Figures and figure supplements

The shape of the bacterial ribosome exit tunnel affects cotranslational protein folding

Renuka Kudva et al
Figure 1. Cotranslational protein folding assay. (A) Front view of the 50S subunit of the *E. coli* ribosome adapted from PDB 3JBU (Zhang et al., 2015), with tunnel proteins uL4 and uL22 indicated in gray. The globular domain of uL23 is indicated in orange with the β-hairpin loop depicted in yellow. uL24 is shown in dark blue, with the loop at the tunnel exit shown in light blue. The exit tunnel, outlined by a stalled SecM nascent chain (purple), is ~100 Å in length. (B) The arrest-peptide assay (Nilsson et al., 2015). The domain to be studied is placed L residues upstream of the critical proline at the C-terminal end of the 17-residue long arrest peptide (AP) from the *E. coli* SecM protein. A 23-residue long stretch of the *E. coli* LepB protein is attached downstream of the AP, allowing us to separate the arrested (A) and full-length (FL) products by SDS-PAGE after translation. Constructs are translated in the PURExpress in vitro translation system supplemented with WT, uL23 Δloop, or uL24 Δloop high-salt washed ribosomes for 20 min. The relative amounts of arrested and full-length protein were estimated by quantification of SDS-PAGE gels, and the fraction of full-length protein was calculated as

$$f_{FL} = I_{FL} / (I_A + I_{FL})$$

where $I_A$ and $I_{FL}$ are the intensities of the bands corresponding to the A and FL products. (C) $f_{FL}$ is a proxy for the force $F$ that cotranslational folding of a protein domain exerts on the AP. At short linker lengths, both $F$ and $f_{FL} = 0$ because the domain is unable to fold due to lack of space in the exit tunnel. At intermediate linker lengths, $F$ and $f_{FL} > 0$ because the domain pulls on the nascent chain as it folds. At longer linker lengths, $F$ and $f_{FL} = 0$ because the domain is already folded when the ribosome reaches the end of the AP.

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Figure 1—figure supplement 1. A260 = 300 units (6.9 μM) of high-salt washed ribosomes were separated on a 12% Bis-Tris gel and transferred by Western blotting onto a nitrocellulose membrane and detected with antibodies against uL24 (panel A) or uL23 (panel B).
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Figure 1—figure supplement 2. Multiple sequence alignment of uL23 and uL24. (A) and uL24 (B). The uL23 and uL24 β-hairpin loops, boxed, respectively, in orange and blue, are conserved among Gram-negative bacteria, but are short or absent in archaea and eukaryotes. In eukaryotes and archaea, part of the function of uL23 is proposed to be fulfilled by ribosomal protein eL39.

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Figure 2. Structural consequences of removing the hairpin loops in uL24 and uL23 modeled after PDB 3JBU of the SecM stalled ribosome. (A) In wildtype ribosomes, the loop in uL24 partially obstructs the exit tunnel (top panel). Its removal in uL24 Δloop ribosomes creates a wide opening into the tunnel (bottom panel). (B) In wildtype ribosomes, the loop in uL23 extends into the exit tunnel (top panel). Its removal in uL23 Δloop ribosomes creates an open space around the area where the ADR1a domain is known to fold (Nilsson et al., 2015). The ADR1a structure is from PDB 5A7U. (C) Cryo-EM structure of the uL23 Δloop 70S ribosome (EMD-4319), fitted to PDB 3JBU (that includes a Gly-tRNA and a 26-residue long arrested SecM AP) to locate uL23 (orange) and the exit tunnel. The enlarged region shows a difference map (in mesh) obtained by subtracting the cryo-EM map of the uL23 Δloop 70S ribosome from a map generated from 3JBU in Chimera. The difference map shows that the only difference in volume between the two maps is the tRNA (in magenta), the SecM AP (in pink), and the loop deleted from uL23. (D) Extracted cryo-EM density (in mesh) for uL23 in the uL23 Δloop ribosome EMD-4319. Wildtype uL23 (orange) and a de novo-built model for the mutant uL23 Δloop protein (PDB 6FU8; red) are shown in ribbon representation.

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Figure 2—figure supplement 1. Resolution map of the uL23Δ loop ribosome. (A) Calculation of the local resolution using cryoSPARC. (B) Resolution histogram at FSC 0.143. (C) Fourier-Shell correlation (FSC) curve of the refined map at 0.143 indicating an average resolution of 3.28 Å.

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Figure 3. Cotranslational folding in WT, uL23 delta-loop, and uL24 delta-loop ribosomes. (A) $f_{FL}$ profiles ($\Delta f_{FL} = f_{FL}(50 \mu M \text{Zn}^{2+}) - f_{FL}(50 \mu M \text{TPEN})$) for ADR1a constructs translated in the PURE system supplemented with in-house purified WT (gray), uL23 $\Delta$loop (red), and uL24 $\Delta$loop (blue) E. coli ribosomes. (B) $f_{FL}$ profiles for spectrin R16 constructs translated in the PURE system supplemented with in-house purified WT (gray), uL23 $\Delta$loop (red), and uL24 $\Delta$loop (blue) E. coli ribosomes. (C) $f_{FL}$ profiles for titin I27 constructs translated in the PURE system supplemented with in-house purified WT (gray), uL23 $\Delta$loop (red), and uL24 $\Delta$loop (blue) E. coli ribosomes. Error bars in panels a-c show SEM values calculated from at least three independent experiments. Dashed lines indicate $L_{\text{onset}}$ and $L_{\text{end}}$ values, c.f., Table 1. $f_{FL}$ profiles for non-folding mutants of R16 and I27 are found in (Nilsson et al., 2017; Tian et al., 2018). (D) Simulated $f_{FL}$ profiles (full lines) for ADR1a, spectrin R16, and titin I27 obtained with WT (gray), uL23 $\Delta$loop (red), and uL24 $\Delta$loop (blue) E. coli ribosomes. The corresponding experimental $f_{FL}$ profiles from panels a-c are shown as dashed lines. The simulated ADR1a $f_{FL}$ profile marked by X’s was obtained with a uL23 $\Delta$loop(70-72) ribosome model. Simulated $f_{FL}$ profiles for ADR1a with uL24 $\Delta$loop ribosomes, and for R16 and I27 with uL23 $\Delta$loop ribosomes, are essentially identical to the corresponding profiles obtained with WT ribosomes, and are shown in Figure 3—figure supplement 10.

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Figure 3—figure supplement 1. SDS PAGE showing ADR1 constructs translated in PURExpress Δ-Ribosome kit supplemented with high-salt-washed ribosomes isolated from HDB140, HDB143 (uL23 Δ-loop), or HDB144 (uL24 Δ-loop) as indicated. Translations were run on 12% Bis-Tris gels with MOPS running buffer.

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Translation of ADR1a with WT ribosomes from strain HDB140 (except gel ‘a’ lane indicated 24*, see note below gel)

a

b

24* = Linker length 24 translated with uL23\(Δ\)loop ribosomes

Translation of ADR1a with uL23\(Δ\)loop ribosomes from strain HDB143

d

e

Figure 3—figure supplement 2. SDS PAGE showing ADR1 constructs translated in PURExpress \(Δ\)-Ribosome kit supplemented with high-salt-washed ribosomes isolated from HDB140, HDB143 (uL23\(Δ\)loop), or HDB144 (uL24\(Δ\)loop) as indicated.

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Figure 3—figure supplement 3. SDS PAGE showing ADR1 constructs translated in PURExpress Δ-Ribosome kit supplemented with high-salt-washed ribosomes isolated from HDB140, HDB143 (uL23Δ loop), or HDB144 (uL24Δ loop) as indicated.

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Figure 3—figure supplement 4. SDS PAGE showing ADR1a constructs translated in PURExpress Δ-Ribosome kit supplemented with high-salt-washed ribosomes isolated from HDB140, HDB143 (ΔuL23 loop), or HDB144 (ΔuL24 loop) as indicated.

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Translation of R16 Spectrin with WT ribosomes

Translation of R16 Spectrin with uL23Δloop ribosomes
Figure 3—figure supplement 5. SDS PAGE showing Spectrin R16 constructs translated in PURExpress Δ-Ribosome kit supplemented with high-salt-washed ribosomes isolated from HDB140, HDB143 (uL23 Δloop), or HDB144 (uL24 Δloop) as indicated. Translations were run on 12% Bis-Tris gels with MES running buffer.

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**Figure 3—figure supplement 6.** SDS PAGE showing Spectrin R16 and Titin I27 constructs translated in PURExpress Δ-Ribosome kit supplemented with high-salt-washed ribosomes isolated from HDB140, HDB143 (uL23 Δ-loop), or HDB144 (uL24 Δ-loop) as indicated. Translations were run on 12% Bis-Tris gels with MES running buffer.

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Figure 3—figure supplement 7. SDS PAGE showing titin I27 constructs translated in PURExpress Δ-Ribosome kit supplemented with high-salt-washed ribosomes isolated from HDB140, HDB143 (uL23 Δ-loop), or HDB144 (uL24 Δ-loop) as indicated.

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Figure 3—figure supplement 8. $f_{FL}$ profiles for ADR1a constructs translated in PURE by WT, uL23 Δloop, and uL24 Δloop ribosomes, either in the presence of Zn$^{2+}$ or of the Zn$^{2+}$ chelator TPEN. Averages and standard errors calculated from three independent translation reactions are shown.

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Figure 3—figure supplement 9. Sequences of the longest and the shortest constructs used for each protein and, a depiction of the location of the sequences the ribosome exit tunnel when the last residue of the AP is in the P-site (lower panel, yellow box). ADR1 is indicated in red, spectrin R16 in Kudva et al. eLife 2018;7:e36326. DOI: https://doi.org/10.7554/eLife.36326.
blue, titin I27 in pink, and the SecM AP in magenta. The linker between each domain and the AP is in gray. The part of LepB added to the N-terminus of ADR1a is in black.

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Figure 3—figure supplement 10. Summary of results from coarse-grained MD simulations. (A) Average forces exerted on the AP by the folded state (first column), fraction folded protein (second column), and $f_{FL}$ values (last column).
Figure 3—figure supplement 10 continued

(A) Snapshots of folded ADR1a, I27, and R16 domains in wildtype (WT), uL23 Δloop, and uL24 Δloop ribosomes at \( L = L_{\text{onset}} \). Note that the folded proteins are located at similar depths in the exit tunnel in the cryo-EM structures and the simulations for WT ribosomes (this holds also for ADR1a in uL24 Δloop ribosomes as well as for I27 and R16 in uL23 Dloop ribosomes, c.f. panel A). The C terminus of folded ADR1a is located –6 Å deeper in the exit tunnel in uL23 Δloop than in WT ribosomes, while folded I27 and R16 are located, respectively, –15 Å and –13 Å deeper in the exit tunnel in uL24 Δloop ribosomes (dashed guide lines). Note that the linker is more stretched in the WT ribosome simulations compared to the Dloop ribosomes, consistent with the higher force and lower fraction folded protein seen for the WT ribosome data at the same \( L \) values (panel a).

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