Roles and interactions of the specialized initiation factors EIF4E2, EIF4E5 and EIF4E6 in *Trypanosoma brucei*: EIF4E2 maintains the abundances of S-phase mRNAs

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Abstract

Trypanosoma brucei has six versions of the cap-binding translation initiation factor EIF4E. We here investigated the functions of EIF4E2, EIF4E5 and EIF4E6 in bloodstream forms. Purification and mass spectrometry of non-RNAse treated extracts, and comparison with EIF4E3, confirmed the specific associations previously found in the procyclic form, which infects the Tsetse fly host. Some co-purification of RNA-binding proteins, especially with EIF4Es 3 and 6 was also observed. Bloodstream forms lacking EIF4E5 grew normally and could differentiate to replication-incompetent procyclic forms. Depletion of EIF4E6, in contrast, inhibited bloodstream-form trypanosome growth and translation.

EIF4E2 complexes with a putative RNA stem-loop binding protein, SLBP2, but has no EIF4G partner, and no association with general translation factors, suggesting that it is not an active translation initiation factor. Bloodstream forms lacking EIF4E2 multiplied slowly, with a slight increase in G2 cells at the expense of G1. They had a low maximal cell density, with expression of the stumpy-form marker PAD1 but no evidence for enhanced stumpy-form signaling. RNAi targeting SLBP2 had similar effects. The EIF4E2 knock-out cells differentiated readily to procyclic forms, which grew normally. mRNA pull-downs revealed strong association of EIF4E2 with mRNAs that are maximally abundant in S phase, three of which were previously shown to be bound and stabilised by the pumilio domain protein PUF9. The same mRNAs had decreased abundances in EIF4E2 knock-out cells. Yeast 2-hybrid results suggest that PUF9 interacts directly with SLBP2, but PUF9 was not abundant in the EIF4E2 pull-downs. A possible interpretation is that the EIF4E2-SLBP2 complex interacts with PUF9, and its bound RNAs, only early during G1/S, stabilising the mRNAs in preparation for translation later in S phase or in early G2.