and a wall of 10 μm were used to reduce radiation damage. The X-ray beam with a size of $0.07 \times 0.20\text{ mm}$, was adjusted to pass through the center of the capillary. The

exposure time was set to 2 s to avoid detector saturation and radiation damage. The scattering signal of the complexes was obtained by subtracting the scattering of the matching buffer from that of the sample solution.

Supplementary information for this article is available online: <http://embor.embopress.org>

Acknowledgements

The work was supported by the MU research board and NSF CAREER award MCB-115343 to P.V.C. P.V.C. is a Pew Scholar in the Biomedical Sciences. The use of the Advanced Photon Source, an Office of Science User Facility operated for the U.S. Department of Energy (DOE) Office of Science by Argonne National Laboratory, was supported by the U.S. DOE under Contract No. DE-AC02-06CH11357. The authors thank Harry Noller and Dmitri Ermolenko for providing fluorescently labeled ribosome constructs, L1/L33 knock out strain and expression plasmids for L1 and L33. We thank Mario Pennella for helpful discussions on the manuscript.

Author contributions

PQ and DY performed single molecule FRET experiments and analyzed data; PQ and PVC designed the project; XZ assisted with the SAXS data collection and analysis; PQ, XZ and PVC wrote the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- Selmer M, Dunham CM, Murphy FVT, Weixlbaumer A, Petry S, Kelley AC, Weir JR, Ramakrishnan V (2006) Structure of the 70S ribosome complexed with mRNA and tRNA. *Science* 313: 1935–1942
- Takay S, Hickerson RP, Noller HF (2005) mRNA helicase activity of the ribosome. *Cell* 120: 49–58
- Korostelev A, Trakhanov S, Laurberg M, Noller HF (2006) Crystal structure of a 70S ribosome-tRNA complex reveals functional interactions and rearrangements. *Cell* 126: 1065–1077
- Namy O, Moran SJ, Stuart DI, Gilbert RJ, Brierley I (2006) A mechanical explanation of RNA pseudoknot function in programmed ribosomal frameshifting. *Nature* 441: 244–247
- Qu X, Wen JD, Lancaster L, Noller HF, Bustamante C, Tinoco I Jr (2011) The ribosome uses two active mechanisms to unwind messenger RNA during translation. *Nature* 475: 118–121
- Giedroc DP, Cornish PV (2009) Frameshifting RNA pseudoknots: structure and mechanism. *Virus Res* 139: 193–208
- Cornish PV, Ermolenko DN, Staple DW, Hoang L, Hickerson RP, Noller HF, Ha T (2009) Following movement of the L1 stalk between three functional states in single ribosomes. *Proc Natl Acad Sci USA* 106: 2571–2576
- Larsen B, Gesteland RF, Atkins JF (1997) Structural probing and mutagenic analysis of the stem-loop required for Escherichia coli dnaX ribosomal frameshifting: programmed efficiency of 50%. *J Mol Biol* 271: 47–60
- Cornish PV, Ermolenko DN, Noller HF, Ha T (2008) Spontaneous intersubunit rotation in single ribosomes. *Mol Cell* 30: 578–588
- Frank J, Agrawal RK (2000) A ratchet-like inter-subunit reorganization of the ribosome during translocation. *Nature* 406: 318–322
- Dunkle JA, Wang L, Feldman MB, Pulk A, Chen VB, Kapral GJ, Noeske J, Richardson JS, Blanchard SC, Cate JH (2011) Structures of the bacterial ribosome in classical and hybrid states of tRNA binding. *Science* 332: 981–984
- Fischer N, Konevega AL, Wintermeyer W, Rodnina MV, Stark H (2010) Ribosome dynamics and tRNA movement by time-resolved electron cryomicroscopy. *Nature* 466: 329–333
- Jin H, Kelley AC, Loakes D, Ramakrishnan V (2010) Structure of the 70S ribosome bound to release factor 2 and a substrate analog provides insights into catalysis of peptide release. *Proc Natl Acad Sci USA* 107: 8593–8598
- Ermolenko DN, Majumdar ZK, Hickerson RP, Spiegel PC, Clegg RM, Noller HF (2007) Observation of intersubunit movement of the ribosome in solution using FRET. *J Mol Biol* 370: 530–540
- Fei J, Richard AC, Bronson JE, Gonzalez RL Jr (2011) Transfer RNA-mediated regulation of ribosome dynamics during protein synthesis. *Nat Struct Mol Biol* 18: 1043–1051
- Munro JB, Altman RB, Tung CS, Sanbonmatsu KY, Blanchard SC (2010) A fast dynamic mode of the EF-G-bound ribosome. *EMBO J* 29: 770–781
- Koch MH, Vachette P, Svergun DI (2003) Small-angle scattering: a view on the properties, structures and structural changes of biological macromolecules in solution. *Q Rev Biophys* 36: 147–227
- Zhang W, Dunkle JA, Cate JH (2009) Structures of the ribosome in intermediate states of ratcheting. *Science* 325: 1014–1017
- Gao H, Sengupta J, Valle M, Korostelev A, Eswar N, Stagg SM, Van Roey P, Agrawal RK, Harvey SC, Sali A, Chapman MS, Frank J (2003) Study of the structural dynamics of the *E. coli* 70S ribosome using real-space refinement. *Cell* 113: 789–801
- Matsumoto A, Ishida H (2009) Global conformational changes of ribosome observed by normal mode fitting for 3D Cryo-EM structures. *Structure* 17: 1605–1613
- Jin H, Kelley AC, Ramakrishnan V (2011) Crystal structure of the hybrid state of ribosome in complex with the guanosine triphosphatase release factor 3. *Proc Natl Acad Sci USA* 108: 15798–15803
- Marshall RA, Dorywalska M, Puglisi JD (2008) Irreversible chemical steps control intersubunit dynamics during translation. *Proc Natl Acad Sci USA* 105: 15364–15369
- Aitken CE, Puglisi JD (2010) Following the intersubunit conformation of the ribosome during translation in real time. *Nat Struct Mol Biol* 17: 793–800
- Wen JD, Lancaster L, Hodges C, Zeri AC, Yoshimura SH, Noller HF, Bustamante C, Tinoco I (2008) Following translation by single ribosomes one codon at a time. *Nature* 452: 598–603
- Guo Z, Noller HF (2012) Rotation of the head of the 30S ribosomal subunit during mRNA translocation. *Proc Natl Acad Sci USA* 109: 20391–20394
- Noller HF, Yusupov MM, Yusupova GZ, Baucom A, Cate JH (2002) Translocation of tRNA during protein synthesis. *FEBS Lett* 514: 11–16
- Bekaert M, Rousset JP (2005) An extended signal involved in eukaryotic -1 frameshifting operates through modification of the E site tRNA. *Mol Cell* 17: 61–68
- Sanders CL, Curran JF (2007) Genetic analysis of the E site during RF2 programmed frameshifting. *RNA* 13: 1483–1491
- Cornish PV, Ermolenko DN, Staple DW, Hoang L, Hickerson RP, Noller HF, Ha T (2009) Following movement of the L1 stalk between three functional states in single ribosomes. *Proc Natl Acad Sci USA* 106: 2571–2576