Selectivity and Robustness in Recognition of Stalled Ribosomes

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Ribosome stalling on mRNAs can decrease protein expression. To decipher ribosome kinetics at stall sites, we measured protein expression from a reporter library of over 100 variants that encoded systematic perturbations of translation initiation rate, the number of stall sites, and the distance between stall sites. Our measurements support a novel model in which abortive termination of translation occurs upon collision of a stalled ribosome with a trailing ribosome. In our computational analysis, ribosome collisions selectively stimulate abortive termination without fine-tuning of kinetic parameters. We propose that ribosome collisions serve as a robust timer for cells to recognize stalled ribosomes.

Ribosomes move at an average speed of 3–20 codons per second during translation elongation \textit{in vivo}. Since this rate is higher than the typical initiation rate of ribosomes on mRNAs [less than 1 s\textsuperscript{-1}], elongation is often assumed not to affect the expression level of most proteins. Nevertheless, the elongation rate of ribosomes can decrease significantly at specific locations on an mRNA due to low abundance of aminoacyl-tRNAs. Ribosome profiling — the deep sequencing of ribosome-protected mRNA fragments — has enabled the identification of additional factors that induce slowing or stalling of ribosomes during elongation (1). An important question emerging from these studies is the extent to which ribosome stalling affects the expression of the encoded protein, since initiation might still be the slowest step during translation.

Several mechanistic models have been proposed to explain how ribosome stalling during elongation might affect the expression of the encoded protein. In the widely used traffic jam model (2), the duration of ribosome stalling is sufficiently long to induce a queue of trailing ribosomes extending to the start codon, thus decreasing the translation initiation rate. Evidence supporting this model has been found in the context of polypyrrole stalls in \textit{E. coli}, and rare-codon induced pausing in \textit{E. coli} and yeast. In an alternate abortive termination model, ribosome stalling causes premature termination without synthesis of the full-length protein (3). This model is thought to underlie the action of various ribosome rescue factors in \textit{E. coli} and yeast.

Despite the experimental evidence supporting the above models, predicting the effect of ribosome stalling on protein levels has been challenging because of uncertainty in our knowledge of \textit{in vivo} kinetic parameters such as the duration of ribosome stalling and the rate of abortive termination.

Further, while we have a detailed understanding of the kinetic steps and structural changes that occur during the normal elongation cycle of the ribosome, the ‘off-pathway’ events that occur at stalled ribosomes have been elucidated in only a few specific cases (4). Thus, development of complementary approaches, which can quantitatively constrain the \textit{in vivo} kinetics of stalled ribosomes without precise knowledge of rate parameters, will be useful for bridging the gap between the growing list of ribosome stall sequences and their effect on protein expression.

Here, we investigated the effect of ribosome stalling on protein expression using amino acid starvation in \textit{E. coli} as an experimental model (5). In this system, we previously found that both ribosome traffic jams and abortive termination occur at a subset of codons cognate to the limiting amino acid (3). Motivated by these observations, here we computationally modeled ribosome traffic jams and abortive termination with the goal of predicting their effect on protein expression. Even without precise knowledge of \textit{in vivo} kinetic parameters, we found that these two processes give qualitatively different trends in protein expression when the initiation rate, the number of stall sites and the distance between stall sites are systematically varied. Surprisingly, experimental measurements support a model in which traffic jams and abortive termination do not occur independent of one another; rather, collisions by trailing ribosomes stimulate abortive termination of the stalled ribosome. We find that this model is consistent with the absence of long ribosome queues in ribosome profiling measurements, and it naturally provides a mechanistic basis for the selectivity of abortive termination towards stalled ribosomes. While these conclusions are limited to the specific context of amino acid starvation in \textit{E. coli}, the integrated approach developed in this work should be generally applicable to investigate other ribosome stalls in both bacteria and eukaryotes.

\textbf{REFERENCES}