Comment on “Principles of ER cotranslational translocation revealed by proximity-specific ribosome profiling”

Jan et al. (Research Articles, 7 November 2014, p. 716) propose that ribosomes translating secretome messenger RNAs (mRNAs) traffic from the cytosol to the endoplasmic reticulum (ER) upon emergence of the signal peptide and return to the cytosol after termination. An accounting of controls demonstrates that mRNAs initiate translation on ER-bound ribosomes and that ribosomes are retained on the ER through many cycles of translation.

J an et al. (1) report that ribosomes engaged in the translation of secretome mRNAs are recruited from the cytosol to the endoplasmic reticulum (ER) membrane early in translation and released back into the cytosol soon after completion of protein synthesis. Although these conclusions are consistent with the established signal hypothesis/signal recognition particle (SRP) pathway model (2, 3), recent work has demonstrated that ER-bound ribosomes translate both signal sequence– and cytosolic protein–encoding mRNAs and that ribosomes associate stably with the ER after termination (4–6). In an attempt to reconcile these discrepancies, we reanalyzed the authors’ data, including control data that were not integrated into their conclusions. We suggest that the data from Jan et al. rather demonstrate that ER-bound ribosomes initiate translation while remaining associated with the ER, translate both secretome– and cytosolic protein–encoding mRNAs, and stably associate with the ER through multiple cycles of translation.

To examine ribosome trafficking and translation dynamics, Jan et al. developed proximity-specific ribosome profiling, where chimeras of resident ER membrane proteins and the biotin ligase BirA were used to selectively label ribosomes bearing BirA acceptor sequence–tagged ribosomal protein RPL16 (9). In one iteration, BirA-Ssh1, where Ssh1 is paralogous to the protein–conducting channel Sec61, was used to label translocon-proximal, ER-bound ribosomes. Jan et al. then determined the location of tagged ribosomes on mRNAs by ribosome profiling. The authors observed that few ribosomes were labeled by BirA-Ssh1 before the emergence of a signal sequence, leading them to conclude that ribosomes are cotranslationally targeted from the cytosol to the ER after the signal sequence is translated, consistent with the signal hypothesis/SRP pathway model.

We were able to reproduce the authors’ primary observation using their data, where BirA-Ssh1–labeled ribosomes were depleted on mRNAs encoding predicted signal sequences (10) until ~30 codons after the signal sequence (Fig. 1A). However, when we analyzed data from control experiments with a BirA-Ubc6 tail-anchor chimera (Ubc6TA), designed to be a reporter for general ER-associated translation, this ribosome depletion was not observed (Fig. 1B). Instead, the BirA-Ubc6TA reporter identified ER ribosomes translating secretome mRNAs throughout the open reading frame, including before the emergence of a signal sequence. This indicates that ribosomes were bound to the ER before signal sequence emergence, and that ribosome localization to the ER is not obligatorily coupled to SRP-signal sequence recruitment. To explain the paucity of ribosomes in the first ~30 codons observed with the BirA-Ssh1 reporter, we suggest a scenario in which ER-bound ribosomes initiate translation from the ER-bound state and subsequently undergo signal sequence–dependent lateral recruitment to BirA-Ssh1 translocons, as an alternative to the cytosol to BirA-Ssh1 translocon ribosome trafficking model the authors propose. Because ER-bound ribosomes are well represented on secretome mRNAs before the emergence of the signal sequence, the data from Jan et al. demonstrate that cotranslational targeting to the ER is unlikely to be the sole, or even primary, mechanism by which mRNAs or ribosomes are localized to the ER.

If ribosomes are not recruited to the ER in a manner coupled to emergence of the signal sequence, how might the authors’ conclusions regarding ribosome exchange rates between the cytosol and ER be viewed? In Jan et al., ribosome exchange rates were determined by tracking the mRNA composition of BirA-Sec63–labeled ribosomes as a function of biotin labeling time. At short biotin pulse periods, BirA-Sec63–labeled ribosomes were highly enriched in secretome mRNAs. This enrichment dissipated within 7 min of labeling, at which point ribosomes translating cytosolic and secretome mRNAs were similarly labeled. These data led to the interpretation that labeled ribosomes that were previously translating ER proteins left the ER to initiate translation of cytosolic proteins, and so the composition of the tagged ribosome-associated mRNAs serves as a proxy for ribosome exchange. For this interpretation to be valid, ER-bound ribosomes must be largely devoted to the translation of secretome mRNAs. However, when analyzing the mRNA composition of labeled ribosomes during this time course, we found that the majority of tagged ribosomes were translating cytosolic proteins even after 1 min of biotin labeling, invalidating the assumption that ribosome subcellular localization can be inferred from the composition of the associated mRNAs (Fig. 2A). This finding is consistent with several studies using diverse organisms and methods (Fig. 2B). The early enrichment for ribosomes translating ER proteins

![Fig. 1. Ribosomes are bound to the ER before signal sequence emergence.](http://science.sciencemag.org/) The enrichment of ribosomes in the biotin-labeled pulldown relative to input was calculated at each codon for mRNAs that encode signal sequences. Plots were generated for (A) BirA-Ssh1 and (B) BirA-Ubc6TA. Shaded area represents mean ± SD across all analyzed genes. Both analyses were performed on data from the 7-min biotin pulse without cycloheximide.

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that the authors reported, instead of representing ribosome exchange, is likely due to a modest kinetic advantage for ribosomes in close proximity to translocon-associated BirA-Sec63. Although the half-life of a ribosome on the ER therefore remains unknown, the bulk of evidence that we have discussed above and elsewhere (11) indicates that it is substantially longer than a single cycle of translation.

Fig. 2. The majority of ER-associated translation is devoted to cytosolic protein-encoding mRNAs. (A) The fraction of BirA-Sec63-labeled ribosomes engaged in the translation of cytosolic or ER proteins was calculated at the indicated labeling times. (B) Stacked bar plot depicting the proportion of ER-associated mRNAs encoding cytosolic proteins. [Data are from (4, 5, 8)].

REFERENCES AND NOTES

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