Ribosomal proteins eL24 and eL19, involved in intersubunit bridges, have the specific roles to ensure the ribosome functionality

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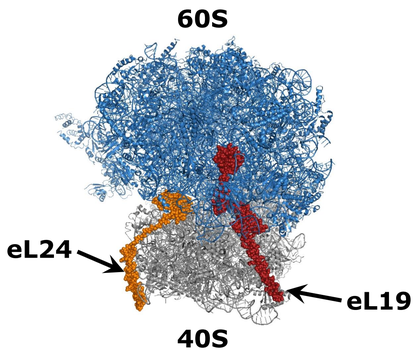
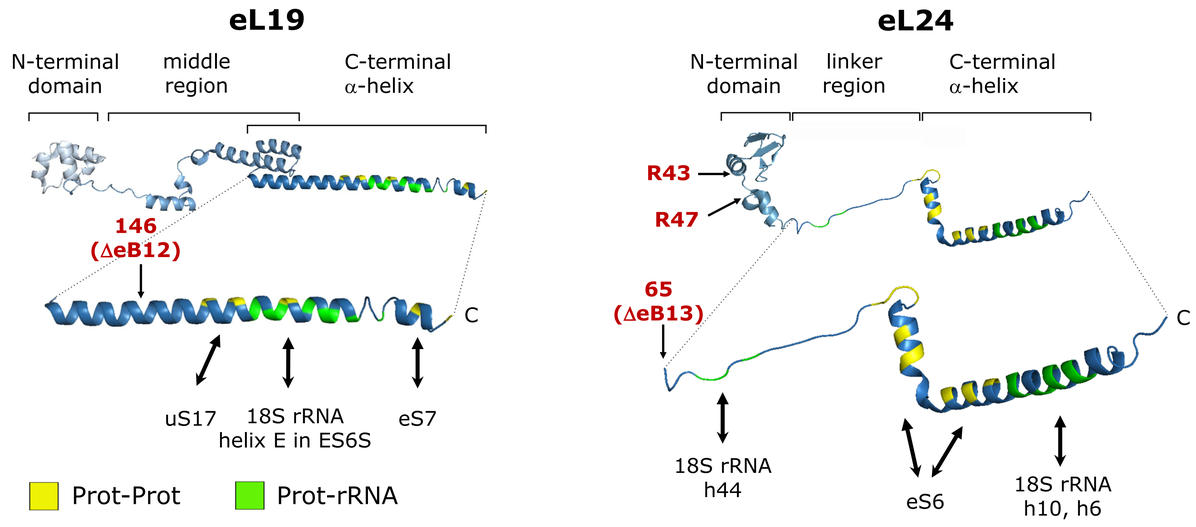
Introduction

- Seventeen bridges are formed during association of yeast ribosome subunits (Ben-Shem *et al.*, 2011). Five intersubunit bridges are eukaryote-specific.
- Two eukaryote-specific bridges, eB12 and eB13, are structurally similar to each other. Both bridges are formed by the long protein α-helices extending from 60S subunit E- and A-site sides, respectively.
- Essential protein eL19 (shown in red) is the main component of eB12 bridge.
- Dispensable for cell viability protein eL24 (shown in orange) participates in formation of two intersubunit bridges: eB13 and B6.
- The C-terminal α-helix and linker region of eL24 form the main part of eB13 bridge.
- The conserved B6 bridge is made of only two contacts between N-terminal domain of eL24 and 18S rRNA.



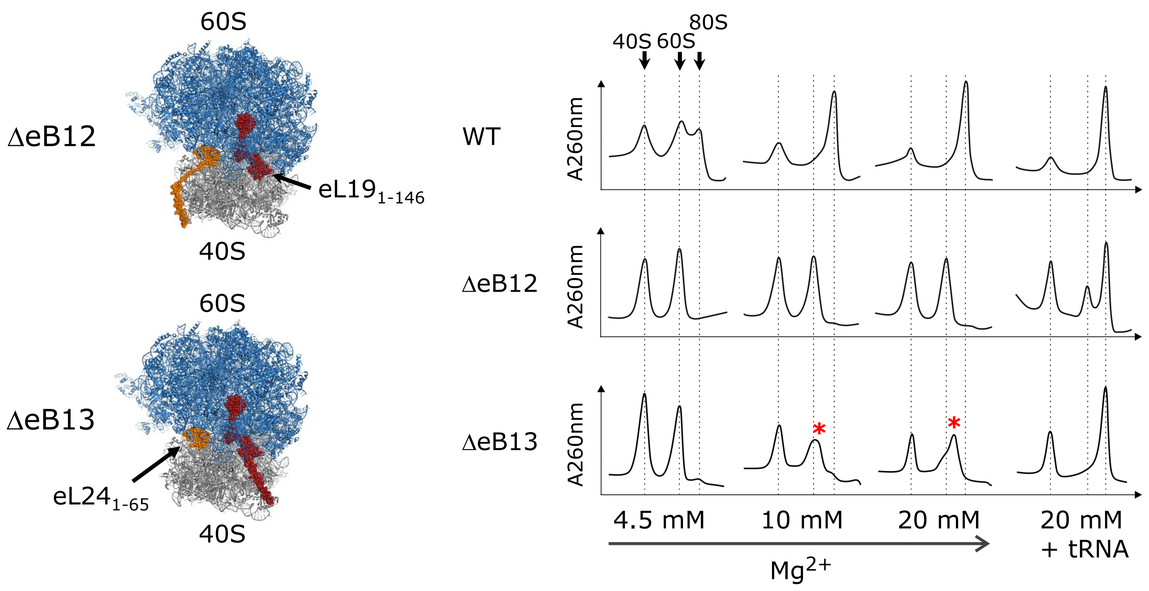
Aim of this study was to evaluate the importance of eukaryote-specific bridges eB12 and eB13 in terms of translation.

1. Ribosomal proteins eL19 and eL24



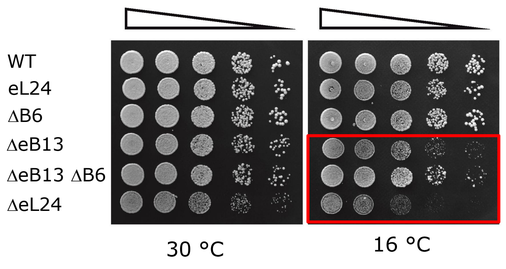
2. Bridges eB12 and eB13 are essential for stable 40S and 60S subunit reassociation *in vitro*

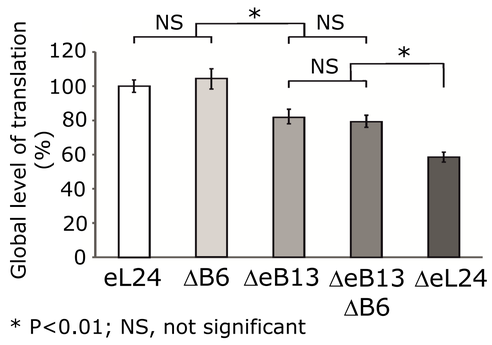
- *In vitro* reassociation of wild-type 40S subunits and wild-type or mutant 60S subunits



3. Loss of bridge eB13 leads to cold sensitivity, reduction of protein synthesis and formation of stalled translational initiation complexes

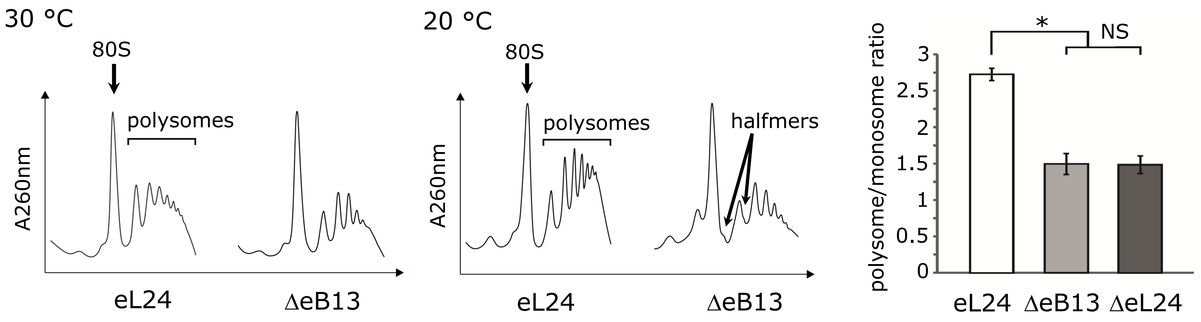
- Serial dilutions spot-test assay on YPD





* P<0.01; NS, not significant

- Ribosome-polysome profile analysis



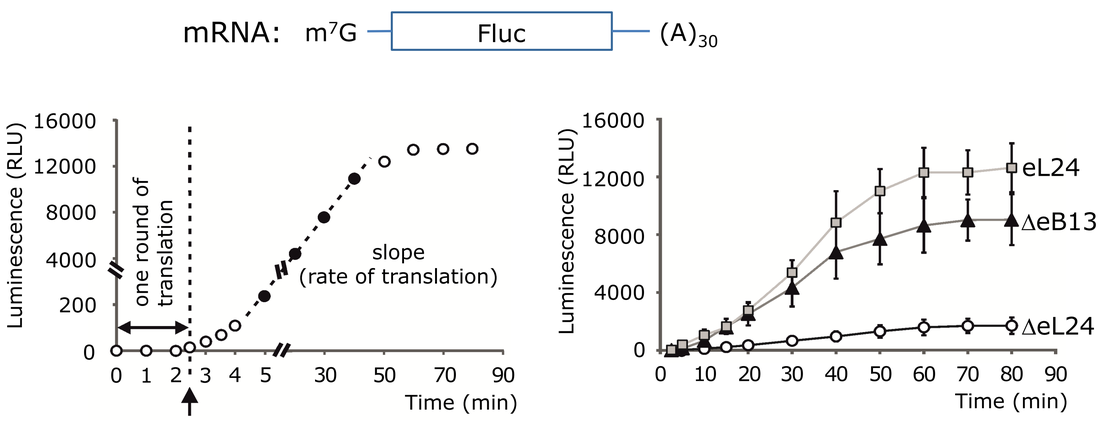
* P<0.01; NS, not significant

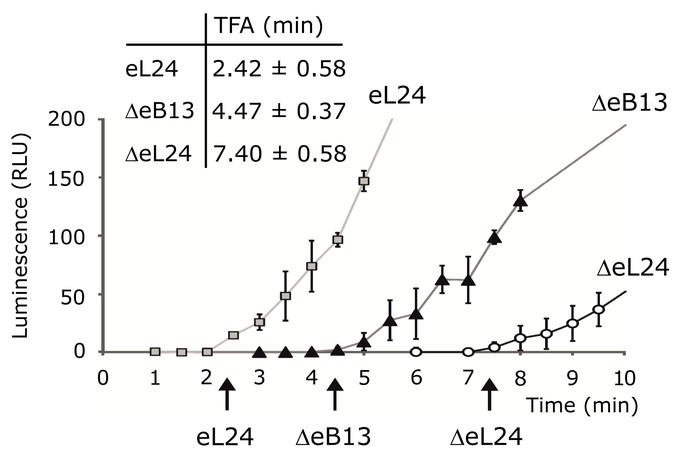
Conclusions

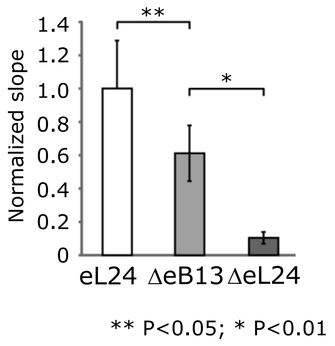
- Essential function of protein eL19, carried by the N-terminal domain and middle region, is in ribosome biogenesis (Kisly *et al*., 2016). Secondary function of eL19, provided by the C-terminal α-helical domain, is eB12 bridge formation.
- Bridges eB12 and eB13 ensure stable/correct subunit interaction.
- Bridge B6 does not play a significant role in ribosome functioning. Loss of this bridge has no apparent influence on the yeast cell growth and global level of translation.
- The eB13 bridge is important for initiation and elongation steps of translation.
- The N-terminal domain of eL24 plays a significant role at the initiation step of translation.

4. eB13 bridge forming region and N-terminal domain of eL24 are required for the efficient *in vitro* translation

- Cap- and polyA tail-dependent translation of Firefly luciferase (Fluc) mRNA in cell-free translation extracts

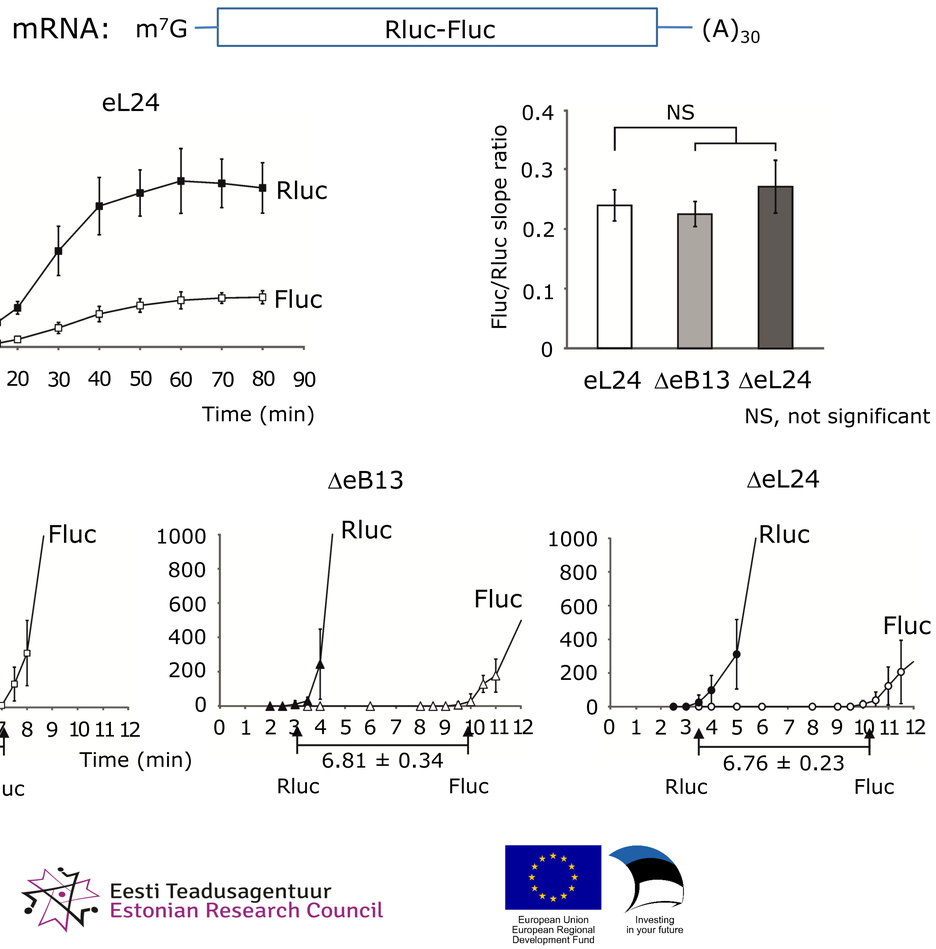






5. Loss of bridge eB13 leads to reduced rate of *in vitro* elongation

- Cap- and polyA tail-dependent translation of fusion Renilla-Firefly luciferase (Rluc-Fluc) mRNA in cell-free translation extracts



Funding: Eesti Teadusagentuur Estonian Research Council

European Union European Regional Development Fund

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