A144 · eIF5A Depletion Increases Ribosome Occupancy at Cotranslational Ssb Chaperone Binding Sites

Presenter: Eimaan Bilal — Stony Brook University

Authors: Eimaan Bilal, Jae Ho Lee

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Cotranslational chaperones help ensure proper folding of nascent polypeptides as they emerge from the ribosome, preventing protein aggregation associated with many diseases. However, how changes in elongation dynamics influence these interactions remains unclear. We investigated whether depletion of eIF5A, a translation factor that facilitates elongation through stall-prone sequences, alters ribosome occupancy around binding sites of the yeast Hsp70 chaperone Ssb. Publicly available ribosome profiling data from Saccharomyces cerevisiae consisting of two wild-type and two eIF5A-depleted replicates, were processed through a computational pipeline including adapter trimming, noncoding RNA removal, coding sequence alignment, and generation of codon level ribosome density profiles. Ssb-binding positions were mapped to their corresponding open reading frames, and ±40-codon windows were extracted around each binding site. Ribosome density was normalized to each site's mean density, with replicates normalized independently before averaging within conditions. RNA-depleted reads showed > 90% alignment rate to the coding sequence reference for all samples. Metagene analysis revealed localized enrichment of ribosome occupancy around Ssb-binding sites in both conditions. eIF5A depletion produced a substantially greater peak near the Ssb-binding position than wild type, reaching approximately 1.65 compared to 1.35 mean site-normalized density. This pattern suggests enhanced translational pausing near sites of cotranslational Ssb interaction following eIF5A depletion. Our findings demonstrate that the perturbation of eIF5A-dependent elongation alters local translation dynamics around Ssb-binding sites. These results allow us to further investigate how translation speed may regulate interactions between ribosomes, nascent polypeptides, and cotranslational chaperones to prevent protein misfolding and aggregation.

eIF5A Depletion Increases Ribosome Occupancy at Cotranslational Ssb Chaperone Binding Sites

Eimaan Bilal*[1,2], Jae Ho Lee[1,2]
[1] Stony Brook University, [2] Department of Biochemistry and Cell Biology

Background: Cotranslational chaperones help ensure proper folding of nascent polypeptides as they emerge from the ribosome, preventing protein aggregation associated with many diseases. However, how changes in elongation dynamics influence these interactions remains unclear. We investigated whether depletion of eIF5A, a translation factor that facilitates elongation through stall-prone sequences, alters ribosome occupancy around binding sites of the yeast Hsp70 chaperone Ssb.
Methods: Publicly available ribosome profiling data from *Saccharomyces cerevisiae* consisting of two wild-type and two eIF5A-depleted replicates [1], were processed through a computational pipeline including adapter trimming, noncoding RNA removal, coding sequence alignment, and generation of codon level ribosome density profiles. Ssb-binding positions were mapped to their corresponding open reading frames, and ±40-codon windows were extracted around each binding site. Ribosome density was normalized to each site's mean density, with replicates normalized independently before averaging within conditions.
Results: RNA-depleted reads showed > 90% alignment rate to the coding sequence reference for all samples. Metagene analysis revealed localized enrichment of ribosome occupancy around Ssb-binding sites in both conditions. eIF5A depletion produced a substantially greater peak near the Ssb-binding position than wild type, reaching approximately 1.65 compared to 1.35 mean site-normalized density. This pattern suggests enhanced translational pausing near sites of cotranslational Ssb interaction following eIF5A depletion.
Conclusion: Our findings demonstrate that the perturbation of eIF5A-dependent elongation alters local translation dynamics around Ssb-binding sites. These results allow us to further investigate how translation speed may regulate interactions between ribosomes, nascent polypeptides, and cotranslational chaperones to prevent protein misfolding and aggregation.

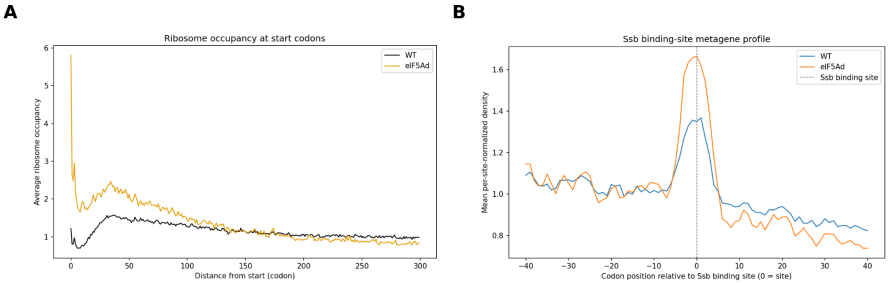

Figure 1. eIF5A depletion alters ribosome occupancy globally and around Ssb chaperone-binding sites.
(A) Start-codon metagene analysis of ribosome occupancy in wild-type (WT) and eIF5A-depleted (eIF5Ad) *S. cerevisiae* replicates. Density at each codon position is normalized to that gene's mean density across the full CDS and averaged across genes. eIF5A depletion produces a sharp accumulation of ribosomes near the start codon relative to WT, consistent with increased translational pausing. **(B)** Ssb-centered metagene profile of ribosome density (±40 codons; n = 2263 sites), per-site normalized and averaged across WT and eIF5Ad replicates. eIF5A depletion produces greater localized ribosome occupancy around Ssb-binding sites compared with WT, suggesting perturbation of eIF5A-dependent elongation alters local translation dynamics at sites of cotranslational chaperone interaction.

[1] Schuller, A. P., Wu, C. C.-C., Dever, T. E., Buskirk, A. R., & Green, R. (2017). eIF5A Functions Globally in Translation Elongation and Termination. *Molecular Cell,* 66(2), 194–205.e5. https://doi.org/10.1016/j.molcel.2017.03.003