Novel elements of translation termination in trypanosomatids

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Accurate termination of translation is essential for efficient gene expression, yet it has not been studied in *Trypanosoma brucei*. In addition, *Blastocrithidia nonstop* is a closely related trypanosomatid that has a non-canonical genetic code with all three stop codons reassigned to encode amino acids. Only UAA can function either as a stop codon, or as a glutamate codon. Hence, it remains unclear how termination of translation is determined in this organism. We investigate translation termination in *T. brucei,* an established model organism, to define the core termination components, and to functionally assess their counterparts in *B. nonstop*. We focus on eukaryotic release factor 1 (eRF1), the protein that recognizes stop codons and triggers translation termination. Its knock-down was generated in *T. brucei* and as expected, TbeRF1 is essential for cell survival. Application of the dual reporter assay in this cell line confirmed that termination at UAA becomes inefficient after depletion of TbeRF1. Next, we demonstrated that the ectopic expression of the *B. nonstop* eRF1 homolog in *T. brucei* knock-down cell line leads only to partial restoration of termination at UAA, corresponding with failure to rescue the growth defect caused by depletion of the endogenous TbeRF1. This can be explained by comparison of sequences of the two eRF1 homologs revealing substitutions in residues that have been already described as crucial for its function. In addition, we also noticed novel, previously unreported substitutions, which will be further examined. We propose that for efficient termination, *Bn*eRF1 may require protein partner(s) to complement its function. Hence, we performed co-immunoprecipitation from *B. nonstop* cells followed by proteomic analysis. Indeed, one of the identified hits was reported to contribute to mRNA stability before, which aligns with RNA binding and potentially termination, a hypothesis that is currently under investigation. In summary, we use trypanosome as a model to study the unique mechanisms of translation in *B. nonstop*, since better understanding of the termination molecular machinery and its alternatives could provide valuable insights for human diseases associated with defects in termination of translation.